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Exploration of Algerian ecosystems for the selection of Actinobacteria belonging to the genus *Streptomyces* developing potentialities of PGPR and antagonists of wheat phytopathogens: Modeling of bioactive metabolites production

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DEDICATION

I dedicate this work to my parents, Zineb NECHMA and Kamel ALLOUN, my sweet brother Borhen Eddine, and all my family, who have given me endless love, support and advice. I would never be here without you. I love you!

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List of Abbreviations and Symbols

°C :	Degree Celsius
T :	Temperature
g :	Gram
mg :	Milligram
µ :	Micro
µg :	Microgram
µL :	Microliter
mL :	Milliliter
PGPR	Plant growth-promoting rhizobacteria
IAA	Indole-3-acetic acid
MiST	Microbial Signal Transduction
PP	Phenotypic plasticity
RSA	Root system architecture
PSMs	Plant secondary metabolites
ATP	Adenosine triphosphate
ABA	Abscisic acid
ML	Machine learning
AI	Artificial intelligence
RSM	Response Surface Methodology
ANN	Artificial neural networks
GA	genetic algorithm
SCG	Spent coffee grounds
CBP	Carob bean powder

Abstract

This work aims to isolate Actinobacteria strains with a growth promotion ability and the biocontrol potential of *Fusarium culmorum*, the wheat root rot-causing fungi. The exploration of terrestrial and aquatic Algerian ecosystems, i.e. the wheat rhizosphere in the Tiffeche region (Souk-Ahras) and the aquatic sediments of Lake Oubeira (El Taref), respectively, resulted in 102 native isolates. Therefore, 37 have morphological and cultural characteristics similar to the genus *Streptomyces*. These isolates were screened for their plant growth-promoting traits. These activities consist of the production of hydrogen cyanide (HCN), ammonia (NH_3), and indole-3-acetic acid (IAA), as well as *in vitro* antagonism against *F. culmorum*. Among the auxin family, IAA constitutes a crucial phytohormone regulating specific tropic responses of plants and functions as a chemical signal between host plants and their symbionts. IAA derived from Actinobacteria grown on agricultural waste represents a more economical alternative than its synthetic homologous. Rhizospheric isolate AW 22 was positive for HCN and NH_3 production, growth inhibition of *F. culmorum* with an index of 67.320±8.99% and high IAA content of 23.999±1.126 µg. mL^{-1} in standard growth conditions on yeast-tryptone broth (YTB) amended with 0.2% (w/v) L-Tryptophan. Thus, the AW22 isolate was selected for a polyphasic chemotaxonomic characterization and the optimization of the production process of this phytohormone. Molecular and phylogenetic analysis identified isolate AW22 as *Streptomyces rubrogriseus*, and its sequence was deposited in Genbank under accession ID OP176004. Analysis of the putative IAA produced by *S. rubrogriseus* AW22 on YTB using thin-layer chromatography (TLC) and (HPLC) revealed R*f* values equal to 0.69 and a retention time of 3.711 min, equivalent to the authentic IAA. Artificial intelligence-based approaches (i.e. Behnken design from response surface methodology (BBD-RSM) with artificial neural networks (ANNs) coupled with the genetic algorithm (GA)) were employed to bioengineer *in vitro* and *silico* a suitable medium for maximum IAA bioproduction. According to the Box Behnken Design matrix, data were based on empirical studies involving the inoculation of AW22 in various cultural conditions and low-cost feedstocks notably, the spent coffee grounds (SCGs). Four input variables comprising L-Trp (X1), incubation T° (X2), initial pH (X3) and SCG concentration (X4) were screened via Plackett-Burman design (PBD) and served as BBD and ANN-GA inputs. The IAA yield constituted the output variable (Y in µg. mL^{-1}). Upon training the model, the optimal conditions suggested by the ANN-GA model were X1= 0.6%, X2= 25.8°C, X3 = 9, X4=30%). An R^2 of 99.98%, adding to an MSE of $1.86x10^{-5}$ at 129 epochs, postulated higher reliability of the ANN-GA approach in predicting responses, compared with BBD-RSM modeling exhibiting an R^2 of 76,28%. Using the process parameters generated by ANN-GA AW 22 achieved a maximum IAA yield of 188.290±0.38 µg. mL^{-1}. This optimization resulted in a 4.55-fold and 4.46-fold increase in IAA secretion after eight days of incubation, corresponding to ANN-GA and BBD-RSM models, respectively. These results confirm the validity of both models in maximizing IAA yield from the multifunctional *S. rubrogriseus* AW22 isolated for the first time in Algeria.

Keywords. *Streptomyces*; Indole-3-acetic acid (IAA); Artificial intelligence (AI); Response surface methodology (RSM); Artificial neural networks (ANNs); Mathematical Modeling.

Résumé

L'objectif de ce travail réside dans l'isolement de souches d'actinobactéries promotrice de la croissance des plantes et ayant des potentialités de biocontrôle du *Fusarium culmorum*, agent causal de la pourriture racinaire du blé. L'exploration des écosystèmes terrestres et aquatiques algériens à savoir, la rhizosphère du blé dans la région de Tiffeche (Souk-Ahras) et des sédiments aquatiques du lac Oubeira (El Taref), respectivement ont abouti à 102 isolats autochtones dont 37 ont présenté des caractéristiques morphologiques et culturales analogues au genre *Streptomyces*. Ces isolats ont été criblés pour leurs activités promotrices de la croissance des plantes qui consistaient en la production d'hydrogène cyanide (HCN), d'ammoniaque (NH_3), d'acide indole-3-acétique (AIA) ainsi que l'antagonisme *in vitro* vis-à-vis de *F. culmorum*. Parmi la famille des auxines, l'AIA constitue une phytohormone déterminante régulant les réponses trophiques spécifiques des plantes et fonctionne comme un signal chimique entre les plantes hôtes et leurs symbiotes. L'AIA produit par Actinobactéries cultivées sur les déchets agricoles représente une alternative plus économique comparé à son homologue synthétique. L'isolat rhizosphérique AW 22 s'est avéré positif par rapport à la production d'HCN et d'NH_3, une inhibition de la croissance de *F. culmorum* avec un indice de 67.320±8.99% et une teneur élevée en IAA dans des conditions standards de croissance estimée à 23.999±1.126 µg mL^{-1} en présence de 0.2% (w/v) L-Tryptophane sur le bouillon yeast-tryptone (YTB). A cet effet, l'isolat AW22 a été sélectionné pour une caractérisation polyphasique chimio-taxonomique ainsi que la modélisation du procédé de production de AIA. L'analyse moléculaire et phylogénétique a identifié l'isolat AW22 comme *Streptomyces rubrogriseus* et sa séquence a été déposée dans Genbank sous le numéro d'accession OP176004. La chromatographie sur couche mince (TLC) et la chromatographie liquide à haute performance (HPLC) de l'AIA présomptif produit par la souche *S. rubrogriseus* AW22 sur milieu YTB a révélé des valeurs R*f* égales à 0.69 et un temps de rétention de 3.711 min, équivalent à l'AIA standard. Les approches d'intelligence artificielle, à savoir le Box Behnken Design de la méthodologie de surface de réponse (DBB-MSR) ainsi que les réseaux de neurones artificiels (RNA) couplés à l'algorithme génétique (AG), ont été utilisées pour la formulation *in vitro* et *in silico* d'un milieu optimal favorisant la production maximale d'AIA. *Streptomyces rubrogriseus* AW22 a été cultivé sous diverses conditions de culture et des substrats à haute valeur ajoutée notamment l'extrait de marc de café (EMC). Quatre variables d'entrée comprenant le L-tryptophane (X1), la température d'incubation (X2), le pH initial (X3) et la concentration du substrat (X4) ont été criblées via la matrice de Plackett-Burman Design (PBD). Ces variables ont constitué les variables d'entrées du BBD et de l'ANN-GA avec le rendement en AIA comme variable de sortie ou la réponse (Y en µg. mL^{-1}). Les conditions optimales suggérées par le modèle ANN-GA étaient X1 = 0.6 %, X2 = 25.8 °C, X3 = 9, X4 = 30 %. Un R^2 de 99.98 % et une MSE de 1.86 x 10^{-5} à 129 époques, postule une meilleure fiabilité du modèle mathématique élaborée par l'ANN-GA dans la prédiction des réponses comparée au BBD-RSM présentant un R^2 de 76.28 %. L'application des paramètres générés par ANN-GA a permis d'obtenir un rendement AIA maximal de 188.290 ± 0.38 µg. mL^{-1}. Les modèles élaborés par ANN-GA et BBD-RSM ont permis d'augmenter le rendement de l'AIA de 4.55 fois et de 4.46 fois la production initiale d'AIA après huit jours d'incubation, respectivement. Ces résultats confirment la validité des deux modèles pour maximiser le rendement en AIA à partir de la souche multifonctionnelle *S. rubrogriseus* AW22 isolée pour la première fois en Algérie.

Mots clés. *Streptomyces*; Acide indole-3-acétique (AIA); Intelligence Artificielle (IA); Méthodologie de la surface de réponse (MSR); Réseaux de neurones artificiels (RNA); Modélisation mathématique.

ملخص

يهدف هذا العمل إلى عزل سلالات أكتينوباكتيريا ذات القدرة على تعزيز نمو النباتات وإمكانية المكافحة الحيوية لفطر *Fusarium culmorum*، المسبب لتعفن جذور القمح. نتج عن استكشاف النظم البيئية الجزائرية البرية والمائية، أي جذور القمح في منطقة تيفيش (سوق أهراس) والرواسب المائية في بحيرة أوبيرا (الطارف) ، 102 و37 عزلة أصلية على التوالي لها خصائص مورفولوجية مشابهة ل *Streptomyces* .تم فحص هذه العزلات لمعرفة خصائصها المعززة لنمو النبات. تتكون هذه الأنشطة من إنتاج سيانيد الهيدروجين (HCN) ، والأمونيا (NH_3) ، وحمض الإندول -3 الخليك (IAA)، وكذلك تثبيط نمو فطر *F. culmorum*.

من بين عائلة auxin، يشكل IAA هرمونًا نباتيًا مهما ينظم الاستجابات التوجيهية و الانحناءات الغذائية المحددة للنباتات ويعمل كإشارة كيميائية بين النباتات المضيفة والكائنات الدقيقة المتعايشين معها. تمثل IAA المشتقة من الأكتينوباكتيريا المزروعة على البقايا الزراعية بديلاً أكثر اقتصادية من نظيرتها الاصطناعية. كانت العزلة AW 22 إيجابية لإنتاج HCN وNH_3، وتثبيط نمو الفطر *F. culmorum* بمؤشر 67.320 ± 8.99٪ ومحتوى IAA مرتفع يبلغ 23.999 ± 1.126 ميكروغرام /مل في ظروف النمو القياسية في مرق التربتون (YTB) المعدل بـ 0.2٪ (وزن / حجم) إل-تريبتوفان. بناءا على هذه الخصائص، تم اختيار عزلة AW22 لتوصيف مورفوكيميائي متعدد الأطوار وتحسين عملية إنتاج هذا الهرمون النباتي. صنف التحليل الجزيئي والتطور الوراثي العزلة AW22 على أنها *Streptomyces rubrogriseus*، وتم إيداع تسلسله في Genbank تحت معرف الانضمام OP176004. كشف تحليل IAA المفترض الذي أنتجته *S. rubrogriseus* AW22 على YTB باستخدام كروماتوغرافيا الطبقة الرقيقة (TLC) و (HPLC) عن قيم R*f* تساوي 0.69 ووقت استبقاء 3.711 دقيقة، أي ما يعادل قيم IAA الأصلي.

تم استخدام الأساليب القائمة على الذكاء الاصطناعي أي تصميم Box Behnken Design من منهجية سطح الاستجابة (BBD- RSM) مع الشبكات العصبية الاصطناعية (ANNs) إلى جانب الخوارزمية الجينية (GA) في الهندسة الحيوية *in vitro* و*in silico* وسيطًا مناسبًا لتحقيق أقصى إنتاج حيوي لـ IAA .وفقًا لمصفوفة BBD ، استندت البيانات إلى دراسات تجريبية تتضمن زرع AW22 في ظروف مختلفة ومواد أولية منخفضة التكلفة متل القهوة المستهلكة (SCGs). تم فحص أربع متغيرات إدخال تشتمل على(X1) L-Trp، حرارة الحضانة (X2) ° T، الحموضة(X3) و (X4) تركيز SCG عبر تصميم Plackett-Burman (PBD) وعملت كمدخلات BBD و ANN-GA. شكل محصول IAA متغير الخروج او الناتج (Y).

عند تدريب النموذج، كانت الظروف المثلى المقترحة من ANN-GAهي X1 = 0.6٪ ، X2 = 25.8°C، X3 =9، 30 ٪=X4 .يفترض R^2 بنسبة 99.98 ٪، إضافة إلى MSE من 1.86 × 10^{-5} في 129 حقبة، موثوقية أعلى لنهج-ANN GA في التنبؤ بالاستجابات، مقارنةً بنمذجة BBD-RSM التي تعرض R^2 بنسبة 28. 76 ٪.

باستخدام معلمات العملية التي تم إنشاؤها بواسطة ANN-GA، حقق AW 22 أقصى عائد IAA 0.38 ± 188.290 ميكروغرام /مل. أدى هذا التحسين إلى زيادة 4.55 ضعفًا و 4.46 ضعفًا في إفراز IAA بعد ثمانية أيام من الحضانة، بما يتوافق مع نموذجي ANN-GA وBBD-RSM، على التوالي. تؤكد هذه النتائج صحة كلا النموذجين في زيادة و تكثيف عائدIAA من *S. rubrogriseus* AW22 متعددة الوظائف المعزولة لأول مرة في الجزائر.

الكلمات المفتاحية. *Streptomyces* -حمض الإندول -3 الخليك- الذكاء الاصطناعي- منهجية سطح الاستجابة (RSM)- الشبكات العصبية الاصطناعية (ANNs)- النمذجة.

State-of-the-art and Statement of novelty

Streptomyces are a genus of ubiquitous Actinobacteria with diverse biotechnological applications. The metabolic versatility of this genus is functional in sustainable agriculture for various applications such as phytohormone production. Developing sustainable solutions to exploit spent coffee grounds (SCGs) is necessary to solve pollution-related problems. SCGs represent a by-product of coffee, a significant municipal solid waste affecting ecosystems. They are rich in nutrients and can serve as a substrate for producing microbial metabolites of interest, such as phytohormones, enzymes, and antifungals. This work presents several original traits. Actinobacteria strain *Streptomyces rubrogriseus* AW22 has been isolated for the first time in Algeria from the wheat rhizosphere. It has been found to produce remarkable amounts of Indole-3-acetic acid (IAA) via an L-Tryptophan-dependent pathway. This study is the first to report on the potential utilization of spent coffee grounds, and carob bean grounds extracts as a high-added value culture medium to optimize actinobacteria-originated IAA production in submerged conditions. The novelty also resides in using Artificial intelligence (AI)-based approaches to optimize the bioprocess. Specifically, Response Surface Methodology (RSM) and Artificial Neural Networks coupled with the genetic algorithm (ANN-GA)-assisted approaches were used as novel methods to optimize IAA production. We tested various by-product mixtures of SCGs and CBGs. The final formulation allows the obtaining of high IAA content. **The scientific novelty and relevance of this study's findings suggest the elaboration of a patent.**

Introduction

Introduction

Plants are constantly subject to biotic and abiotic stressors, threatening their optimal growth. Hence, plants deploy strategies to adapt to these demanding conditions: activating metabolic pathways and selectively recruiting a microbiome in their rhizosphere. During this time, the role of roots in overcoming these shifting environmental factors cannot be overstated (Pascale et *al*., 2020).

Plant-microorganisms multilateral interactions are driven by the plant growth-promoting rhizobacteria (PGPR) and their capacity to colonize root surfaces and benefit their hosts (Kudjordjie et *al*., 2019; Maggini et *al*., 2020). The integration of eco-friendly management techniques including biopesticides and biofertilizers constitutes a safer and cleaner alternative to chemical agroactives required for optimal plant growth (Alloun et *al*., 2023; Boubekri et *al*., 2021).

Plant growth promoting Actinobacteria possess potential bioactivity in terms of disease-suppressiveness and the biocontrol of a broad spectrum of fungal phytopathogens. Actinobacteria produce a wide array of natural agroactive compounds such as plant growth regulators (PGRs) and plant growth stimulation and biofortification (Borah and Thakur, 2020; Toumatia et *al*., 2016).

Indole-3-acetic acid (IAA) is a representative member of the Auxin family. It is an endogenous phytohormone modulating various essential plant physiological activities, from its role in shaping plant morphology to regulating physiological growth activities (Grones et *al*., 2018; Rakusová et *al*., 2019). IAA acts as a chemical signal coordinating the plant's specific phenotypic and tropic responses to environmental stimuli and controls the differentiation and cell division of vascular tissue, the initiation of lateral and adventitious roots, and the stimulation of stem and root extension (Duca and Glick, 2020). IAA also contributes to alleviate abiotic stress and contributes to the resistance of plants (Huang et *al*., 2020; Zhou et *al*., 2020).

Streptomyces is a representative genus of the Actinobacteria phylum synthesizing physiologically active IAA to be oriented for industrial Auxin production (Boubekri et *al*., 2021; Myo et *al*., 2019). Plants and microorganisms synthesize IAA via several interrelated pathways, including the tryptophan-dependent pathway (Duca and Glick, 2020; Robert et *al*., 2018).

In recent investigations, economic carbon and nitrogen sources were employed as substitutes for expensive laboratory-grade medium components (Al Farraj et *al*., 2020; Alloun et *al*., 2023).

Spent coffee grounds (SCGs) constitute a brewing process's derivate containing 75% of the original coffee bean (Wu et *al*., 2019). They are toxic pollutants rich in polyphenols, flavonoids, chlorogenic acid, and protocatechuic acid, with essential antioxidant activity (Esquivel et *al*., 2012) disturbing many life processes, adding to the massive oxygen quantity required for their decomposition (Hardgrove and Livesley, 2016; Lessa et *al*., 2018). The total polysaccharides in SCGs constitute about 45.3% (w/w, dry weight) and are non-reducing sugars (Pasin et *al*., 2011). Heat treatment, microbial degradation, and aerobic metabolism could reduce SCG toxicity (Hao et *al*., 2018).

Microbial growth and metabolic profiling, including IAA production, are complex, less predictable, and closely linked with nutritional and physiological parameters. Subsequently, machine learning (ML) modelling and optimization approaches may provide prospective substitutes for controlling or simulating targets such as microbial metabolites.

The media engineering using empirical models, such as response surface methodology (RSM) and Artificial intelligence (AI) assisted methods like artificial neural networks (ANNs), can respectively generate non-linear quadratic models, accurately predict and optimize *in silico* process parameters, study their significance and simultaneous interactions with universal approximation capability (Dragoi and Vasseghian, 2021). Moreover, these approaches can mathematically describe the phenomenon (Schmidt et *al*., 2019). ANNs are often coupled with adaptive meta-heuristic optimization algorithms, such as the genetic algorithm (GA), to increase their performance while reducing their complexity (Fan et *al*., 2018; Smaali et *al*., 2021).

The fundamental approach followed in this investigation consists of:

1. Isolating an actinobacterium with the ability to produce a considerable amount of IAA.
2. Performing a polyphasic characterization of the potent strain including morphological, physiological and molecular identification.
3. Describing its multifunctionality related with plant growth promotion and biocontrol effect, such as lytic enzymes production, phosphate solubilization, ammonia production, *in vitro* antagonism to phytopathogenic *Fusarium* species.

4. Formulating an appropriate, cost-effective medium composition to achieve maximum IAA output with SCGs as a high value-added feedstock substrate using Plackett-Burman experimental design (PBD) and Box-Behnken design (BBD) for screening significant process variables and defining their adequate range, respectively, followed by the development of a regression model.
5. Mathematically modeling the production of IAA on the optimal medium using RSM combined with ANN-GA to properly optimize the low-cost, nutritionally adequate process parameters in submerged fermentation and improve the low-cost bioprocess to generate IAA from *Streptomyces* sp.

It is the first research that investigates the efficient use of SCG or CBP as media components for the production of IAA originated from a newly isolated actinobacterium. This study provides insight into the critical operating conditions influencing the bioprocess for the semi-pilot or large-scale synthesis of agro-active compounds, including IAA, for further sustainable use in agriculture.

1-Literature Review

1. Literature review

1.1. Plant-microbiome interactions in the rhizosphere

1.1.1. Plant roots fundamental roles

As sessile organisms, plants are continuously exposed to several biotic and abiotic stresses (osmotic stress, drought, edaphic stress, heavy metals, and nutrient scarcity). These stresses constitute significant threats to crop yield and participate in constantly reducing the amount of arable land that is fit for food production through rapid deterioration of soil quality, thus menacing food security (Mahalingam, 2015). The development of abiotic stress-tolerant varieties and resistance to pathogens has gained attention (Mickelbart et *al*., 2015). However, breeding technologies require generations of trials to achieve genetic stability to reach the desired result. It is imperative to emphasize the plant roots' vital role in essential nutrient absorption, including nitrogen, phosphorus, potassium, and other micronutrients required for plant metabolism and survival.

The interaction of plants with soil microorganisms is ensured via roots. Additionally, plant roots are responsible for overcoming these changing stressful environmental conditions disrupting the plant growth process. This response can involve changes in root morphology, such as developing more profound or extensive root systems that allow them to access water and nutrients more effectively in dry or nutrient-poor soils.

Furthermore, plant roots also engage in complex metabolic and signaling pathways that involve the production and regulation of hormones and other signaling molecules, resulting in Stress-mediated physiological responses. Plant roots link between soil environments and shoot through the transduction of signals and transmission of all information from belowground to the aerial parts of the plant.

1.1.2. Plant microbiome, rhizosphere and rhizosphere competence

Plant microbiota is a designation that encompasses all the plant-associated microbes living freely or in symbiosis with their host plants (Pascale et *al*., 2020). The sum of this microbiota and their genomes are referred to as *plant microbiome*. Microbiomes are mainly composed of free-living or symbiotic bacteria (Ahmad et *al*., 2013; Nadeem et *al*., 2014; Zhang et *al*., 2011). These microbiomes may be situated in the phyllosphere, endosphere (endorhiza/inner tissues as vascular tissues or in specific structures called nodules) or the ectosphere (including rhizosphere and other outer surfaces rhizoplane) of their host plants (Pang et *al*., 2021).

Plant microbiome effectively colonizes root surfaces, thus enhancing growth and reproduction of host plants through various direct and indirect mechanisms (Kudjordjie et *al*., 2019; Maggini et *al*., 2020).

In soil, it is referred to as *rhizosphere*, the narrow zone of the soil which is in direct contact with plant roots and soaked in their particular exudates. Plant rhizosphere is essential for root activity and metabolism, where the most critical plant-microbe interactions occur (Chandra et *al*., 2018).

Subsequently, root colonization ability and development in the plant rhizosphere, known as *rhizosphere competence* (RC), appears as one of the most specific criteria for selecting plant microbiome that influences the efficacy of plant growth promotion and biocontrol of pathogens (Pascale et *al*., 2020).

1.1.3. Complex dialogue linking plants to their microbiomes

Plant-microorganism interactions are multilateral and involve chemical signaling between both parties. These interactions are significant crop influents on health, quality and productivity in a natural and sustainable agroecosystem and biosafety program (Berg et *al*., 2013).

The synthesis microbial metabolically active substances is generally dependent on environmental stimuli such as climate factors (pH, temperature, redox), nutrients availability (major and minor minerals, carbon source), and inter and intra-species interactions (Asaf et *al*., 2017; Berg et *al*., 2013; Bhatia et *al*., 2016; Compant et *al*., 2010; Mutturi et *al*., 2016).

Likewise, Microbial Signal Transduction (MiST) is involved in linking the environmental stimulus and plant chemical signaling molecules (input) with a regulatory response through gene expression, enzyme activity, and antibiotic production (output) (Ulrich and Zhulin, 2007). Secondary metabolites, such as microbial phytohormones, mediate the chemical communication between plants and their microbiome during their interaction.

1.1.4. Root exudates selectively recruit microbiomes

Belowground, a high concentration of soil microorganisms observed in rhizosphere is related to the presence of nourishing substances released from root cells in the rhizospheric space after plant metabolism**.** These substances, *root exudates* or *rhizodeposits*, are profitable for microorganisms associated with plants. Root exudates are plant photosynthates that form up to 30%–60% of the secondary metabolites PSMs. They mainly contain low-molecular-weight compounds (such as phenolics, amino acids, including L-Tryptophan, nucleotides, sugars,

terpenoids, organic acids and lipids) and high-molecular-weight compounds (such as nucleic acids, polysaccharides, and proteins). These compounds may be secreted into the rhizosphere or surrounding soils either actively using ATP as the energy source or passively through diffusion. Depending on the nature of the PSM itself, some molecules may be instantly assimilated by soil microorganisms, while others act as chemical signals among organisms (Sugiyama and Yazaki, 2014).

Notably, the active and/or passive release of PSMs vigorously participates in modelling the plant microbiome by the selective attraction of beneficial microbes and repulse to the deleterious ones forming a particular microbiome that is specific for each crop (Badri et *al*., 2013; Bulgarelli et *al*., 2012; Lundberg et *al*., 2012; Viaene et *al*., 2016). Indeed, these carbon-rich photosynthesis products create a unique and complex chemical environment and sink energy sources for the plant microbiome (Badri et *al*., 2009).

The diversity and activity of plant microbiomes are not static and remain relatively unique and specific to each crop and this variation comprising the composition in terms of diversity and density, activity and proportion of some specific species.

Interestingly, several factors affect plant microbiome including root exudates profile (Sasse et *al*., 2018; Vives-Peris et *al*., 2019), rhizodeposits (Sudha and Masilamani, 2012; Tian et *al*., 2020), host plants species and cultivars (Compant et *al*., 2019), their development stages (Schlechter et *al*., 2019) environmental stimuli, including abiotic stresses and biotic factors (Pang et *al*., 2021).

The mechanism underneath this selective recruitment is based upon the ability of featured microorganisms to metabolize specific PSMs secreted by roots, supporting their proliferation in the rhizosphere and highlighting the function of PSMs as mediators of plant-soil microbiome interactions (Sasse et *al*., 2018).

This phenomenon has been demonstrated using stable isotope tracked between plants and their associated microbes (Haichar et *al*., 2012). However, only a few reports have attempted to integrate the chemical basis and molecular mechanism into the PSMs microbiome association (Pang et *al*., 2021). Following this logic, depending on the secreted PSMs profile, some microorganisms will be advantaged over others.

Alterations in root exudates profile also affects microbial metabolism, leading to a different chemical signalling traffic linking the microbiome to its host plant (Bressan et *al*., 2009; Haichar et *al*., 2012). Several factors may influence the production, the dynamic alterations in the root exudates composition, and their relative abundances. Indeed, the exudation profile depends on location along the root, plant developmental stage, species and cultivars, and genetic modification. Moreover, the soil quality, the bioavailability of essential mineral cues vital for photosynthesis, interactions with soil microorganisms, climate conditions and the eventual presence of stresses are also to consider (Korenblum et *al*., 2020).

Root exudates are not only responsible for the selective recruitment assembly of plant microbiome and involved in plant–microbe chemical interactions in the rhizosphere (De Vries et *al*., 2020; Sasse et *al*., 2018; Yuan et *al*., 2018). But on a larger frame, they participate in what is known as the "rhizosphere effect", presented as the contribution of root exudates in shaping and assembling microbial communities between bulk soil and rhizosphere (Berendsen et *al*., 2012; Reinhold-Hurek et *al*., 2015; Sasse et *al*., 2018).

1.1.5. Recruited microbiome regulates host metabolism

According to the previous section speculations, the plant rhizosphere constitutes a key reservoir of microorganisms susceptible to selection and development as bioinoculants in modern sustainable agriculture practices. Their suitability relies on their ability to establish close contact with the plant roots (Xiang et *al*., 2012). Nevertheless, the exact mechanism by which plants endorse colonization by beneficial bacteria, including Actinobacteria, is still broadly investigated. Plant secondary metabolites are significant actors in microbe assembling and colonization of the rhizosphere, being at the same time chemical signaling molecules and nutrient sources for microbes (Berendsen et *al*., 2012). However, only a few metabolites essential for microbial assembly are identified.

Further, spotting the light on the effective participation of plant-originated compounds contribute to recruiting beneficial bacteria to their root microbiomes, their characterization, and their precise role as signaling molecules and mediators between these associates is the first step to elucidating the exact nature of the plant-microorganisms communicative network (Bodenhausen et *al*., 2013; Bulgarelli et *al*., 2012; Lundberg et *al*., 2012). The perfect coordination of complex processes in the rhizosphere is fundamental for the benefit of both parties. This coordination is established on the subtle synergetic interaction between plant

physiological balance and the accommodation of its microbiome (Berendsen et *al*., 2012). Non-surprisingly, this will require the elaboration of a descriptive and detailed multilateral chemical pathway that combines all the components involved in the forward and backward plant-microbe interactions.

These dynamic exchanges of chemical signals, including plant and microbial bioactive metabolites, and molecular pathways correspond to their specific chemical dialogue. These may constitute the same signals building up this complex, repetitive, reciprocal pattern of the synergistic interaction between plants and microbiomes and their environmental physical and chemical conditions. The realization is growing that some microbes can modulate the production of specific PSMs, including plant bioactive phytometabolites, which can influence the microbiome metabolism (Mastan et *al.,* 2019).

In function of particular chemical signals and specific features of the plant root niche, the plant-microbiome transduces the stimuli generating a *response* consisting of gene expression to provide beneficial services to host plants and perform vital multifunction. Most advantageous traits consist of disease suppressiveness (Carrión et *al*., 2019), facilitation of nutrients uptake (Zhang et al., 2019) and subsequently alleviating both biotic and abiotic stresses (Berg et *al*., 2013; De Vries et *al*., 2020; Ryan et *al*., 2008). The stimulation of the plant immune system by the plant microbiome has also been reported (Pascale et *al*., 2020; Stringlis et *al*., 2018; Vannier et *al*., 2019). However, little is known about the contribution of plant microbiomes to host PSMs production (Pang et *al*., 2021).

Nonetheless, mechanisms by which specific microbial metabolites trigger plant metabolism are elaborated and provide an initial insight into the process. Following this reasoning, microbial auxin helps to modulate the plant microbiome during any stage of plant growth by actively controlling the root exudation activity (frequency, content and quantity) through the regulation of the root phenotypic plasticity (PP), either during optimal or stress conditions.

1.2. Soil dwelling-Actinobacteria

1.2.1. Taxonomy and Genomic Diversity

Actinobacteria are filamentous Gram-positive aerobic, facultative anaerobic, or anaerobic bacteria with a high G+C content in their DNA, ranging from 51 to more than 70% (Ventura et *al*., 2007). According to Ludwig et *al*. (2012), *Actinobacteria* represents one of the largest taxonomic units among the 18 critical lineages currently recognized within the domain of

Bacteria in terms of the number and variety of identified species. The phylum *Actinobacteria* includes 5 subclasses, 6 orders, and 14 suborders. The genomic diversity of Actinobacteria reflects their biodiversity, which could have great biotechnological applications (Ventura et *al*., 2007).

However, a research update based on 16S rDNA trees by Gao and Gupta (2012) eliminated the taxonomic ranks of subclasses and suborders, elevating the former subclasses and suborders to the ranks of classes and orders, respectively. The phylum "Actinobacteria" is thus divided into six classes: Acidimicrobiia (01 order) (Norris, 2012), Actinobacteria (include 20 orders) after the classification based on the whole genome (Nouioui et *al*., 2018), Coriobacteriia (02 order) (Gupta et *al*., 2013), Nitriliruptoria (02 orders) (Ludwig et *al*., 2012), Rubrobacteria (01 order) (Suzuki, 2015), and Thermoleophilia (02 orders) (Suzuki and Whitman, 2012). Based on the Actinobacteria classification (Parte et *al*., 2020), the cited classes above include 73 families and 443 unequally distributed genera. The majority of these families (394) are within the class Actinobacteria.

1.2.2. Ecology and Habitat Distribution

Actinobacteria are ubiquitous and present in various ecosystems and adopt different lifestyles. Adapting Actinobacteria to a wide range of ecological niches including soil, fresh and salt water, and the air has increased their diversity.

Most of the Actinobacteria are saprophytes, or soil-dwelling organisms. Some species form mutualistic or parasitic associations with higher organisms including plant pathogens (e.g., *Streptomyces scabiei*, *S. acidiscabies*, and *S. turgidiscabies*) (Wanner, 2006), plant commensals (*Leifsonia* spp.), soil inhabitants (*Streptomyces* spp.) as reported by (Ventura et *al*., 2007), nitrogen-fixing symbionts (*Frankia*) (Franche et *al*., 2009). *Actinobacteria* class are associated with plants growing in different habitats and under extreme environments (Goudjal et *al*., 2013; Sahay et *al*., 2017; Singh et *al*., 2016).

1.2.3. Physiology

The population density and diversity of Actinobacteria depend on their habitat and their influencing climatic conditions. Environmental factors, such as temperature, pH, and soil moisture, influence the growth of Actinobacteria. The vegetative growth of Actinobacteria in the soil is favoured by low humidity, especially when the spores are submerged in water. In dry soils with greater moisture tension, growth is limited and may be halted.

Actinobacteria can survive mesophilic, but thermophilic conditions reaching 60°C is an enabling trait for their use as inocula (Edwards, 1993). They are considered aridity-winners by (Marasco et *al*., 2021), who stated that aridity changes the composition and interactions of the plant-microbial community. By modulating the distribution of aridity-tolerant (winners), Actinobacterial inoculations protect the plants from the deleterious effects of drought and significantly boost their measured physiological parameters (Chukwuneme et *al*., 2020).

Likewise, acidophilic Actinobacteria may be necessary to inoculate plants that grow in acidic soil (Bull, 2010). Most Actinobacteria grow in soils with a neutral pH with an optimal growth between 6 and 9, with maximum growth around neutrality. Many halotolerant Actinobacteria have been isolated from saline environments and proved effective crop protection agents in harsh settings (Qin et *al*., 2018; Siddikee et *al*., 2010; Zhou et *al*., 2017). Some Actinobacterial strains' extremophile nature could be a valuable tool for rehabilitating degraded areas under extreme environmental conditions, and they can boost crop production under a variety of stress conditions, including extreme temperatures, pH, salinity, and drought (Qin et *al*., 2011).

1.2.4. Biotechnological roles

Fay et *al*. (2015) indicate that soil is complex, diverse, and nutrient-limited. It has a high microbial diversity and a wide range of microbial interactions. Secreting various secondary metabolites allows soil-derived bacteria to compete, communicate, or form mutualistic interactions with other species (Tyc et *al*., 2017). Actinobacteria play an essential role in soil microbial interactions due to their considerable secondary metabolic ability and relative abundance in soil. The production of various secondary metabolites, particularly antibiotics, is Actinobacteria's most useful potential, accounting for nearly 40% of the bioactive microbial metabolites found in the last 70 years (Bérdy, 2012).

Actinobacteria frequently outcompete other microbes in the community by antibiosis (Abrudan et *al*., 2015; Kinkel et *al*., 2014). Antibiotics, conversely, may operate as signals or cues for inter-microbial communication, according to another study (Ratcliff and Denison, 2011; Romero et *al*., 2011). Furthermore, the generation of secondary metabolites is typically dependent on resource levels. Nutrient deficiency can indicate high cell density and triggers secondary metabolism (Frank, 1994). In contrast, adequate nutrients are required for a strain to afford the cost of antibiotic synthesis (Abrudan et *al*., 2015). These perspectives highlight the complexities of Actinobacterial interactions.

1.2.5. *Streptomyces*, a representative genus

The *Streptomyces* genus belongs to the most prominent family Streptomycetaceae (order Actinomycetales). This family comprises more than 700 species. They are Gram-positive, neutrophilic, facultative aerobic, mesophilic filamentous bacteria with a growth temperature between 25 and 35°C, whose DNA has a G + C content higher than 70% (Botas Muñoz, 2013; Law et *al*., 2017) and linear, moderately large genomes (8-10 Mb), regarded as special features among bacteria (Hopwood, 2019).

The ecologically and industrially important genus *Streptomyces* is a bacterial taxon commonly found in soil and host-associated microbiomes. Interestingly, some *Streptomyces* bacteria exist also in a variety of environments, such as extreme environments and underexplored habitats, terrestrial and marine regions, marine symbionts, plant root symbionts in the rhizosphere, growing on gamma-irradiated surfaces or thermal springs, endophyte and mangroves. Over 850 *Streptomyces* species have been studied (Aryal et *al*., 2020; Kemung et *al*., 2018).

Streptomyces-derived bioactive natural compounds possess ecological and medical importance. These biomolecules function as antimicrobial, antiviral, cytotoxic, antitumor, antihypertensive, immunosuppressive, insecticide, antioxidative, plant growth-promoting, and herbicidal agents (Pham et *al*., 2021; Sharma et *al*., 2021).

1.2.6. Complex genome, complex Life Cycle

Species of the *Streptomyces* genus also have a well-studied, complex life cycle that classically encompasses three morphologically differentiated growth stages (Elliot et *al*., 2008). *Streptomyces* species are spore-producing bacteria. The bacteria life cycle begins with the germination of spores. The germ tubes grow by extending the hyphae's tips, then branch. Eventually, a dense network of vegetative cells forms the vegetative mycelium (Jones and Elliot, 2017).

Streptomyces' growth cycle has several stages: vegetative growth, aerial hyphae formation, and sporulation. These stages are subject to different genetic controls. The *bld* genes regulate the formation of aerial hyphae. The *bld* genes regulate the production of specific proteins of aerial hyphae and, in some cases, activate a specialized metabolism (Hengst et *al*., 2010). Subsequently, *whi* genes are activated that encode regulatory proteins, which promote cell division, chromosomal segregation, and spore maturation (Bush et *al*., 2013). The filamentous life cycle is particularly efficient in the soil environment.

Streptomyces have multiple biosynthetic gene clusters (BGCs) on each genome, which are the source of numerous bioactive compounds with medical or agricultural use (Nicault et *al*., 2021; Ward and Allenby, 2018). The biosynthesis secondary metabolites in *Streptomyces* species is regulated through multiple regulatory pathways induced by nutritional and environmental stimuli (Nah et *al*., 2021; Sun et *al*., 2017).

1.3. Multifunctionality of Actinobacteria and specific interactions with their symbionts

1.3.1. Actinobacteria's specific interactions with plant symbionts

The abundance of the *Streptomycetaceae* family, which is directly related to the vast array of secondary metabolites provided by their large genome, justifies the enrichment of Actinobacteria in the plant rhizosphere. Furthermore, to take advantage of their root exudates and rhizosdeposits, rhizospheric and plant-associated Actinobacteria actively colonize root surfaces and establish adjacent associations with their host referred to as *actinorhizal*-type associations and providing beneficial services to their hosts (Rey and Dumas, 2017).

While certain Actinobacteria strains that coexist with plants in a mutualistic symbiosis can interact with them as free-living, non-symbiotic bacteria (rhizospheric, exophytic), others dwell intracellularly when they adopt a (endo)symbiotic type of association and play an important role in the endosphere (Kunova et *al*., 2016; Viaene et *al*., 2016).

The penetration of endophytic Actinobacteria inside plant tissue via the lateral root hair openings is ensured with a hyphae structure that allows a solid cleavage for the rhizospheric soil particles forming a powerful bond with the plants. Curiously, pathogenic Actinobacteria species penetrate plant cell walls using the exact mechanism (Bonaldi et *al*., 2015; Seipke et *al*., 2012). Nonetheless, the precise biology of endophytic species living inside plant roots, as well as the genetic foundations regulating their strategies of penetration and avoidance of the host immune response, are yet unexplained.

Likewise, genes involved in the symbiosis of *Streptomyces* spp. with their host plants and the plant genetic background regulating these interactions still need to be discovered, contrary to *Frankia* spp. (Seipke et *al*., 2012; Sellstedt and Richau, 2013).

Actinobacteria's high survival rate and resilience to adverse environmental factors are based on their complex developmental program represented by their filamentous morphology, which also allows them to grow by hyphal-tip extension. As a result, they are qualified to compete

with the rest of the plant microbiome for space and nutrients in root exudates (microbe-microbe interactions).

Furthermore, their filamentous biomass contributes to improving the soil's texture and structure (Hamedi and Mohammadipanah, 2015). The ability of sporulating Actinobacteria to withstand prolonged periods of biotic (the presence of other microbial competitors) and handle abiotic stress (drought, heat, irradiation, and nutritional deprivation) is conferred by the formation of distinctive, thick-walled, latent spores (van der Meij et *al*., 2017).

1.3.2. Plant growth promoting activities of Actinobacteria

Production of siderophores by Actinobacteria competitively increases the bioavailability of ferric ion for their host plants while depriving pathogenic fungus of it (Rungin et *al*., 2012). Additionally, through the *rhizosphere effect*, they support the selective assembling of advantageous bacteria and mycorrhiza in the plant microbiome as they convert root exudates into other metabolites absorbed by other rhizospheric microorganisms (Kurosawa et *al*., 2008). There have been reports of lytic enzyme synthesis by Actinobacteria strains. Some of these enzymes contribute in organic matter degradation by decomposing complex natural polymers like lignocellulose (cellulose, hemicellulose, and lignin), while others, like chitinases, participate in the destruction of fungal cell walls, thus having a biocontrol effect (Sivakala et *al*., 2021; van der Meij et *al*., 2017).

Additionally, it has been suggested that several Actinobacteria species contribute to the cycling of minerals and nutrients by enhancing their bioavailability in the rhizosphere, which benefits plant nutrition. In trees and shrubs, actino-nodules formed by nitrogen-fixing Actinobacteria, such as *Frankia* species and *Micromonospora* species, have been reported (Kucho et *al*., 2010).

Actinobacteria isolated from the plant root environment, such as *Streptomyces* species, assimilate the nutrients present in the rhizodeposits and, in return, they boost their plant symbionts' resistance to biotic stressors in two distinct ways.

Figure 1 demonstrates the mechanisms by which Actinobacteria boost plant growth and the immune system and protect pathogenic microorganisms, which constitute the multifunctionality of these filamentous bacteria.

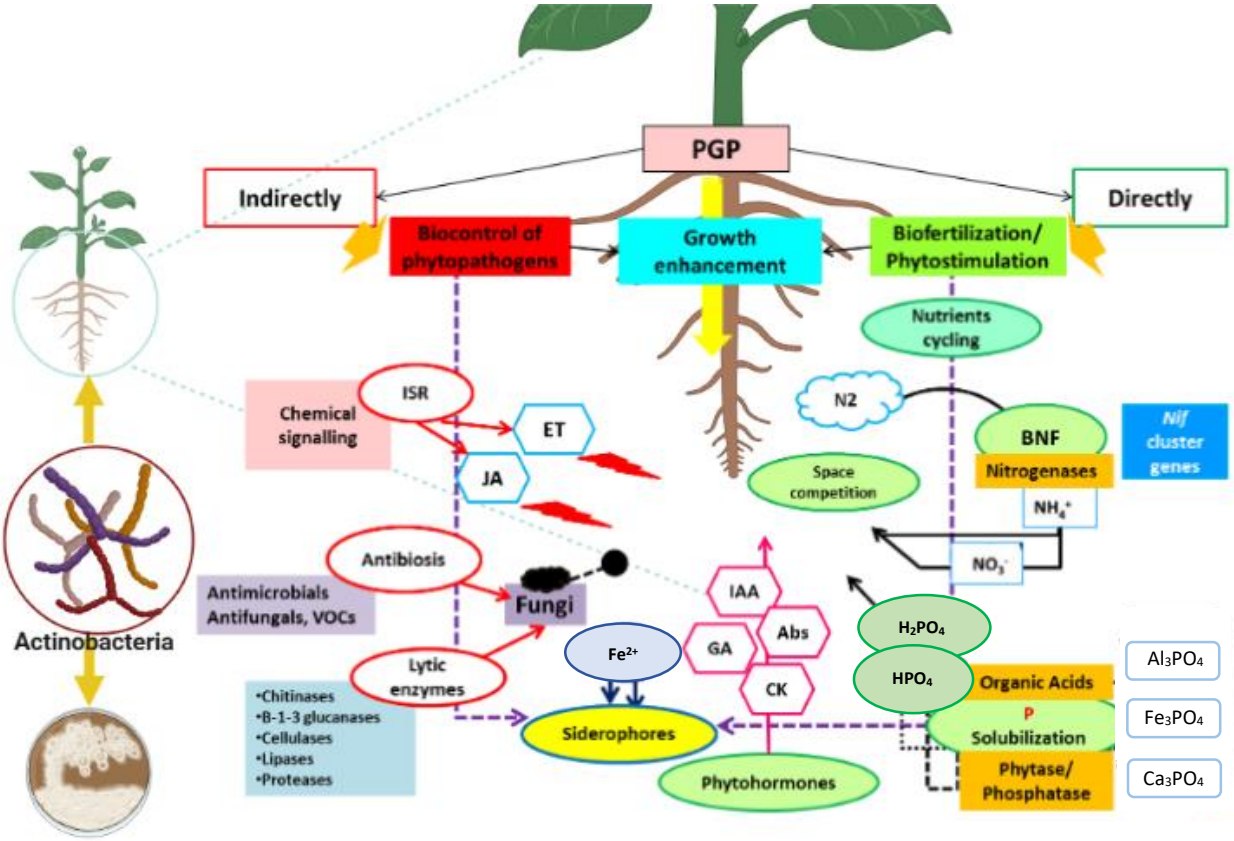


Figure 1. Multi-functionality of Actinobacteria in plant growth promotion (Personal figure, made with www.Biorender.com).

They directly provide what is known as plant disease suppressiveness and the biocontrol effect, which is the first line of defense against microbial pathogens (Cha et *al*., 2016). The biocontrol of phytopahogens may involve antibiosis and hyperparasitism. The second strategy consists of eliciting an *induced systemic resistance* (ISR), a plant immune response, which enables the plant to shift into a state of enhanced defensive aptitude (Pascale et *al*., 2020; Rey and Dumas, 2017).

The release of moderate amounts of phytohormone/phytohormone-like molecules, such as auxins, cytokinins and gibberellins, and abscisic acid (Jog et *al.*, 2016). These molecules serve as a chemical signaling between Actinobacteria symbionts and their host plants, further underlines the importance of *Streptomyces* spp. as root-associated Actinobacteria in plant health and physiology.

Phytohormones production is typical to several plant growth-promoting rhizobacteria are regarded as an asset for stress alleviation activities and significant improvement of plant growth (Rashad et *al*., 2015). Thus, plant-Actinobacteria communication also relies on releasing

various phytohormones, Actinobacteria-originated enzymes inhibiting ethylene biosynthesis and contributing to stress alleviation (Bhattacharyya and Jha, 2012; Palaniyandi et *al*., 2011, 2014).

Some Actinobacteria species and more specifically *Streptomyces* spp. contribute to mitigate eventual adverse environment restricting plant growth, thereby protecting plants and preventing the proliferation of soil-borne plant pathogens. The multifunctionality of Actinobactria features them as interesting metabolic engineering targets for the formulation of biopesticides and biofertilizers to stimulate plant growth (Olanrewaju and Babalola, 2019).

1.3.3. Root exudates modulation of secondary metabolite regulation in Actinobacteria

Actinobacteria's abundance in the rhizosphere correlates positively with the concentration of root exudates, thus selectively recruiting species over others. Since root exudates are carbon and nitrogen-rich components, their large quantities constitute an excellent nutrient source for plant microbiome and root-associated-Actinobacteria Actinobacteria (Haichar et *al*., 2008; Huang et *al*., 2014). They act as chemoattractants of Actinobacteria strains. Salicylic acid and jasmonate plant signaling pathways may be implicated in regulating *Streptomyce*'s symbiosis with plants (Lebeis et *al*., 2015).

The role of salicylic acid, the pathogenic stress phytohormone, has been suggested to attract antagonistic Actinobacteria to plant roots when exudated into the soil. This relationship has been elaborated based on evaluating the effect of salicylic acid absence on the incidence of specific antibiotic-producing species in the disrupted salicylic acid pathway mutants of *A. thaliana*. However, the exogenous application of salicylic acid positively increased the occurrence of particular species, including *Terracoccus* and *Streptomyces*. It emphasized the role of root exudates in the recruitment of multifunctioning Actinobacteria for root colonization and indirect plant growth promotion (Lebeis et *al*., 2015). These authors suggest the involvement of salicylic acid in the recruitment of beneficial antibiotic-producing Actinobacteria highlighting its role as a chemical mediator of the communication pathway between plants and Actinobacteria during exposure to pathogenic stress.

Root exudate composition affects the abundance and assemblage of the Actinobacteria community in the rhizosphere, a significant part of their microbiome. In return, Actinobacteria stimulate their host plant's immune pathways via recognizing general microbe-associated

molecular patterns (MAMPs), increasing the resistance of the plants to pathogens systemically induced plant immunity (Conn et *al*., 2008; Pieterse et *al*., 2012). However, the specific metabolites produced by *Streptomyces* spp. triggering plant immunity has not been identified yet. Nonetheless, the exact mechanism by which this chemical signaling influences the diversity and incidence of rhizospheric Actinobacteria species by regulating their attraction to root microbiome, root colonization and inducing the expression of secondary metabolites, such as antimicrobials, bacterial auxin, siderophores and lytic enzymes to assist the plant in their growth and tolerance during challenging conditions remain unclear.

Hypothesis on how plant-originated carbon sources influence secondary metabolite production in Actinobacteria by inducing or repressing gene expression has gained interest. Several reports are precise that beneficial and pathogenic Actinobacteria strains are both attracted by plant rhizodeposits (Loria et *al*., 2006). For instance, *Streptomyces scabies* causes common scab disease in potato and taproot crops by synthesizing the phytotoxin thaxtomin, a nitrated dipeptide that inhibits cellulose leading to plant cell death. However, the production of thaxtomin by *S. scabies* responds to root exudates-originated chemoattractants. Thus, it links the chemical environment set by the root exudates components and its effect on the metabolic response in Actinobacteria.

Furthermore, root exudates-originated organic acids implicated in the phosphorus solubilization were found to attract specific plant growth-promoting microorganisms to the plant microbiome (Bais et *al*., 2006). However, the exclamation resides in whether some specific exudates participate in the dynamic recruitment of Actinobacteria species to the root microbiome because of their utilization by these specific species or their role as chemoattractants (Viaene et *al*., 2016). Besides, essential elements deficiency caused by their absorption by plant roots may trigger antibiotic production in Actinobacteria for competition for these vital elements. Moreover, the composition and dynamic of root exudation by maize plants are affected by the scarcity of macronutrients which certainly affects the dynamism in the primary and secondary metabolism of its microbiome (Carvalhais et *al*., 2015).

Nonetheless, it is necessary to understand better the specific interactions between plants and their associated Actinobacteria and the involvement of root exudates originated sensing-molecules in chemical pathways. Thus, the metabolic response will allow us to conceive a spatiotemporal dynamics model of the bidirectional signal exchange from the exudation of

specific molecules to the regulation of related secondary metabolites in Actinobacteria in the root system in real-time *in vivo*. These investigations may improve this beneficial multifunctioning bacteria's biocontrol and phytostimulation potential.

1.4. Plant growth regulators (Phytohormones)

Every molecule that is involved in the regulation of plant physiological and morphological responses during their development is referred to as a *phytohormone* (Pang et *al*., 2021). Phytohormones play a prominent role in controlling plant metabolism and growth (Kazan, 2013) and their responses to various environmental stresses (Hu et *al*., 2013). The chemical structures of the plant growth regulators classes are represented in Figure 2.

Phytohormones stand as an integral part of the plant defense system, commonly known as the plant's systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Chen et *al*., 2020). Phytohormones are also implicated in the interactions between plant and their microbiomes (Pang et *al*., 2021).

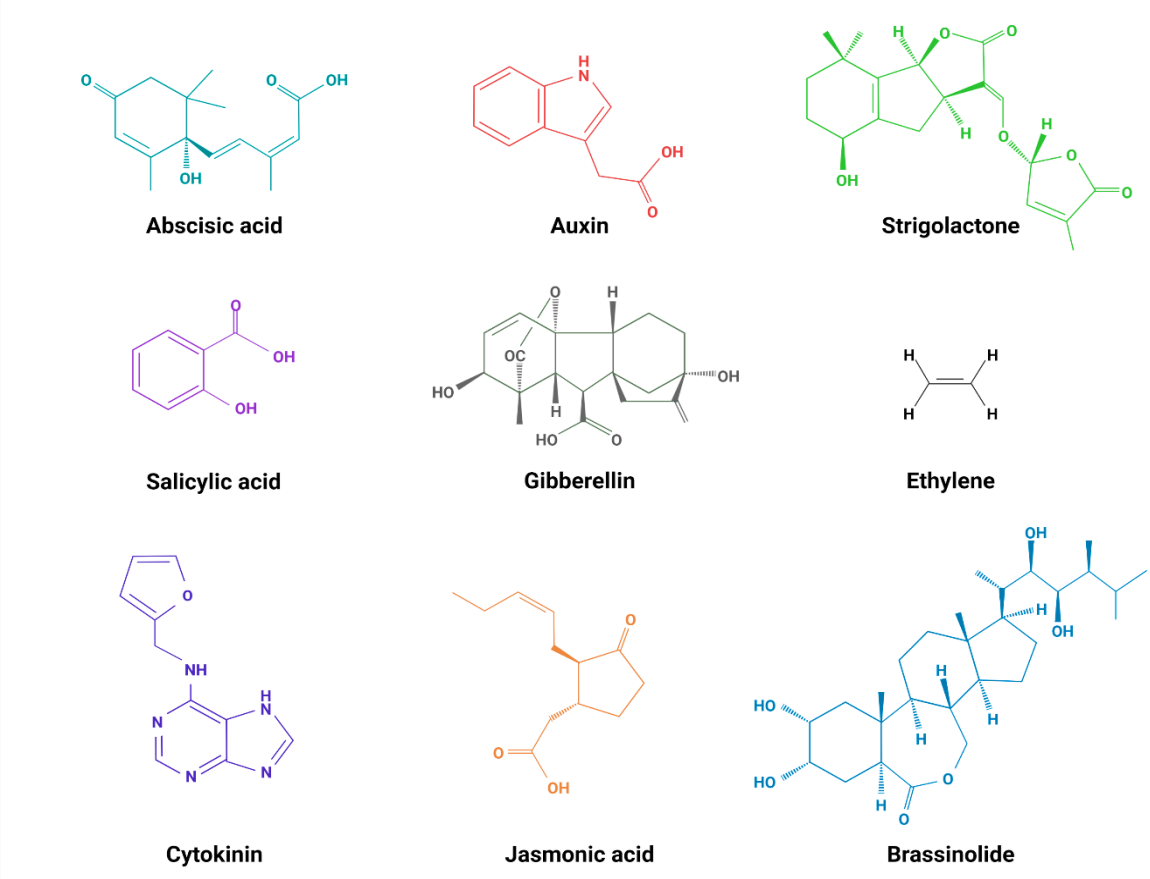


Figure 2. Chemical structures of the main classes of plant hormones Personal figure, made with www.Biorender.com).

1.5. Indole 3-Acetic Acid

1.5.1. Chemical properties of Auxin, the growth hormone

Auxins are a family of plant hormones possessing indole ring compounds (Khamna et *al*., 2010) and controlling gene expression during several processes of plant cell growth and development (Bhoi et *al*., 2021).

Indole-3-acetic acid ($C_{10}H_9NO_2$) (IAA; also designated by auxin) is the most abundant, naturally occurring and physiologically active endogenous form in plants (Lebrazi et *al*., 2020). IAA is a monocarboxylic acid (acetic acid) with the molecular weight of 175.18g/mol and in which 1H indol 3-yl group substitutes one of the methyl hydrogens present in this acid. IAA is responsible for most auxin-mediated effects inducing plant growth. IAA is produced mainly from tryptophan, an essential aromatic amino acid. Four other forms of natural endogenously produced auxins constitute of indole-3-butyric acid (IBA), indole-3-propionic acid (IPA), 4-chloroindole-3-acetic acid (4-CI-IAA), and 2-phenylacetic acid (PAA). In its active form, IAA generally makes up only ~ 25% of the total IAA level present in a plant. However, this percentage depends on the plant species, the type and age of a particular plant tissue (Duca and Glick, 2020).

1.5.2. Root phenotypic plasticity and specific plant tropic responses

Auxin activity is related to low concentrations. This phytohormone activates of signal transduction pathways for the induction of genes, synthesis of proteins and metabolites required for the plant tolerance to stressors. Local endogenous auxin accumulation and biosynthesis in plant's tissues, referred to as *auxin maxima*, drive root and shoot tropisms (Figure 3) (Ding et *al*., 2011). Plant tropism includes *phototropism*, *gravitropism*, *halotropism* (Galvan-Ampudia et *al*., 2013) and *thigmotropism (obstacle avoidance)* (Zhang et *al*., 2020).

In addition, *auxin maxima* were directly connected to embryogenesis and organogenesis, i.e. vascular tissue development, cell elongation, tissue expansion and apical dominance and fruit formation (Etchells, 2016). However, auxin is involved in the regulation of genes (Gupta, 2018). In addition, phytohormones production, such as ethylene (Woodward and Bartel, 2005), have also been reported. With auxin maxima (Brumos et *al*., 2018), the asymmetrical distribution of auxin is established by a directional active cell-to-cell polar auxin traffic.

Auxin transport plays critical functions in auxin homeostasis and is mediated by PIN-FORMED (PIN) proteins (Park et *al*., 2017). PINs are auxin-efflux carriers belonging to a class

of transmembrane proteins. They exhibit polar localization on the plasma membrane (PM) or on the endoplasmic reticulum (ER) to intermediate the direction of auxin flow. Thus, they preserve inter and intracellular auxin balance (Łangowski et *al*., 2016; Nodzyński et *al*., 2016).

PINs control root tropism in response to environmental cues. Indeed, external signals trigger the intracellular traffic of PIN2, creating asymmetrical auxin accumulation and cell extension (Galvan-Ampudia et *al*., 2013). For instance, the asymmetrical degradation of PIN2 mediated by proteasome in cells modulates root gravitropism. Halotropism and negative phototropism were also modulated by PIN2 (Galvan-Ampudia et *al*., 2013).

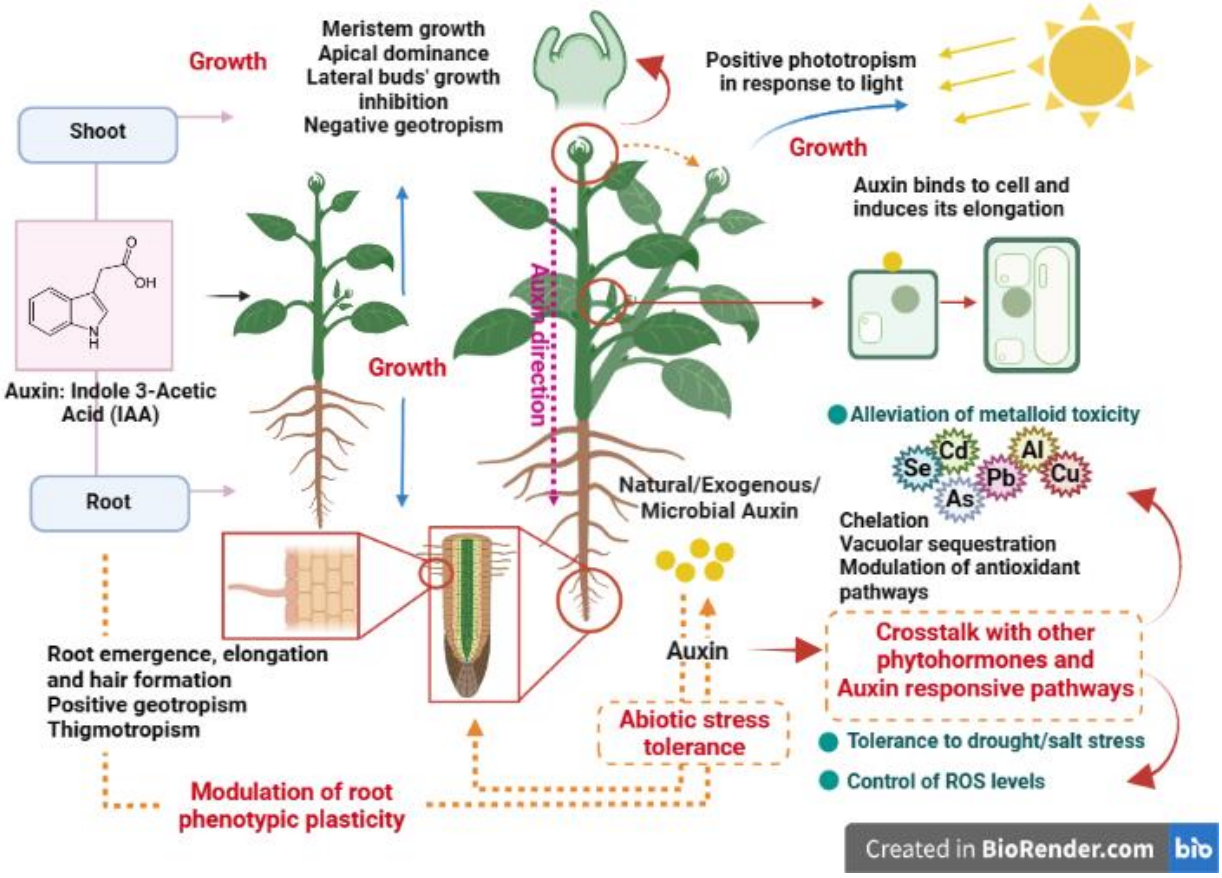


Figure 3. Specific phytotropic responses mediated by auxin signaling and modulation of root system architecture (RSA) (Personal figure, made with www.Biorender.com).

Another essential role involving PIN2 is the avoidance of roots to mechanical obstacles, which has been demonstrated by (Lee et *al*., 2020; Zhang et *al*., 2020). They indicated that PIN2 mediates the root bending during thigmotropic responses and obstacle avoidance through the asymmetrical accumulation of auxin in the concave side when roots are touching the barrier; for accelerating root growth.

1.5.3. Auxin-improved stress tolerance and adaptability in plants

Auxin can alter and modulate RSA, expressed in roots' developmental PP (Huang et *al*., 2020; Zhou et *al*., 2020). These changes are directly or indirectly related to abiotic stress alleviation via alterations in auxin gene expression patterns (Korver et al, 2018). However, explaining the signaling pathways driving abiotic stress tolerance and changes in endogenous hormone levels is essential. Auxin crosstalk with other phytohormones has also been described. Nonetheless, its implications in activating the auxin signal transduction cascade and regulating stress-responsive gene expression still require verification.

Individual or combined (multi) stress may affect plants' synthesis, transport, and pool of auxin, inducing stress tolerance (Bouzroud et *al*., 2018). Plants respond to adverse/suboptimal conditions by activating the synthesis of several enzymes and triggering antioxidant activities in order to lessen stress-related damage (Yadav et *al*., 2021). Abscisic acid (ABA) and IAA cooperate to maintain root growth. Reactive oxygen species (ROS) levels are regulated by auxin. Nevertheless, ascorbate peroxidase 6 controls the interaction between ROS, auxin, and ABA and boosts tolerance to drought stress (Jogawat et *al*., 2021).

Table 1 summarizes plant response and auxin regulation during the exposure to some metalloids and other abiotic stresses.

Metallic stress

The function of IAA as a modulator of various aspects of plant growth and tolerance to heavy metals-related toxicity has been the subject of speculation (Sytar et *al*., 2019). Numerous microRNAs, including miR393 (Yuan et *al*., 2019) and miR160 (ARF4), imply auxin signaling for drought stress in response to environmental stimuli (Bashir et *al*., 2019). Metallic triggers alter the expression of miRNAs 164, 167, and 390 influence the regulation of auxin (Singh et *al*., 2021). For instance, as a negative impact of aluminum (Al), Wang et *al*. (2016) connected the restriction in IAA transport from shoot to root with the reduction of root growth and PP in *Medicago sativa*.

However, the exogenous application of IAA as a priming substance could enable the plant to reduce Al stress by sustaining the expression of the *AUX1* and *PIN2* genes. Uncertainty still exists regarding the precise mechanism underlying the reduction of heavy metal stress. Though,

using exogenous auxin as a treatment restored the endogenous hormonal equilibrium, attenuating heavy metals' negative impacts on auxin metabolism (Table 1).

Drought stress

Tryptophan-2-Monooxygenase overexpression increases auxin levels under water deficits, improving plants' tolerance to drought (Bielach et *al*., 2017). During drought stress, DREB2A/B overexpression is followed by increased transcription of three AUX/IAA suppressor proteins, which regulate stomatal closure and decreased expression of WRKY63 and regulates the formation of GLs aliphatic compounds (Salehin et *al*., 2019). To ensure plant halotropism, AUX /IAA influx carriers compete with phosphatidic acid (Han et *al*., 2017).

The exogenous application of IAA on white clover (*Trifolium repens*) resulted in IAA-mediated physiological alterations under drought stress conditions. Plants treated with exogenous IAA and L-2-aminooxy-3-phenypropionic acid (L-AOPP) exhibited an increased relative water content (RWC) and total chlorophyll content (Chl) compared to control suggesting a substantial improvement in white clover's drought tolerance induced. This tolerance is due to the eventual alteration of the endogenous plant hormone pool, which was followed by an increase in ABA and jasmonic acid (JA) levels and the modulation of drought-responsive genes and the genes associated with senescence (SAG) (Y. Zhang et *al*., 2020).

Salinity stress

Salt stress alters the cellular localization of PIN2 and inhibits *PIN2* expression (Sun et *al*., 2008). Auxin-mediated response to salinity is mainly expressed by an increase in lateral root number while concurrently decreasing their elongation. This ability was suppressed in auxin signaling mutants *axr1*, *axr4* and *tir1*, while inhibiting the lateral root emergence in the auxin influx mutant *aux1* (Wang et *al*., 2009). IAA signaling has been linked to the modulation of the membrane-bound transcription factor NTM2, which attenuates salinity-related damages, according to the studies on the overexpression of the *IAA30* gene of the NTM2 conducted by Jung and Park (2011).

Flavonoid accumulation in plant tissues is accredited to auxin-regulating activity and has been reported to inhibit auxin degradation (Blakeslee et *al*., 2019). During the salt stress response, flavonoids bound to Glutathione 5-transferase phi-2 transcripts to control auxin trafficking

(Blakeslee et *al*., 2019; Salem et *al*., 2020). Moreover, salinity changes the selective absorption of ions, resulting in a low Na^+ buildup in the shoot tissues (Kumar Sharma, 2018).

Secondary messengers are crucial for osmotic stress resistance, including -stomatal closure, including Ca^{2+} released by the plasma-membrane protein OSCA1 (Kumar Sharma, 2018). Elevated sodium concentrations activate the Na^+/H^+ antiporter and regulate ionic homeostasis by triggering a cytosolic calcium signal. Controversy, phospholipase D alpha 1 action leads to an accumulated phosphatidic acid and stimulates the MPK6 to remove sodium from the cell via SOS1 antiporter. Heat stress also impacts membrane fluidity and causes enhanced Ca^{2+} influx and fast --ROS generation (Kumar Sharma, 2018).

Oxidative stress

Osmotic, ionic, and metalloid stressors generate ROS signals (Landi et *al*., 2020), destabilising the redox balance and eliciting oxidative stress by inducing the oxidative degradation of biomolecules (Ogbe et *al*., 2020). The induction of *YUC6* gene expression associated with the thiol-reductase activity suppresses ROS activity and preserves IAA (Fattorini et *al*., 2017).

Response to metabolic leaks in plants helps mitigate stress's effects and restrict ROS production in the mitochondria and chloroplast (Choudhury et *al*., 2017). In response to drought, H_2O_2 and O_2 levels drop through an auxin-dependent pattern, accompanied by an increase in auxin levels (Bielach et *al*., 2017).

Plants synthesize secondary metabolites, including enzymes like peroxidases, lactases, and catalases (Hasanuzzaman et *al*., 2019; Vaish et *al*., 2020) and non-enzymatic antioxidants such carotenoids, phenols, and ascorbate to mitigate oxidative stress (Hasan et *al*., 2019). IAA peroxidases, for instance, control IAA levels during root growth and hypocotyl elongation, while carotenoids, phenols, and ascorbate affect auxin stability (Bielach et *al*., 2017).

Temperature and shade stresses

The amount of heat shock proteins (HSPs) increases in the cytoplasmic pool to enable the stabilization of auxin receptors TIR1 for proper high-temperature-induced responses in hypocotyl and root development (Wang et *al*., 2016).

Further, YUC2, YUC5, YUC8, and YUC9 levels augmented after shade treatment which increases auxin production before returning to the baseline after 96 hours (Müller-Moulé et *al*., 2016), and auxin production increased in response to shade stress (Mroue et *al*., 2018). Moreover, during shade stress, the cytokinin oxidase *AtCKX6* gene overexpression stimulates auxin synthesis for modulating cytokinin levels (Mroue et *al*., 2018).

Table 1. Auxin-mediated plant responses to various stressors.

Stress	Auxin-mediated plant responses to stress	Reference
Selenium (Se) and Arsenic (As) stress	The restriction in auxin and ethylene production impacts root development and primary metabolism.	Malheiros et *al*. (2019)
Cadmium (Cd)-induced stress	Se improved tobacco plant development by increasing auxin levels.	Luo et *al*. (2019)
Aluminium (Al)	Boron (Bo) generates increased Al accumulation while reducing Al toxicity (Singh et *al*. 2021) by boosting auxin-mediated root surface alkalization and regulating the proton pump H^+-ATPase, therefore improving Al assimilation.	Li et *al*. (2018)
Cd^{2+}	Exogenous NAA application increases hemicellulose 1 content in *Arabidopsis* root cell wall, thus the retention of Cd^{2+} in roots blocking its translocation to shoot parts and resulting in an auxin-induced decrease in Cd^{2+} toxicity.	Zhu et *al*. (2013)
Lead (Pb)	An exogenous supply of IAA at low concentrations (10^{-10} M) was found to alleviate the toxic effect of Pb on sunflower *Helianthus annuus*. The auxin-induced plant response resulted in an increased root volume, surface area and diameter, suggesting the plant's IAA-induced phytoextraction of lead and zinc.	Fässler et *al*. (2010)

Cd and salt stress	The auxin conjugate (IAA-Asp) modulates catalase and peroxidase activity and induces protein carbonylation in Cd stress-exposed pea plants (*Pisum sativum* L.).	Ostrowski et *al*. (2016)
Drought or osmotic stress	The root system architecture modulates the hydrolase IAR3 (IAA-Ala Resistant3) to convert inactive conjugate auxin form (IAA–amino acid) to bioactive auxin form stimulating lateral root development.	Kinoshita et *al*. (2012).
Salinity	A significant decrease in IAA levels was followed by an increase in other phytohormones, such as ABA, suggesting the crosstalk between IAA and ABA and providing helpful information on IAA-improved salinity tolerance in plants.	Iqbal and Ashraf (2013)
Drought stress	ABA modulates auxin transport to control primary root and root hair growth in both *Arabidopsis* and rice and thus induces drought-mediated alterations in root architecture involving a higher proton secretion process through the action of plasma membrane-located H-ATPases, required for maintaining root elongation.	Xu et *al*. (2013)
Cd	Exogenous IAA application on *C. camphora* leaves subjected to cadmium stress increased the net photosynthetic rate, enhanced chlorophyll a, total chlorophyll and carotenoid contents, reduced the respiration rate, and the concentrations of proline, soluble sugar, soluble protein and malondialdehyde, reducing ROS damage, alleviating Cd stress compared to Cd-stressed plants without the addition of IAA.	Zhou et *al*. (2020)

1.6. Microbiome-originated auxin

1.6.1. Microbiome-mediated alteration of plant endogenous IAA homeostasis

The IAA pool is considered suboptimal or optimal for plant growth. IAA may be acquired as chemically synthesized IAA or microbe-originated (organic) compounds as a product of the metabolism of L-tryptophan and is essential for plant development (Habibi, 2014) through the modulation of plant IAA pools. The *quantitative abundance* of auxin in the rhizosphere is attributed to its biosynthesis by the plant microbiomes.

Various bacterial IAA biosynthesis pathways are similar to plant biosynthesis pathways (Spaepen, 2007). Exogenous bacterial IAA has been found to evoke similar effects as endogenous or synthetic auxin in stimulating plant root system development (Shahzad et *al*., 2016).

Exogenous IAA (synthetic or microbial) has the potential to alter the plant's endogenous IAA levels, inducing physiological responses in the root system. Likewise, microbial IAA produced *in situ* acts in conjunction with endogenous IAA in the plant. The bioactivity of the exogenous IAA depends on its concentration and the susceptibility of plant tissue to its varying levels.

Exogenous IAA positively affects root elongation, but its positive impact is limited to a small concentration. Rhizobacteria produce IAA in response to a plentiful supply of substrates in the rhizodeposits to alter the auxin pool, perturbing their host physiological processes for their benefit (Khoi Nghia, 2017). Thus, bacterial IAA contributes, directly or indirectly, to plant fitness and growth also by creating a competitive environment for pathogens.

Bacteria-originated IAA indirectly influences photosynthesis by expanding water and nutrient research and absorption by increasing root biomass, lateral root number, and surface area available for uptake from the surrounding soil, leading to increased metabolism and the release of nutrient-rich root exudates in the rhizosphere, providing a plentiful supply of substrates for synthesizing secondary microbial metabolites including IAA (Drake et *al*., 2013; Upreti and Sharma, 2016).

Microbial production of auxin contributes to the colonization of plant roots and the establishment of plant-associated bacteria via suppressing the plant's defence mechanisms against the invading microbes.

Several auxin signaling pathways may be responsible for bacterial infection and colonization susceptibility of plants. Thus, the enhancement of these auxin signaling pathways leads to the

inhibition of phytopathogens activity thereby protecting the plants from infectious diseases which is related effect of IAA to improving colonization (Patten et *al*., 2013).

Likewise, the downregulation of auxin signaling F-box receptors and auxin-responsive genes in *Arabidopsis thaliana* led to decreased disease symptoms and susceptibility to *P*. *syringae* DC3000 (Navarro et *al*., 2006). However, the colonization and disease incidence of *P*. *syringae* increased by 20-fold via exogenous auxin administration and up-regulation of auxin signaling and biosynthesis (Wang et *al*., 2007). Moreover, via the activation of *expansins*, including cell wall loosening proteins, bacterial auxin weakens the protective, fibrous extracellular matrix of roots, enabling bacterial colonization (Ding et *al*., 2008).

Higher IAA concentrations inhibit the proliferation of beneficial and pathogenic plant-associated bacteria due to their weak acid nature. Unexpectedly, more elevated amounts of IAA inhibit their ability to grow by causing an upsurge in the synthesis of the stress hormone ethylene (Woodward and Bartel, 2005). According to Zúñiga et *al*. (2013), the reverse action, which implies the degradation of IAA by beneficial bacteria, may effectively neutralize the limit or adverse effects of the exogenous IAA from pathogenic microbes and significantly improve plant growth.

Interestingly, some plant-associated microorganisms can modulate IAA levels to benefit the host plant owing to their dual ability to generate and degrade IAA. As a result, plants with low amounts of endogenous IAA are more susceptible to bacterial IAA (Glick, 2012).

Nevertheless, it has been reported that Indole-3-acetic acid does not function as a regular hormone in bacterial cells like plants. Accordingly, their ability to produce the same metabolite may have evolved as a reciprocal chemical signaling molecule in plant-microbiome communication, affecting gene expression in many bacteria (Raheem, 2018; Belimov, 2015). A higher level of IAA in nodulated roots than in non-nodulated ones (Theunis, 2004; Ghosh, 2006), suggested to be at the origin of root nodule functionality, supported this hypothesis.

Consequently, the continuous release of this phytohormone by plant microbiome with low but required quantities contributes to the IAA's physiological role in plant growth promotion and hormonal balance for optimal development (Verma et *al*., 2019).

Therefore, screening, identifying and incorporating efficient IAA-producing rhizobacteria strains that utilize the rich source of substrates released from roots in modern agricultural systems becomes necessary. Thus, research is underway globally to exploit the potential for

developing IAA-producing Actinobacteria as plant inoculants to assist plants during their growth and protection for sustainable agriculture.

1.6.2. Low-cost large-scale organic auxin production

The chemical manufacturing of auxins has several disadvantages, such as complicated operation and reduced purity. The substance is also highly toxic to farmers and irritant to the skin, eyes, and respiratory system, which decreases demand for it (Keswani et *al*., 2020). Contrary to the negative attributes of synthetic IAA, microbial biotechnology for IAA production represents a sustainable, balanced alternative that benefits both farmers and consumers (Bunsangiam et *al*., 2021; Mushtaq, 2021). The approach yields products with improved bioactivity, purity, and decreased process costs (Subramaniam et *al*., 2016).

Through the efficient identification of potent IAA producers, *in vitro* and *in silico* process studies, and medium engineering using organic wastes as carbon sources, specifically those rich in tryptophan, the development of bioprocesses for large-scale microbe-originated IAA production, is currently gaining interest. Using such substrates minimizes the fermentation cost, and the bioprocess could be expanded to create plant-biostimulants suitable for organic farming. These materials are also favourable for the environment and the economy (Luziatelli et *al*., 2021).

Organic IAA is the most promising method for designing and formulating green fertilizers for agriculture. The production of affordable IAA from lignocellulosic wastes, such as oat straws or hulls, is a cost-efficient substitute for other expensive media components that also require the incorporation of tryptophan (Table 2) (De Oliveira et *al*., 2017).

Table 2. Organic wastes as substrates for laboratory and large-scale IAA production from various microorganisms in optimal conditions.

Bacteria strain	**Fermentation substrate**	**Production scale**	**IAA yield**	**References**
Streptomyces fradiae NKZ-259	Corn starch and KNO_3 as nitrogen source	Laboratory-scale / submerged conditions	42.345 µg. mL^{-1}	Myo *et al*. (2019)
Pichia fermentans	Wheat straw Pretreatment of substrate with *P. chrysosporium*	Laboratory-scale / broth and submerged conditions	150 µg. mL^{-1}	Giri and Sharma (2020)
Rhodosporidiobolus fluvialis DMKU-CP293	Crude glycerol and feed-grade tryptophan	Pilot-scale	3569.32 mg. L^{-1}	Bunsangiam et *al*. (2021)
Kasakonia pseudosacchari TCPS-4	Corn flour and soy bean meal	Laboratory-scale	18.71 mg. L^{-1}	Chaudhary et *al*. (2021)
Enterobacter sp.	Vegetable peptone	Large-scale / submerged batch /benchtop fermenter	17.2 mg [IAAequ]/L/h)	Luziatelli et *al*. (2021)
Saccharothrix texasensis MB15	Wheat waste (leaves and roots)	Laboratory-scale / submerged conditions	148 µg. mL^{-1}	Benadjila et *al*. (2022)
Streptomyces rubrogriseus AW22	Spent coffee grounds and carob beans power	Laboratory-scale	188,290 µg. mL^{-1}	Alloun et *al*. (2023)

1.6.3. Auxin-producing Actinobacteria

Actinobacteria possess the ability to synthesize physiologically active Auxin. IAA synthesis was attributed to both *Streptomyces* and non-*Streptomyces* species. Nevertheless, compared to other bacterial species associated with plants, concentration ranges are still moderate (0.2–15 mg/L).

Actinobacteria-originated auxin works as exogenous supply, thus may significantly influence the endogenous IAA's homeostatic control represented by the biosynthesis, accumulation, degradation/oxidation, and conjugation of IAA, signaling patterns, and relative expression of auxin-responsive genes. The modulation of endogenous auxin level results in changes in plant response to external stimuli and acclimation to the unpredictably unfavorable environmental cues that can be deleterious for the plant.

Table 3 summarizes all the IAA producing Actinobacteria, their origin and their *in vitro* and/or *in vivo* plant growth promoting activities on host plants.

Table 3. Auxin producing Actinobacteria and their plant growth promoting activities.

Strain Taxa	Origin/Host plant	IAA yield	PGP Traits/effects on plants	References
Streptomyces mutabilis IA1	Saharan soil	74.39 µg. mL^{-1}	Wheat Seedling growth stimulation and increased shoot growth by more than 27% Biocontrol of *Fusarium culmorum* by reducing seedling blight occurrence (64.7%) and decreased severity (79.6%) Rhizosphere competence and colonization of internal plant tissues such as the roots and caryopsis.	Toumatia et *al*. (2016)
Endophytic *S. rochei* strain PTL2	Inner root tissues of *Panicum turgidum*, a Saharan native plant in Algeria	100.3 µg. mL^{-1}	A broad spectrum of *in vitro* antifungal activities Suppress *R. solani* tomato damping-off by reducing the disease incidence from 89.3% to 14.1% compared to Thiram® treatment (16.7%). Significant improvement in the root length, shoot length and dry weight of tomato (*cv. Marmande*) seedlings	Zamoum et *al*. (2017)
Actinobacteria isolates ABC21, ANU34, ABC33 and ANC48	Rhizosphere of sugarcane	[5.33-60.28] µg. mL^{-1} after 21 days.	IAA production was detected after 14 days, indicating late presentation of this phytohormone.	Rodrigues, (2018)
Actinobacteria *Streptomyces* isolates (48, S1, S3, S4, S4-1)	Arbuscular mycorrhizal *Funneliformis mosseae* CMU-RYA08 spores	[0.74-11.12] µg. mL^{-1}	IAA production during reduced water activity at up to a_w = 0.919.	Lasudee et *al*. (2018)
Streptomyces sp. MNC-1	Merzouga desert, Morocco	Up to 75.54 µg. mL^{-1} after eight	IAA and siderophore production, P and K solubilization, N-fixation	Nafis et *al*. (2019)

		days of incubation		
Streptomyces sp.	Rhizosphere soil of cucumber and tomato	[7.0-40.9] µg. mL^{-1}	Biocontrol *F*. *oxysporum* Schlecht. f.sp. *lycopersici* (Sacc.) race 3 causing tomato wilt PGP activities (IAA and siderophore production).	Abbasi et *al*., (2019)
Nocardiopsis dassonvillei strain MB22	Algerian Sahara	109.8 µg. mL^{-1}	A significant increase in the durum wheat (cv. Vitron) dry weight, root, and shoot lengths of seedlings Siderophores, chitinases and HCN production P solubilization	Allali et *al*. (2019)
Streptomyces spp. Strain ASR 46, ASR 58, ASR 75 and ASR 76	Soybean rhizosphere (*Glycine max*)	0.46-30.6 mg. mL^{-1}	Boosting hypocotyl and the radicular length, the number of lateral roots, and dry weight Siderophores and Chitinase production P solubilization Nitrogen fixation	Fatmawati et *al*. (2019)
Streptomyces strains (Car21t, Cal31t, Crc32t, Dbi28t, Cal24h)	Sponges from Taman Nasional Kepulauan Seribu, Indonesia.	Up to 5 mg. L^{-1}	Improved shoot and root lengths Augmented root number Siderophores production N fixation	Retnowati et *al*. (2019)
Nocardia BMG 111209 and *Nocardia* BMG51109	Root nodules of *Casuarina glauca*	18.43 µg. mL^{-1} of protein	Alterations in root hair Increased plant biomass and length Activated infection and nodulation process	Ghodhbane-Gtari et *al*. (2019)
Streptomyces 7.1 - *Streptomyces* strain *A20* and	Colombian soils	[4.07-7.98] µg. mL^{-1}	Increased root and shoot fresh weight in cultivar F733. Root endosphere colonization.	Suárez-Moreno et *al*. (2019)

Streptomyces strain 5.1			*In vitro* PGP traits, including cellulolytic and proteolytic activities, inorganic P solubilization, IAA and aminocyclopropane-1-carboxylate (ACC) deaminase production, and Siderophore production).	
Streptomyces sp. and *Nocardiopsis* sp.	Date Palm trees rhizosphere (Phoenix dactylifera L.)	Up to 5.8 mg. L^{-1}	Increased primary metabolites production, including sugars, amino acids and unsaturated fatty acids. Enhanced flavonoids and vitamins levels. IAA and siderophore production.	Abd Elgawad et *al*. (2019)
Streptomyces sp. (ARK 13, ARK 63, ARK 86, ARK 94, ARK 116)	Soybean Rhizosphere	[2.08-16.70] ppm	Increased hypocotyl and radicular length Enhanced lateral roots number Inorganic P solubilization	Wahyudi et *al*. (2019)
Streptomyces thermocarboxydus isolate BPSAC147	Root tissues of *Alstonia scholaris L*.	Up to 39.4 µg. mL^{-1}	Improved germination rate Enhanced shoot and root lengths and dry weight Enhancement of the chlorophyll fluorescence parameters Solubilization of inorganic P Cellulase and Amylase production	Passari et *al*. (2017) and Passari et *al*. (2019)
Arthrobacter arilaitensis strain (MG547869) and *Streptomyces pseudovenezuelae* strain (MG547870)	Maize plantations	9.44 ± 0.01 µg. mL^{-1} and 8.96 ± 0.03 µg $.ml^{-1}$, respectively	Siderophores, HCN and Ammonia production P solubilization ACC desaminase activity Improved seedlings emergence and growth Enhanced shoot and root lengths, increased germination rate and vigor index. Increased dry shoot and root weights, chlorophyll contents, and leaves number.	Chukwuneme et *al*. (2020)

Nocardiopsis dassonvillei subsp. *dassonvillei* T45, *Streptomyces xantholiticus* G22 and *Streptomyces iakyrus* G10	Salty water (Sebkha) in Northeast of Algeria	[7.44-21.4] µg. mL^{-1}	PGP traits (IAA, ACC deaminase, siderophores, lytic enzymes) Biocontrol of tomato pathogens *F solani and F. oxysporum* f. sp. *lycopersici*	Djebaili et *al*. (2020) Smati and Kitouni (2019)
Streptomyces sp. strain SA1 and *Streptomyces* sp. strain S43	*Camellia* spp. roots and leaves	[4.4-46.5] µg. mL^{-1}	*In vitro* PGP characteristics (P solubilization, ammonia, siderophores and chitinases production). Enhancement of tea clones' growth, fresh and dry biomass, in nursery conditions during seedlings bioassays.	Borah and Thakur (2020)
Streptomyces griseorubens BC10	Morrocan desert soils	Up to 128.44 µg. mL^{-1}	IAA production and stimulation of plant growth by inducing the rooting process from emergence to elongation.	Boubekri et *al*. (2021)
Streptomyces rubrogriseus AW22	*Triticum durum* rhizosphere, Tiffeche region of Souk-Ahras, Algeria	41,79 µg. mL^{-1}	*In vitro* PGP traits (IAA production, ammonia, cellulases, and chitinases, proteases, lipases and laccases production).	Alloun et *al*. (2023)

1.7. Machine-learning assisted optimization of auxin production

Synthetic IAA and other chemical agroactive substances pose severe environmental and farmer safety risks. Furthermore, particularly in modern agricultural systems, they are expensive regarding production yield. IAA synthesis follows a pricey, occasionally unstable, and dangerous chemical process. Its microbial production, in contrast, necessitates some specific traits of microorganisms, most notably the genetic background of species or strains (Lebrazi et *al*., 2020), which is greatly influenced by environmental conditions in the soil. Microbe-originated IAA, notably Actinobacteria-originated IAA, may be as effective as synthesized homologous IAA as a secure and environmentally beneficial substitute (Bunsangiam et *al*., 2021).

Actinobacterial proliferation, the quantity and profile of secondary metabolites, and the overall metabolic pathway can all be impacted by changes in media composition. Similarly, physiological cultural parameters like temperature, pH, fermentation duration, and nutritional conditions, including L-tryptophan (the precursor) and carbon and nitrogen sources, determine *in vitro* IAA generation and yield (Zerrouk, 2019).

Despite the enormous number of auxin-producing Actinobacteria identified in the literature, no strain has, as far as we know, been exploited explicitly in the industrial manufacture of auxin or commercialized. Thus, the production of auxin is, therefore, not an established attribute. Under specific nutrient and cultural conditions, it can increase. Recently, experimental models have been exploited to predict microbial growth and the synthesis of its metabolites. Artificial neural networks (ANNs) and response surface methodology (RSM) are some of these techniques. All the reports implicating machine-learning approaches for optimizing specific microbial metabolites are represented in the Table 4.

1.7.1. Response surface methodology (RSM)

To process complex data, RSM incorporates mathematical and statistical modeling into the experimental design (Qin et *al*., 2012; Saini et *al*., 2020). The real benefit of RSM is that it only involves a few tests for modeling. RSM enables quick completion of these tests and high-precision answers based on the regression equation (Kavitha et *al*., 2016). RSM makes it possible to simultaneously evaluate several interactions between the chosen parameters and suggests the best value for each (Roy et *al*., 2018).

The use of RSM prevents the superfluous addition of components to the culture medium despite being simply a second-order polynomial (Feng et *al*., 2017). RSM increases the auxin yield and decreases production costs by choosing the most critical variables, combining their effects, and integrating their interactions. Additionally, RSM uses statistical significance analysis to predict the highest auxin yield in line with the optimum fermentation medium and growth circumstances recommended by the mathematical design.

Two approaches are recommended; the Plackett- Burman experimental design (PBD) first screens and classifies the essential elements impacting the fermentation process in the fractional factorial design. PBD only has two levels, a low (-1) and a high (+1), as opposed to full factorial designs. Full factorial designs, however, demand a corresponding rise in the number of trials and components.

The next phase entails applying RSM to optimize variables based on the broad ranges of the ideal fermentation conditions predicted by PBD for increased IAA yield. Central composite design (CCD) and Box-Behnken design (BBD) from RSM constitute a commonly adopted statistical method for elaboration quadratic models and attempt at an effective auxin production maximization (Ribeiro et *al*., 2003). For instance, BBD and CCD enable industrial-scale Actinobacteria biomass preparation utilizing inexpensive substrates (Al Farraj et *al*., 2020).

1.7.2. Artificial Intelligence (AI)-based approaches

Parallelly, some bioengineering applications rely on AI-based methods such as artificial neural networks (ANNs). ANNs respond as the primary method for modelling and treating complex nonlinear or challenging connections when developing phenomenological or traditional models evolves difficult or unfeasible (Lahiri and Ghanta, 2008). ANNs are iterative computational techniques inspired by the brain's structure and made up of interconnected cells known as neurons.

The ANNs model design comprises numerous data processing systems of many units (neurons) (Dragoi and Vasseghian, 2021; Vasseghian et *al.*, 2020). Each neuron possesses effective internal communications that enable them to work harmoniously to resolve particular challenges. Afterwards, it can be trained by building a network of connected artificial neurons, developing a training algorithm for the network, and then using the algorithm on the network.

The multi-layer perceptron (MLP), consisting of the input layer, one or more hidden layers, and the output layer, is the feed-forward ANN used most commonly (Ali and Abd, 2021).

ANNs are often trained using the Backpropagation (BP) optimization function (Al-Kharashi and Skitmore, 2009) through Levenberg-Marquardt (LM) and Bayesian Regularization procedures (BR). The first algorithm is a rapid BP approach (Chou et *al*., 2013) that randomly splits the data into training, validation, and testing phases. The second necessitates two phases: testing and training (Khalid et *al*., 2017).

The complexity and performance of the chosen ANN model dictate the number of hidden neurons, which is an essential element while building the network. Low numbers of hidden neurons can lead to lower prediction performance, whereas too many neurons can cause overfitting and long computing delays (Pandey et *al*., 2016). Therefore, choosing the adequate number of neurons that offers the best predictive accuracy is critical.

The correlation coefficient (R), determination coefficient (R^2), and mean squared error (MSE) are used to estimate the model's accuracy. An analysis of a model's prediction performance can be made when the MSE is at its lowest and R^2 is high, R^2>0.98~0.99.

The most popular transfer functions are tangent sigmoid (*tansig*) and logarithmic sigmoid (*logsig*), which are employed for the created network to deliver more exact and accurate prediction results, especially when the phenomenon is nonlinear (Ali and Abd, 2021).

1.7.3. Importance of ANN integrated approaches in Microbial Biotechnology

The use of ANNs in microbial biotechnology to create process models is only occasionally documented in studies. ANN-based models have some advantages over traditional methods, including the capacity to forecast and optimize any complicated data process parameters *in silico* (Desai et *al*., 2008). ANN can be created without foreknowledge, can handle incomplete data from the inputs and outputs material and does not involve a mathematical description of the phenomenon examined (Azizi et *al*., 2016). Thus, this approach generates appropriate predictions of the best bioprocessing methods (Aghaeinejad-Meybodi et *al*., 2019; Dhanarajan et *al*., 2014; Mourabet et *al*., 2014).

The exceptional generalization capabilities of ANN, along with its capacity for handling noise, allow it to anticipate outputs accurately for fresh input data sets. In high-dimensional space, it learns designs and anticipates problems' repercussions (Nguyen et *al*., 2021). It cannot ensure the success of the ideal global solution (Rajendra et *al*., 2009).

1.7.4. Meta-heuristic algorithms as optimization tools

Meta-heuristic optimization algorithms, based on the fundamentals of natural phenomenon (Jiang et *al*., 2014; Poh et *al*., 2016). These algorithms do not easily get trapped in a local minimum (Agarwal et *al*., 2016). According to literature, the most commonly solicitated algorithms are the Genetic Algorithm (GA) and Particle Swarm Optimization (PSO).

GA relies on the Darwinian genetic evolution principle and uses genetic operators such as selection, mutation and/or inversion, and crossover to find the appropriate solution to the problem (Yahya et *al*., 2020). This procedure is called the fitness function (Ghaedi and Vafaei, 2017). This operation is repeated several times over generations to generate the fittest chromosomes, constituting the solutions or the optimal operating variables for the studied bioprocess.

Thus, GA represents a suitable evolutionary, adaptive optimizer often coupled with ANN and finds the precise or approximate optimal operational parameters for a single exclusive target, such as IAA production, with satisfying performance while reducing ANN complexity (Fan et *al*., 2018). Few studies have been reported concerning the ANN-GA for optimizing IAA production from *Streptomyces* strains using low-cost substrates.

Table 4. Statistical and AI-based optimization approaches for specific microbial metabolites.

Approach	**Design**	**Microorganism**	**Metabolite**	**Reference**
RSM	CCD	*Bacillus subtilis*	Jiean-peptide	Zhong et *al*. (2014)
RSM	CCD	*Streptomyces*	Oxytetracycline	Singh and Rai (2012)
NMDS, ANN	CCD	*Streptomyces*	Actinomycin D	Tripathi et *al*. (2012)
RSM	BBD, PBD	*Streptomyces*	Antibiotic	Rajeswari et *al*. (2014)
RSM	CCD, PBD	*Streptomyces olivaceus*	Olivanic acid	Singh and Tripathi (2008)
RSM	BBD, PBD	*Streptomyces*	Milbemycin	Baoxin et *al*. (2011)
ANN, PSO, RSM	CCD	*Bacillus licheniformis*	Ɛ-Polylysine	Bhattacharya et *al*. (2017)
RSM	BBD, PBD	*Streptomyces* sp. 1-14	Antibacterials	Yun et *al*. (2018)
RSM	CCD, PBD	*Streptomyces fradiae* NKZ-259	IAA	Myo et *al*. (2019)
RSM	CCD	*Rhizobium* sp.	IAA	Lebrazi et *al*. (2020)
RSM	BBD	*Pichia fermentans*	IAA	Giri and Sharma (2020)
ANN, MOGA	CCD	*Kazakonia pseudosacchari* TCPS-*4*	IAA	Chaudhary et *al*. (2021)
RSM	BBD	*Rhodosporidiobolus fluvialis DMKU-CP293*	IAA	Bunsangiam et *al*. (2021)
RSM	CCD	*Saccharothrix texasensis MB15*	IAA	Benadjila et *al*. (2022)
RSM, ANN-GA	BBD, PBD	*Streptomyces rubrogriseus AW22*	IAA	Alloun et *al*. (2023)

2-Materials and Methods

2. Materials and Methods

2.1. Isolation of *Streptomyces*-Like isolates

Actinobacteria were isolated from soil samples originating from two different sites in Algeria (Appendix A: Figure S1). The first consists of a semi-arid, nonsaline rhizospheric soil collected from wheat-growing fields in the Tiffeche Region (36° 9′ 24″ N; 7° 41′ 56″ E) of Souk-Ahras Province. The second was the aquatic sediments of Lake Oubeira, El Kala region of Taref, Algeria (36°50'26.3"N 8°23'07.9"E).

After detaching the healthy wheat roots from the soil without visible damage, the bulk soil was withdrawn by shaking the roots. The still firmly adhered soil was recovered as rhizospheric soil and safely transferred. Soil samples of aquatic sediments were collected at 15 cm depth from different points, merged, transported to the laboratory, and kept at 4°C until use.

Upon processing, after a pre-incubation of 1 h at 45°C three replicates of 4-5 g of soil samples were mixed with 1 g of $CaCO_3$ and processed for actinobacteria isolation (Suárez-Moreno et *al*., 2019). Starch Casein Agar (Starch 10 g/L, Casein 1 g/L, K_2HPO_4 0.5 g/L, Agar 13 g/L, pH 7.2) and yeast extract-malt extract agar (International *Streptomyces* Project medium 2 [ISP 2] (Malt extract 10.0 g, yeast extract 4.0 g, glucose 4.0 g, agar 18.0 g, and distilled water 1000 mL; pH 7.0 ± 0.2) were used as the isolation media, and plates were incubated for 7-21 days at 28°C, as described previously by Kusuma et *al*. (2020). *Streptomyces*-like isolates were selected based on their microscopic and macroscopic features, purified by subculturing, and subsequently characterized on International *Streptomyces* Project media (ISP2, ISP3, and ISP4) (van der Aart et *al*., 2019; Yan et *al*., 2018). Pure colonies were maintained on yeast extract-malt extract agar (International *Streptomyces* Project medium 2 [ISP 2] (Shirling and Gottlieb, 1966) and as mixtures of hyphal fragments and spores in 20%, v/v glycerol at -20°C and -80°C.

2.2. Plant growth-promoting traits

All isolates were investigated for their plant growth-promoting activities including their ability to produce catalase, IAA, ammonia (NH_3) and hydrogen cyanide acid (HCN) and *in vitro* biocontrol potential.

2.2.1. Antagonism assay

The antifungal activity of the Actinobacteria-like isolates was assessed *in vitro* against *Fusarium culmorum* according to the dual culture method. The wheat root-rot causing fungi was obtained from the LaMyBAM culture collection. Briefly, a six-day old fungal plug of 6

mm was deposited 3cm apart from the Actinobacterial streak performed 3 days before on PDA. After 7 days of incubation at 30°C, and until fungal mycelia completely covered the agar surface in the control plate, the percentage of inhibition of fungal growth was calculated according to Montealegre et *al.* (2003).

$$I\% = (Rc-Ra/Rc) \times 100\%$$

Where, Rc = Radial growth of fungus in control plates (mm). Ra = Radial growth of fungus on the plate inoculated with Antagonist (mm). Results are expressed as means ±standard deviation of three replicates of inhibition rates (%) of the fungal growth in the presence and absence of the potential Actinobacteria antagonist.

2.2.2. Auxin production and quantification assay

IAA production of Actinobacteria isolates was screened on yeast extract-tryptone broth (YTB) [in g L^{-1}: Yeast extract, 5 ; Tryptone, 10 ; NaCl, 5; pH 7.0±0.2] in the presence of 0.2% (w/v) L-tryptophan. Flasks were inoculated with a spore suspension of a concentration of (~ 10^8 spores ml^{-1}) followed by incubation at 28±0.2°C for 8 days under the agitation of 150 rpm (Alloun et *al*., 2023). The higher IAA producer was selected for further studies.

The eight days culture supernatants were obtained via filtration through Whatman No. 1 filter paper, followed by centrifugation (30 min at 4000×g). The auxin colorimetric assay was performed according to Sadeghi et *al*. (2012) using the Salkowski reagent at a ratio of (1:2) (v/v).

The development of a pink-red colour after 30 min of incubation in the darkness indicates indole compound production by the Actinobacteria. The optical density of the samples was measured at 530 nm using a Helios epsilon UV-vis spectrophotometer (Germany), and IAA concentration was estimated using a standard curve prepared with a synthetic IAA purchased from Sigma, USA (Passari et *al*., 2015).

2.2.3. Ammonia production

Ammonia production was investigated as described by Cappuccino and Sherman (1992). Briefly, 100 µL of Actinobacteria cultures (~10^6 spores.ml^{-1}) were inoculated into 10 ml peptone water and cultures were incubated on a rotary shaker (150 rpm, 30°C) for five days. After the addition of Nessler's reagent (0.5 mL), the development of a yellowish to brownish colour indicated ammonia production.

2.2.4. Hydrogen Cyanide production

Hydrogen Cyanide production by Actinobacteria isolates was monitored following the procedure described by Farda et *al*. (2022) and Lorck (1948). Briefly, five days-old-cultures were inoculated on HCN medium (Nutrient agar supplemented with 4.4 g L^{-1} of glycine) to investigate Cyanide production. A Whatman No. 1 filter paper (9 mm in diam.) was impregnated with picric acid (0.5%) in 2% sodium carbonate solution for 1 min and placed underside of the Petri dish lids. The plates were sealed with parafilm and subsequently incubated for seven days at 30°C. The development of orange to red colour on the filter paper margins indicated the production of HCN (Passar et *al*., 2015).

2.3. Polyphasic characterization of isolate AW22

Based on metabolic and bacteriological characterization, and plant-growth promoting traits screening, isolate AW22 was selected and further investigated for morphological, biochemical, and PGP (i.e., IAA quantification, lytic enzymes production) traits. The isolate was also identified by molecular approach.

2.3.1. Bacteriological characterization

Morphological and cultural characteristics, such as the pigmentation of aerial and substrate mycelia colony size, growth rate and the presence of the diffusible and melanoid pigments, were examined after 8 days of incubation at 28°C on different culture ISP media (ISP1, ISP2, ISP3, ISP4, ISP5, ISP7) according to the descriptions provided by Bergey's Manual of Systematic bacteriology (Goodfellow et al., 2012; Shirling and Gottlieb, 1966).

2.3.2. Carbon sources utilization profile

Determination of sugar utilization profile of selected isolate was adapted to a 96-well microplate using Minimal medium (MM) [in g L^{-1}: $NaNO_3$, 1.2 ; K_2HPO_4, 6 ; KH_2PO_4, 3 ; $MgSO_47H_2O$, 0.2 ; $CaCl_2$, 0.05 ; $MnSO_47H_2O$, 0.01 ; Zn SO_47H_2O, 0.001 ; pH 7.0±0.2] amended with 0.2% (w/v) of Fructose, Mannose, Maltose, Xylose, Arabinose, Lactose, Galactose, Sucrose, Mannitol, Ribose and Rhamnose. Phenol red (PR) was used as a pH indicator instead of Bromocresol purple (BCP). All sugars were filter-sterilized into the MM after autoclaving. The positive control consisted of MM supplemented with 0.2% (w/v) glucose, while MM devoid of any added carbon source was employed as a negative control. 96 well Microplates were UV-sterilized for 20min before utilization.

Each well was filled with 180µl MM (with and without C source) completed to a volume of 200µl with spore suspension (10^6 spore/ml). The assay was performed in triplicates. For five days, parafilm-sealed microplates were incubated at 28°C under slow agitation (100 rpm) and examined periodically. The colour change of the PR into yellow indicated a positive result.

2.3.3. Nitrogen source utilization profile

The utilization of amino acids as the sole nitrogen source by the selected Actinobacteria strain was also evaluated (Williams et *al*., 1983). Each nitrogen source (proline, glycine, leucine, and L-asparagine) was introduced to the basal medium (pH of 7.0±0.2) at final concentration of 0.1% (w/v) and incubated for 14 to 21 days at 30°C.

2.3.4. Physiological and biochemical characterization

The biochemical and physiological characteristics were evaluated according to Williams et *al*. (1983) with modifications. The ability of Actinobacteria isolate to grow at different temperatures (4, 15, 20, 25, 30, 35, 40 and 45°C), at different pH (5.0, 7.0 13.0 ±0.2 pH unit) and to tolerate Phenol 0.5%, Tellurite 0.5%, Sodium azide 0.1% and NaCl concentration from 0–10% (w/v) at 1.0 NaCl unit intervals) were examined. These characteristics were evaluated on GYM agar after 14 days of incubation. The assessment of nitrate reduction, urease production and skim milk peptonization were also performed.

2.4. Lytic enzymes production

2.4.1. Protease assay

Actinobacteria isolates were spot inoculated on 10% (v/v) skimmed milk agar (SMA) to detect extracellular proteases. SMA consists of a stock solution of skimmed milk and agar solution autoclaved individually at 115°C for 10 minutes and 121°C for 20 minutes, respectively. The two solutions were mixed at 60°C to a 1% final concentration of skimmed milk. After incubation at 30°C for 48–72 hours, the formation of clear zones around the colonies indicated extracellular caseinase production (Abdelmoteleb et *al*., 2017).

2.4.2. Cellulase production

The cellulolytic activity, the cleavage of amorphous cellulose, was assessed semi-quantitatively on the minimal medium agar amended with 1% (w/v) Carboxymethyl cellulose (CMC) as the sole energy and carbon source [in g/L: $NaNO_3$, 1.2; K_2HPO_4, 6; KH_2PO_4, 3; $MgSO_47H_2O$, 0.2; $CaCl_2$, 0.05; $MnSO_47H_2O$, 0.01; Zn SO_47H_2O, 0.001; Agar, 15; pH 7.0].

CMC plates were spot inoculated with 5-day-old cultures in the petri dish centre, then incubated for five days at 30°C (Ahirwar et *al*., 2017). The CMC degradation ability of the strain was detected after flooding plates with 0.1% Red Congo (aqueous) solution for 30 minutes, then destained using 1 M NaCl solution to make the hydrolyzed zone visible and clear. Cellulase activity was revealed by the colonies developing a visible halo (Slama et *al*., 2019; Suárez-Moreno et *al*., 2019).

2.4.3. Lignin oxidation activity

The ability of the strain to develop on purified lignin was evaluated on the minimal medium containing 0.5% (w/v) craft Lignin as the sole carbon source [in g L^{-1}: $NaNO_3$, 1.2; K_2HPO_4, 6; KH_2PO_4, 3; $MgSO_47H_2O$, 0.2; $CaCl_2$, 0.05; $MnSO_47H_2O$, 0.01; Zn SO_47H_2O, 0.001; Agar, 15; pH 7.0]. The colony growth after incubation at 30°C for five to seven days indicates a positive result (Alloun et *al*., 2023).

2.4.4. Amylolytic activity

Spot inoculation of the Actinobacteria isolate on Starch-Casein Agar media containing 1% (w/v) of soluble starch was performed [in g L^{-1}: Starch, 10; Casein, 0.3; KNO_3, 2; $MgSO_47H2O$, 0.05; K_2HPO_4, 2; NaCl, 2; $CaCO_3$, 0.02; $FeSO_47H_2O$, 0.01; Agar, 15; pH 7.0]. This Method enables the assessment of starch hydrolyzation mediated by Amylase activity. After incubation for three to four days at 30°C, Lugol's iodine solution [in (w/v) iodine, 5% and KI/L, 10%) was poured on the plates' surface. Prominent halo development around the colonies is an indicator of a positive amylolytic activity of the isolated (Slama et *al*., 2019).

2.4.5. Chitinolytic activity

The chitinolytic activity of *Streptomyces*-like strain was detected according to Gonzalez-Franco et *al*. (2003). The isolate (10µL of five-day-old cultures, 10^6 spore/mL) were spot inoculated on colloidal chitin agar medium (0.4%) (w/v) then incubated for five to seven days at 30 °C.

The colloidal chitin agar media (pH 7.0± 0.2) consisted of [in g L^{-1}: (K_2HPO_4, 0.7; KH_2PO_4, 0.3; $MgSO_45H_2O$, 0.5; $FeSO_47H_20$, 0.01; $ZnSO_4$, 0.001; $MnCl_2$, 0.001; agar, 15), supplemented with 0.4% moist colloidal chitin as the sole carbon supplier (Gómez Ramírez et *al*., 2004; Murthy and Bleakley, 2012). A halo surrounding the colonies revealed the chitinolytic activity of the test strain (Murthy and Bleakley, 2012; Zamoum et *al*., 2015).

2.4.6. Lipolytic activity

Lipase, Lipoproteinases, and Lecithinases production were evaluated on Braid Parker agar after 4 days of incubation at 28°C. Esterase production was examined on Twain 20 and 80 after incubation at 28°C for 8 days.

2.5. Molecular identification

The isolate AW22 was identified taxonomically using 16S rRNA gene sequencing ALVALAB, Spain). Genomic DNA was extracted and the 16S rRNA gene was amplified by PCR using the universal primers 27F (5′-GAGTTTGATCCTGGCTCAG-3′) and 1492R (5′ TACGGYTACCTTGTTACGACTT-3′) as described by (Girão et *al*., 2019)**.** Purified PCR products were sequenced by Sanger method. The 16S rRNA gene sequences were processed, and the isolate's taxonomic affiliation was determined using the NCBI BLAST tool and validated using the identity tool from EzTaxon and the sequence match tool from the Ribosomal Database Project. In addition, a phylogenetic tree was constructed to supplement the taxonomic assessment of the isolates. BLAST results were used to determine the nearest neighbor sequences in GenBank. MUSCLE was used to align all sequences that met these requirements (a Geneious software package). The phylogenic tree was then built with 1000 bootstraps using the Maximum Likelihood approach and the Tamura-Nei model. The tree was created using the Molecular Evolutionary Genetics Analysis software Version 11 (MEGA11) (Kumar et *al*., 2016).

2.6. Time course of IAA and biomass yields from strain AW 22

The optimal incubation period for IAA and biomass yield by AW 22 was determined over ten days on GYM broth supplemented with 0.2% (w/v) L-tryptophan. The conventional oven method measured biomass development (Buono and Erickson, 1985). The AW 22 cultures were filtered using Whatman No. 1 filter paper and dried at 70 °C for 12 hours. All tests were carried out in triplicate with an inoculum size of 3.8×10^6 CFU/mL of AW 22 cells to obtain average values.

2.7. Extraction and TLC confirmation of IAA

The synthesis of IAA was confirmed by thin-layer chromatography (TLC), as Goudjal et *al*. (2013) reported. Fractions were extracted with Ethyl Acetate (EA) at a ratio of (1:3) (v/v) and vacuum evaporated at 40°C. Further, EA fractions of 10–20 µl were spot-deposited on TLC

plates (silica gel GF254, thickness 0.25 mm, Merck, Germany) and developed in ethyl acetate: chloroform: formic acid (55:35:10, by volume). TLC plates were treated with Ehmann's reagent before their visualization under UV light (254 nm), displaying spots with identical Rf values to the standard IAA.

2.8. High-Performance Liquid Chromatography (HPLC) for IAA quantification

EA fractions were subjected to a High-Performance Liquid Chromatography (HPLC, Agilent Technologies, USA) equipped with a UV detector and a column model Cosmosil SC18-MS-II (Nacalai Tesque, Japan). The elution system and the flow rate were optimized and adapted from (Bunsangiam et *al*., 2021; Kaur and Kaur, 2021; Myo et *al*., 2019; Nutaratat et *al*., 2015).

The mobile phase's solvent system consisted of acetonitrile: water: acetic acid (35:65:1 v/v/v) at a 1 mL/min flow rate (Nakurte et *al*., 2012) with a 20 µL injection volume. Thus, the isocratic elution method was preferred over gradient elution while the column temperature was sustained at 25°C. As the standard, authentic IAA (Sigma, USA) was used to quantify IAA in the sample. IAA detection was monitored at 280 nm.

2.9. Medium preparation and culture conditions

Samples of SCG were collected from coffee shop consumption of Robusta coffee beans at 85°C, Algeria. Carob extracts were obtained from freshly collected carob beans. Substrates were either air-dried for fifteen days or heat-dried for eight hours at 60°C for, and Carob beans were chopped, ground and sieved. Extracts were obtained from air-dried SCG by hydrothermal method at 121°C at a concentration of 0.2% (w/v) of distilled water. Subsequently, aqueous solutions were filtered using 8 layers of cheesecloth and Whatman No. 1 filter paper to obtain clear extracts and stocked at 4°C (Wu et *al*., 2019).

2.10. Screening for the best-suited carbon source for IAA yield

Different culture media were tested, consisting of the minimal medium (MM1) [in g L^{-1}: $NaNO_3$, 1.2; K_2HPO_4, 6; KH_2PO_4, 3; $MgSO_47H_2O$, 0.2; $CaCl_2$, 0.05; $MnSO_47H_2O$, 0.01; Zn SO_47H_2O, 0.001; Agar, 15; pH 7.0] supplemented with different carbon sources at 1% (w/v). These substrates consist of carboxymethyl cellulose (CMC), collected carob beans powder (CCB), spent coffee grounds (SCG), oatmeal (Oat), wheat straw fibres (W.S) and starch (Sta). The MM containing glucose as a carbon source was used as the positive control. All media were supplemented with 0.2% (w/v) L-tryptophan (Khamna et *al*. 2010). Culture media were

inoculated with one millilitre of spore suspensions (~ 10^8 spores.ml^{-1}) and incubated under permanent agitation at 150 rpm and 30 °C for eight days.

2.11. Effect of selected carbon source on IAA production

The effect of substrate concentration on the isolate AW 22's ability to generate IAA was investigated. Glucose at 0.2%, 0.5%, and 1% (v/v), Carob Beans Powder extract at 10-50% (v/v), SCG extract medium at 10-50% (v/v), and L-Trp at 0.2% (w/v) were added to the MM. The negative control consisted of a MM devoid of L-Trp, while the positive control was Glucose 0.5%. AW 22 cultures were inoculated and incubated at 28 °C for 8 days under permanent shaking at 150 rpm.

2.12. Statistical optimization of IAA generation

2.12.1. Significant parameters screen using PBD

After obtaining the maximal IAA production using the time course experiment, applying the Plackett-Burman experimental design aims to screen fourteen independent variables. This approach attempts to determine the most influential media components in the IAA generation (Khosravi-Darani and Zoghi, 2008; Purama and Goyal, 2008). Eleven nutrient factors (L-Tryptophan, SCGE, CBPE, $CaCO_3$, Yeast extract, soluble starch, Tryptone, NaCl, K_2HPO_4, $MgSO_4$), three culture conditions (pH, growth temperature, incubation time), and inoculum amount (in%) (v /v) are included in these parameters (Zhao et *al*., 2013).

All independent factors were examined at two widely-spaced intervals, represented as negative values (low level, − 1) and positive values (high level, + 1) in 20 experiments. Each row represents a trial with a response value consisting of IAA yield.
The factors' actual levels are listed in Table 5, while the PBD matrix in uncoded units is summarized in Table 6.

All experiments were performed in triplicate, and the mean value constituted the response. The statistical metrics of the model were determined via the analysis of variance (ANOVA). The variables' significance was estimated by calculating the *p*-value and confidence levels using the standard regression analysis. Factors presenting a 5% level of significance ($p < 0.05$) were further optimized with BBD to increase IAA yield.

The PBD first order model is given in equation (1):

$$Y = \beta_0 + \sum \beta_i x_i \qquad \text{(Eq. 1)}$$

where Y stands for the dependent variable (response = IAA production), β_0 corresponds to the model' intercept, β_i represents the regression coefficient, and x_i is the independent variable. Minitab 19.0 statistical software package was used for the PBD and results analysis.

Table 5. Actual values of independent variables screened by PBD.

Facto label	**Variables**	**Unit**	**level**	
			-1	**1**
X1	CBP	%	30	50
X2	SCG	%	30	50
X3	Starch	g L^{-1}	2	5
X4	Tryptone	g L^{-1}	3	6
X5	Yeast E	g L^{-1}	2.5	5
X6	L-Trp	%	0.3	0.6
X7	NaCl	g L^{-1}	1	5
X8	K_2HPO_4	g L^{-1}	0.3	0.7
X9	$MgSO_4$	g L^{-1}	0.2	0.5
X10	$CaCO_3$	g L^{-1}	1.0	1.5
X11	Incub. Time	Day	6	10
X12	T	°C	26	35
X13	pH	-	7±0.1	9±0.1
X14	Inoc. amount	%	2	4

Note: X_1-X_{14} correspond to various impact variables; "1" and "-1" are two different levels.

Table 6. Plackett–Burman experimental design matrix in uncoded units.

Run N°	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14
1	+	-	+	+	+	+	-	-	+	+	-	+	+	-
2	-	+	-	+	+	+	+	-	-	+	+	-	+	+
3	+	-	+	+	-	-	-	-	+	-	+	-	+	+
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	+	+	-	-	-	-	+	-	+	-	+	+	+	+
6	+	-	-	-	-	+	-	+	-	+	+	+	+	-
7	+	-	+	-	+	+	+	+	-	-	+	+	-	+
8	+	+	-	+	+	-	-	-	-	+	-	+	-	+
9	+	-	-	+	+	-	+	+	-	-	-	-	+	-
10	-	+	-	+	-	+	+	+	+	-	-	+	+	-
11	-	+	+	-	-	-	-	+	-	+	-	+	+	+
12	-	-	-	-	+	-	+	-	+	+	+	+	-	-
13	-	-	+	+	-	+	+	-	-	-	-	+	-	+
14	+	+	+	+	-	-	+	+	-	+	+	-	-	-
15	-	+	+	-	+	+	-	-	-	-	+	-	+	-
16	-	+	+	+	+	-	-	+	+	-	+	+	-	-
17	-	-	-	+	-	+	-	+	+	+	+	-	-	+
18	+	+	-	-	+	+	-	+	+	-	-	-	-	+
19	-	-	+	-	+	-	+	+	+	+	-	-	+	+
20	+	+	+	-	-	+	+	-	+	+	-	-	-	-

Note: X1 ~ X14 represent the impact variables; "+" and "-" correspond to two different levels; 1 to 20 represent 20 different sets of fermentation conditions.

2.12.2. RSM-BBD: Model elaboration

The four highly significant process parameters obtained from PBD (SCG, L- tryptophan concentration, incubation temperature and pH) were later subjected to RSM analysis using the Box-Behnken design (Lanka and Latha, 2015). Twenty-eight trials with four central points were generated with the statistical software package Minitab 19.0 to maximize response and correlate mathematically independent variables (Vasseghian et *al*., 2017).

Each element was evaluated on a level of +1, 0 and -1, where 0 represents the central coded value, +1 represents a high value, and -1 represents a low value (Actual and coded values are

given in table 7 and 8, respectively). The actual response of the IAA yield was selected as the triplicate set's mean. Further, each response was fitted to an independent second-order polynomial model represented in the equation (2):

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i x_j + \sum \beta_{ij} x_i x_j \qquad \text{(Eq. 2)}$$

where the forecasted response value Y represents IAA production, β_0 is the constant term coefficient, β_i is the primary term coefficient, β_{ii} is the quadratic term coefficient, and x_i is the independent variable.

The following equation (Eq. 3) describes the relationship between xi the independent variable and its actual value X_i:

$$x_i = \frac{X_i - X_0}{\delta X} \qquad \text{(Eq.3)}$$

where X_0 and δX stands for the actual value of the independent variable at the test center point, and the step change of the independent variable, respectively.

The regression equation was also used to calculate predicted response values and the statistical competency of the model was resolved using variance analysis (One-way ANOVA). The significance of each factor and the regression model are assessed using the F- and *p*-values. As a result, a high Student's t-test with a low *p*-value attests to the regression model's high reliability (Vasseghian et *al*., 2020). The coefficient of determination (R^2) and the adjusted R^2 statistically assessed the precision of the polynomial model equation.

2D surface plots were generated to demonstrate the interconnections between the responses and the experimental levels of each independent variable. The software's response optimizer tool aimed to determine the best value for each variable to provide the greatest IAA.

Table 7. Actual and coded values for the independent variables evaluated in the BBD.

	Factors			
Level	**L-Trp (%)**	**SCG (%)**	**Temperature (°C)**	**pH**
-1	0.2	30	26	7.1
0	0.4	40	30	8.05
1	0.6	50	35	9

Note: "1", "0" and "-1" correspond to three different level.

Table 8. Experimental matrix of BBD in uncoded units and experimental data.

Run	Variables				IAA yield (μg. mL^{-1})		
	A	B	C	*D*	*Y* (Test value)	*Y* (Fit value)	Residual
1	1	0	1	0	173.06±6.23	179.99	-6.93
2	0	0	0	0	177.18±6.34	120.29	56.88
3	0	1	1	0	86.28±2.74	102.29	-16.01
4	0	1	0	1	74.11±4.63	82.33	-8.22
5	1	0	-1	0	64.20±1.82	98.62	-34.41
6	0	1	-1	0	80.87±3.57	54.46	26.41
7	-1	0	1	0	77.37±10.03	46.89	30.48
8	-1	0	-1	0	34.36±1.54	31.37	3.00
9	0	0	0	0	76.99±4.96	120.29	-43.31
10	-1	0	0	1	31.20±1.37	50.43	-19.22
11	-1	1	0	0	18.56±1.26	22.21	-3.66
12	1	0	0	-1	184.36±7.85	184.02	0.35
13	0	0	1	-1	159.27±6.94	138.15	21.12
14	1	1	0	0	115.35±7.31	92.36	22.99
15	0	-1	0	1	94.23±4.82	76.65	17.58
16	0	0	0	0	155.68±9.42	120.29	35.38
17	-1	0	0	-1	25.31±2.88	35.73	-10.42
18	1	-1	0	0	176.60±7.12	150.14	26.47
19	0	-1	-1	0	78.71±5.71	81.58	-2.87
20	0	-1	1	0	85.37±1.96	130.66	-45.29
21	0	0	-1	-1	129.96±2.80	123.77	6.19
22	0	0	0	0	71.34±5.05	120.29	-48.96
23	0	0	-1	1	57.97±6.20	56.28	1.69
24	0	1	0	-1	60.80±9.14	82.32	-21.52
25	0	0	1	1	155.44±9.00	138.81	16.63
26	-1	-1	0	0	19.76±2.61	19.93	-0.18
27	1	0	0	1	94.04±3.59	102.49	-8.46
28	0	-1	0	-1	147.77±12.37	143.49	4.28

Note: 1 to 28 represent 28 different sets of fermentation conditions.

2.13. Machine learning modeling of IAA generation

The feed-forward back propagation (BP) or Levenberg-Marquardt (trainlm) algorithm was used to generate and train an ANN model using the previously reported BBD data. The distinct divisions of the several neurons that comprise an ANN are the input, hidden, and output layers. The hidden layers, single or multi-architecture, are the operating units serving as feature detectors and introducing nonlinearity into the network (López et *al*., 2017). The development of an ANN model is divided into multiple phases—the training and validation phases (*input feed-forward multilayer* and *error backpropagation*).

Nevertheless, as indicated by (Maji et *al*., 2014), the total number of data points was increased to 200 and generated using the second-order polynomial equation because the insufficient experimental data sets from BBD that doesn't allow to develop an effective network architecture. During the training phase, the validation technique intends to reset the built-in model's reliability. The model can be used in subsequent applications only when the validation findings match expectations.

2.13.1. Artificial neural network (ANN) modeling

At this level, the conventional input feed-forward multilayer ANN (MLP) is combined with a training algorithm for ANN model development. MLPs are simple universal approximators aiming at modeling physicochemical processes (Jasso-Salcedo et *al*., 2017; Moghri et *al*., 2017).

The datasets are divided into three subdivisions. Each subset includes 70% of the data for testing, 15% for validating, and 15% for training the network. In the ANN topology (architecture), inputs and outputs are fixed components. At the same time, the number of hidden layers and their neurons form a series of variable elements. Bias and weights enable the expression of the connections between each layer. In addition to network architecture, internal parameters are chosen based on empirical data to achieve the best ANN identification. As a result, 11 alternative training algorithms and a five-fold cross-validation strategy were employed to determine the ANN core parameters and the optimal number of hidden neurons.

The current process's inputs are the initial concentrations of L-Trp (X1), operating temperature (X2), initial pH (X3), and SCG concentration (X4), and the system's output is the yield of IAA. Two hidden layers were chosen, each with a neuron number ranging from [1-10].

Figure 4 shows the present ANN simple structure, and Table 9 **s**ummarizes the design parameters adopted in developing the present ANN model.

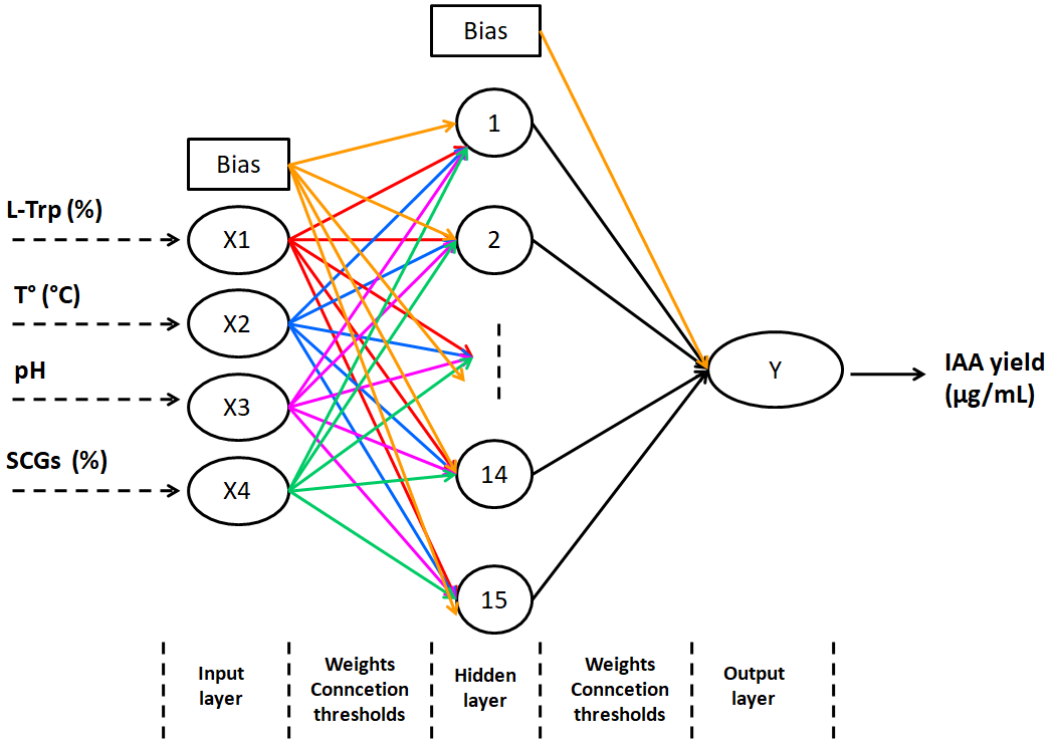


Figure 4. Typical design of the current artificial neural network (ANN).

Table 9. Parameters adopted in creating the ANN model.

Type	**Description**
Inputs layer	4 neurons (L-Trp concentration, SCG concentration, T°, pH)
Hidden layer	n= 2 layers; m= 12 neurons
Output layer	1 neuron (IAA yield)
Learning rate	0.01
Epoch	1000
MSE goal	0.001
Algorithms	Levenberg-Marquardt (trainlm)
	Sigmoid (tansig): preferred between input and hidden layers
Function	Linear: preferred between hidden and output layers

This work developed a three-layered BP-ANN. An activation function operates to generate simulated output on a network that has been trained using random weights and bias values. The

activation functions generate the output by converting the weighted sum of the input (Çelekli et *al*., 2013).

As indicated by equation (4), the signals emitted by the hidden layer are expressed in weights and thresholds via a transferring function.

Still, the hidden-to-output layer-oriented signals constitute the predicted value that may be expressed by the equation (5).

$$x_j = F\left(\sum_{i=1}^{n} x_i \times w_{ij} + P_j\right) \qquad \text{(Eq. 4)}$$

$$y_{i-pred} = F'\left(\sum_{i=1}^{n} v_j \times x_j + Q\right) \qquad \text{(Eq. 5)}$$

where:
xi, bj represent the input and the hidden neuron values, respectively.
wij and *vj* correspond, respectively, to the weights between *xi* (input neuron) and *xj* (hidden neuron) and *xj* (hidden neuron) and the output neuron.
Pj and *Q* represent the connection thresholds of the hidden and output neurons, respectively.
F means the transfer function between *xi* and *xj*; *F'* means the transfer function between *xj* and the output neurons.
$Y_{i\text{-}pred}$ is the IAA concentration predicted value.
wij, *Pj*, *vj*, and *Q* are first arbitrarily designated low values for subsequent readjustments during the feedback process.

The output and hidden layers have *linear* and *tangent sigmoid* transfer functions, respectively. All the ANN model's training values were normalized between 0 and 1 via the min-max method expressed in equation (6) to prevent numerical overflows brought on by larger or smaller weights:

$$x_i = \frac{X_i - X_{min}}{X_{max} - X_{min}} \qquad \text{(Eq. 6)}$$

where X_i, X_{min} and X_{max} represent the normalized, least and highest values of *X* (original value), respectively.

Upon training, the BP algorithm is executed with the *error backwards propagation* to minimize the prediction mean squared error (MSE) iteratively between experimental and simulated output data and constantly adjust weights and biases between neurons.

The MSE (Eq. 7) and the determination coefficient (R^2) (Eq. 8) served as metrics for performance to develop an ideal ANN model capable of evaluating the accuracy of predictions made between the ANN outputs and the targets (Dhanarajan et *al*., 2014; Sivapathasekaran et *al*., 2010; Vasseghian and Dragoi, 2018). The number of hidden neurons was determined based on the minimum value of MSE (Rajendra et *al*., 2009). An optimum network architecture was chosen based on the least MSE and maximum R-values to prevent data over-fitting and improve output accuracy and predictability (Dhanarajan et *al*., 2014; Huang et *al*., 2007).

$$MSE = \sum_{i=1}^{n} \frac{(y_i - y_{i-pred})^2}{n} \qquad \text{(Eq. 7)}$$

$$R^2 = \frac{\sum_{i=1}^{n}(y_i - y_{i-pred})^2}{\sum_{i=0}^{n}(y_{i-av} - y_{i-pred})^2} \qquad \text{(Eq. 8)}$$

where $y_{i\text{-}pred}$ and y_i correspond to the predicted and provided output values, respectively, and n is the respective number of provided data points for the corresponding phase (training, testing, or validation), $y_{i\text{-}av}$ is the average value.

The hidden neuron weight *(Wj)* can be computed according to equation (9), which can be put forward:

$$W_j = \sum_{i=1}^{k} w_{ij} x_i \qquad \text{(Eq. 9)}$$

where *k* represents the input layer-related number of neurons, w_{ij} is the connection weight between *x* (output neuron) and *b* (hidden neuron).
i is the input layer neuron, and x_i represents its value.

Similarly, the output neuron weight *(Wk)* can be computed according to equation (10):

$$W_k = \sum_{j=1}^{z} w_{jk} x_j \qquad \text{(Eq. 10)}$$

where *z* represents the hidden layer-related number of neurons, w_{jk} is the connection weight between *j* (hidden neuron) and *k* (output neuron).

j is the hidden layer neuron, and x_j represents its value.

The activation function generates the predicted output using the neuron's weight in the hidden or output layer according to equation (11).

$$y = f(W + B) \qquad \text{(Eq. 11)}$$

Where:

y stands for the predicted output.

f is the activation function.

W and *B* represent the weight and bias in the hidden or output layer.

The cross-validation procedure was performed ten times to improve predictability and accuracy, with the results averaged.

2.13.2. Sensitivity analysis

The connection weights were examined, and the relative influence of the input factors on the output was evaluated using the Garson algorithm's sensitivity analysis (Zhang and Pan, 2014). The Garson equation (Eq. 12) and potential variable combinations were employed for this type of analysis (Aleboyeh et *al*., 2008; Yetilmezsoy and Demirel, 2008).

$$Q_{ik} = \frac{\sum_{j=1}^{L}\left(\frac{|w_{ij}|}{\sum_{r=1}^{N}|w_{rj}|}|v_{jk}|\right)}{\sum_{i=1}^{N}\left(\sum_{j=1}^{L}\left(\frac{|w_{ij}|}{\sum_{r=1}^{N}|w_{rj}|}|v_{jk}|\right)\right)} \qquad \text{(Eq. 12)}$$

The Q_{ik} stands for the impact percentage of the input variable. The connection weight between *i* and *j*, the input and the hidden neuron, respectively, is indicated by w_{ij}.

The connection weight between *j* and *k* the hidden and the output neuron, respectively, is represented by v_{jk}, and the connection weight between the input neuron N and the hidden neuron *j* is defined by w_{rj}.

N, L, and M are the neurons' numbers in the input, hidden, and output layers.

w and *v* are the connection weights between the input and the hidden layers and between the hidden and the output layers.

2.13.3. Genetic algorithm (GA)

The genetic algorithm constitutes an AI-based stochastic nonlinear optimization formalism that simulates natural selection and genetic patterns (Jiang et *al*., 2014). GA prevents models from

being trapped by local optima by selecting appropriate initial weights and thresholds for the previously created ANN model and applying it as a fitness function (Eq. 13).
The fitness function can be expressed as follows:

$$F = Purelin\left(JW * \tan sig\left(KW * [x_1; x_2; x_3; x_4] + b_j\right) + b_k\right) \qquad \text{(Eq. 13)}$$

Where *F* denotes the IAA yield, b_k and *JW* stand for the output layer's bias and weight, and b_j and *KW* represent the hidden layer's bias and weight, respectively.

The GA algorithm optimized the IAA production bioprocess in numerous steps, including initialization, selection, crossover, and mutation, using varied parameters and rules based on specific characteristics.

In MATLAB, the default "ga" function interprets and treats the elementary data of the parameters requiring optimization, such as initial weights and thresholds, as chromosomes. The best-fitting chromosomes will be chosen through genetic reproduction, which includes crossover and mutation, whereas the least-fitted ones are substituted (Jiang et *al*., 2014). GA is reportedly adept at global searching to achieve convergence, independent of the initial value.

A) The GA starts by providing an initial population of solutions or individuals using initial operating temperature, pH, and L-Trp and SCG concentrations as optimization inputs. The ANN model-related initial weight and threshold were obtained and encoded into binary strings forming the chromosomes.

B) Then, a subsequent selection of outstanding chromosomes with high fitness from the present population is based on the coefficient of fitness for every chromosome. This operation helps to propagate excellent offspring and eliminates the low fitness chromosomes (Jiang et *al*., 2014).

C) The remaining chromosomes are treated using evolutionary operators crossover and mutation to improve the offspring of parents and produce the next generation (Yetilmezsoy and Demirel, 2008). Crossover aims to exchange data and genes among individuals, whereas mutation randomly affects individuals from the population and alters their genes (Bagheri et *al*., 2015).

D) The fitness function (step B) is carried out iteratively until the chromosomes have attained the maximum fitness level, and the convergence forms the optimal solution.

E) The last step entails decoding all chromosomes and substituting the ANN model's starting weights and threshold with these upgraded ones.

For this work's purpose, the settings considered are summarized in Table 10. Nonetheless, the selection function output is multiplied by-1 since the GA algorithm aims to minimize and not maximize outcomes.

Table 10. Configuration of the GA for the IAA production model implementation.

Parameters	Value
Population size	200
Number of elite	2
Crossing fraction	1
Migration fraction	0.2000
Migration interval	20
Direction of migration	forward
Stall generation limit	20
Stall time limit	20
Plot Interval	1
Generations	100

2.14. Validation of the postulated model

The postulated model validation was investigated empirically through a triplicate set of fermentations in the optimized conditions suggested by BBD with desirability 1 and by ANN-GA. The *in silico*-optimized culture conditions and the target response are recorded in the Table 9.

2.15. Statistical analysis and metrics

All experimental data were expressed as the mean of three replicates ± standard deviation (SD). Statistical significance of differences between groups was analyzed by one-way analysis of variance (ANOVA) followed by a Tukey post hoc test, comparing mean values at a 5% level of significance ($p < 0.05$). Differences between the two groups were investigated by Tukey's test. All statistical calculations and graphical representations for data visualization were performed using the Python 3.11 software and the package ggplot2 implemented in RStudio.

3-Results and Discussion

3. Results and Discussion

3.1. *Streptomyces*-like isolates

The morphological and physiological properties of Actinobacteria isolates, which enable the determination of the genus, are of interest. According to previous research, all isolates that were Gram-positive filamentous rods and exhibited the odor of wet soil were kept (data not shown). Wrinkled and colored colonies with waxy, powdery, or velvety surfaces were among the traits obtained from both isolation sites.

Upon enrichment with $CaCO_3$, isolation, purification, and initial PGP screening, soil samples yielded nine isolates from Lake Oubeira, and twenty-eight from wheat rhizospheric soil samples from Tiffech province of Souk-Ahras, Algeria. These isolates were putatively identified as *Streptomyces* spp. based on morphological and bacteriological characteristics.

Streptomyces represents the dominant genus of Actinobacteria in soil ecosystems (Thakur et *al*. 2007). This genus' members are typically isolated from plant tissues and the rhizosphere, indicating their highly compatible nature with various host organisms and contributing to improving their growth (Goudjal et *al*., 2014; Verma et *al*., 2009). Our findings revealed that the prevalence and distribution of *Streptomyces*-like isolates in the newly uncovered province of Souk-Ahras deviate slightly from other known niches, such as the river sand environment, regarding metabolism. Previous research has shown that the environment affects Actinobacteria diversity in soil more than microbial communities (Abbasi et *al*., 2019). Interestingly, various nutrient constraints in the soil play an essential role in the diversification dynamics of soil microbiomes due to natural selection.

3.2. Plant growth promotion and biocontrol activities

Table 11 displays the HCN and NH_3 production ability and *in vitro* antagonism against *F. culmorum* of *Streptomyces*-like isolates from Lake Oubeira and the wheat rhizosphere.

According to Table 11, most isolates showed good HCN and NH_3 production and were all catalase-positive. These abilities are usually described for Actinobacteria (Chukwuneme et *al*., 2020; Jog et *al*., 2012) and have been reported for isolates obtained from Algerian ecosystems (Djebaili et al., 2020).

Table 11. Plant growth promoting activities and *in vitro* biocontrol of *F. culmorum* of *Streptomyces*-like isolates.

Isolation site	Isolates	HCN	NH_3	Catalase	Anti- *F. culmorum*		
					Mean	*SD*	Sig (p <0.05)
Wheat rhizosphere	RZB	+	+	+	0.000	0.00	f
	RZB 11	+	-	+	35.817	0.68	bc
	RZB 13	+	+	+	0.000	0.00	f
	RZB 24	+	+	+	39.031	0.74	b
	RZBF	+	+	+	0.000	0.00	f
	RZB A 10	+	+	+	35.817	0.68	bc
	RZB G 12	+	+	+	37.124	0.68	bc
	RZBC	+	+	+	32.621	6.58	cd
	RZB D	+	-	+	0.000	0.00	f
	AW 11	+	+	+	0.000	0.00	f
	AW 12	+	+	+	36.209	1.80	bc
	AW 18	+	-	+	0.000	0.00	f
	AW 17	+	+	+	33.740	1.22	bcd
	AW 16	+	+	+	32.418	12.69	cd
	RZB G	+	+	+	0.000	0.00	f
	RZB G100	+	+	+	0.000	0.00	f
	AW 22	+	+	+	67.320	8.99	a
	S27	+	+	+	0.000	0.00	f
	S28	+	+	+	0.000	0.00	f
	RPN102	+	ND	+	34.118	3.11	bcd
	AW 25	+	+	+	0.000	0.00	f
	AW 29	+	+	+	0.000	0.00	f
	AW 28	+	+	+	0.000	0.00	f
	AW 01	+	+	+	0.000	0.00	f
	AW 2A	+	+	+	38.431	4.08	bc
	AW 21	+	+	+	0.000	0.00	bcd
	AW 1F2	+	ND	+	0.000	0.00	f
	AW 08	+	+	+	29.114	0.00	de
	AW68	+	+	+	37.255	2.04	bc
	AW34	+	+	+	0.000	0.00	f
	AW42	+	+	+	0.000	0.00	f
Lake Oubeira sediments	AW13	+	-	+	0.000	0.00	f
	AW63	+	+	+	34.940	0.00	bcd
	AW59	+	+	+	0.000	0.00	f
	AW37	+	-	+	24.706	0.00	e
	AW05	+	+	+	32.922	2.14	cd
	AW21	+	+	+	34.771	1.80	bcd

Note 1: "+" indicates positive result, "-" indicates negative result and Sig stands for Significance.
Note 2: Each assay was done three times with three biological replicates, and values represent their means and standard deviation (*SD*). Values followed by the same letter are not statistically different according to Tukey post hoc test (p <0.05) (Rstudio).

In vitro antagonism assay

Several studies advocated that *Streptomyces* species have emerged as biocontrol agents that are safe alternatives to synthetic fungicides for managing phytopathogens. Out of twenty-eight isolates, twelve (43%) rhizospheric Actinobacteria isolates displayed a positive inhibitory effect against *F. culmorum*, with isolate AW22 exhibiting the highest I (%) of 67.320±8.99. Meanwhile, five (55%) aquatic Actinobacteria isolates originated from Lake Oubeira sediments were antagonists. According to Lee and Hwang (2002), these isolates are promising since most antagonists with *in vitro* antifungal activity also exhibit *in vivo* activity. Concerning *F. oxysporum*, Rios-Muñiz and Evangelista-Martínez (2022) have also reported the *in vitro* sensitivity of these pathogens when facing rhizospheric *Streptomyces* sp. CACIS-2 15CA isolate.

Similarly, Le et *al.* (2022) have revealed the broad-spectrum activity of *Streptomyces* sp. AN090126, isolated from agricultural soil in Korea, against various phytopathogenic fungi. This study also revealed that a higher proportion of native rhizospheric Actinobacteria exert solid antagonistic activity than aquatic isolates.

Auxin production

Figure 5 (a, b) represents IAA production form rhizosphere and the aquatic Actinobacteria isolates, respectively.

In the present study, the quantitative assessment of IAA synthesis by *Streptomyces*-like isolates found that all the wheat-associated isolates and the aquatic isolates (100%) possess the ability to yield IAA ranging between 1.8534± 0.1724-23.999±1.126 μg. mL^{-1} and 12.553±0.06 - 26.116±0.61 μg. mL^{-1} after eight days of incubation, respectively.

Our results agree with previous studies (0.2–15 mg L^{-1}). However, IAA production from both *Streptomyces* and non-*Streptomyces* spp. with L-tryptophan remain moderate compared to other plant-associated bacterial phyla (Nimnoi et *al*., 2010).

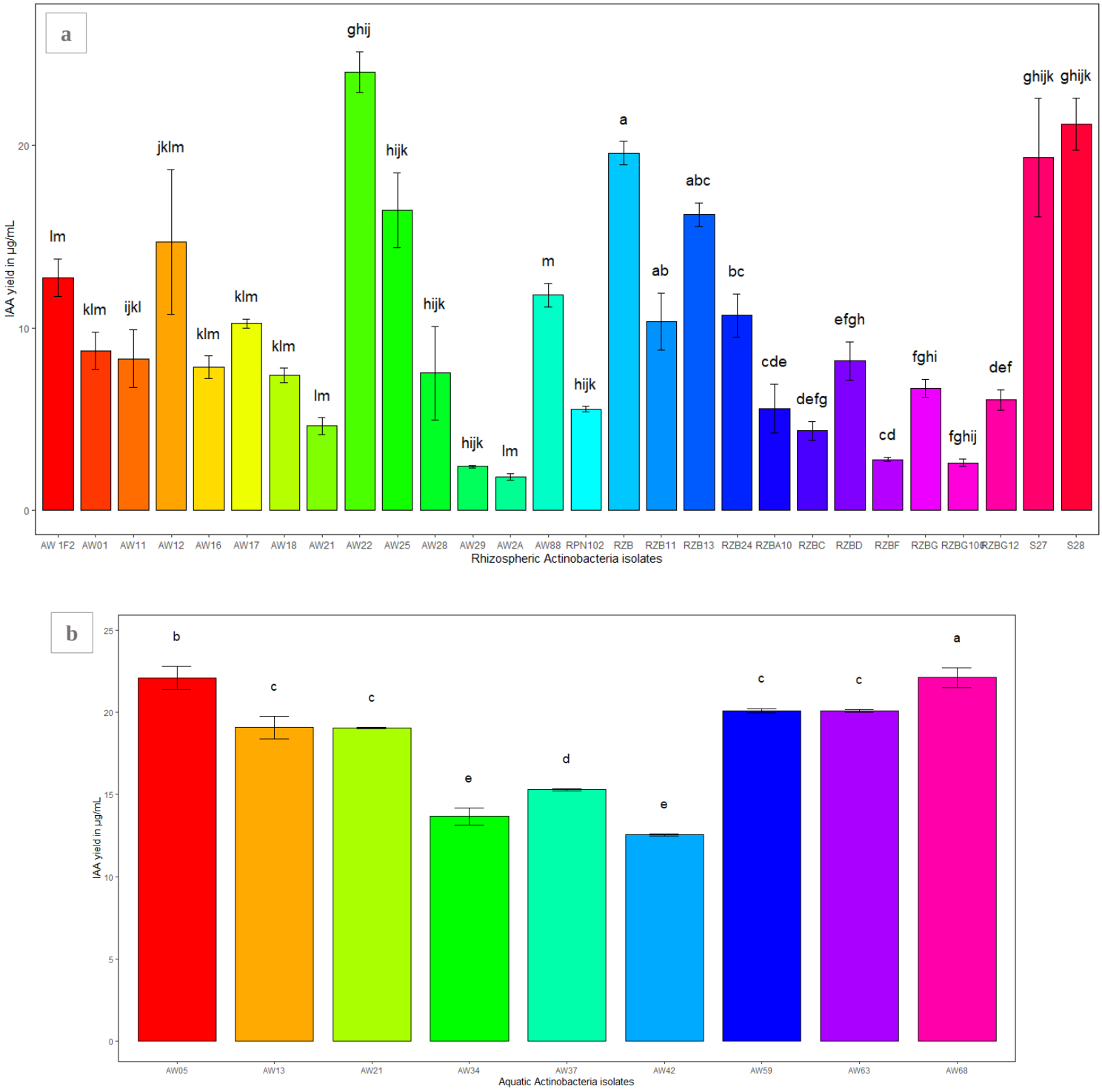


Figure 5. Auxin yields from rhizospheric (a) and aquatic (b) *Streptomyces*-like isolates on YTB (µg. mL^{-1}). One-way ANOVA was used to compare means. Each assay was done three times with three biological replicates, and values represent their means ± standard deviation. Values followed by the same letter are not statistically different according to Tukey post hoc test (p <0.05) (Rstudio).

From the wheat rhizosphere, six isolates comprising RZB13, AW25, S27, RZB, S28 and AW22 were the highest producers (in the range 16.44±2.05-23.999±1.126 µg. mL^{-1}). Isolate AW22

exhibited maximum IAA production with 23.999±1.126 µg. mL^{-1} with 0.2% (w/v) L-Trp added. Thus, AW22 was selected for further studies. Moreover, nine isolates, RZBD, AW11, AW01, AW17, RZB11, RZB24, AW88, AW1F2 and AW12 were moderate producers (in the range 8.185±1.045-12.735±1.038 µg. mL^{-1}) and the rest were the least producers (in the range1.8534± 0.1724- 7.843±0.612 µg. mL^{-1}).

Accordingly, among the Lake Oubeira sediments isolates, AW68 exhibited maximum IAA production. Isolates AW13, AW59, AW63, AW05, and AW21 were moderate producers (in the range 19.076±0.69 - 20.436±0.14 µg. mL^{-1}). Isolates AW34, AW37, and AW42 were minor producers (in the range 12.553±0.06 - 15.302±0.06 µg. mL^{-1}).

Several studies have reported the capacity of rhizospheric *Streptomyces* to synthesize IAA (Ali et *al*., 2021; Oleńska et *al*., 2020) and can be considered a common feature amongst rhizospheric *Streptomyces* species. However, few studies reported the production of IAA from aquatic ecosystems-originated Actinobacteria.

According to Passari et *al*. (2016), *S. thermocarboxydus* DBT219, a tomato-associated endophyte (*Solanum lycopersicum*) yielded 43.8 µg. mL^{-1} of IAA. This production was slightly higher than the minimal concentration reported by Goudjal et *al*. (2016), ranging from 35.9-117 µg. mL^{-1}. Abbasi et *al*. (2019) reported production ranges of 7.0 - 40.9 µg. mL^{-1} of IAA from cucumber and tomato rhizosphere-originated Actinobacteria.

Nafis et *al*. (2019) reported IAA concentrations ranging from 6.70 to 75.54 µg. mL^{-1} within eight days of incubation with *Streptomyces* sp. MNC-1 produces the most.

Several investigations have described the synthesis of IAA by Actinobacteria strains isolated from Algerian environments. Toumatia et *al*. (2016) described the potential of the Saharan soil-derived *Streptomyces mutabilis* IA1 to release a considerable amount of IAA at a maximum level of 74.39 (µg. mL^{-1}), adding to its biocontrol properties against *Fusarium* and rhizosphere competence.

Djebaili et *al*. (2020) reported significant IAA synthesis from a Saharan soil-derived *Streptomyces* strain and salty water (Sebkha) in Northeast Algeria. For example, *Nocardiopsis aegyptica* H14 had the highest score, followed by *Nocardiopsis dassonvillei* subsp. *dassonvillei* T45, *Streptomyces xantholiticus* G22, and *Streptomyces iakyrus* G10 had 14.75, 12.37, and 12.25 µg. mL^{-1}, respectively. *Streptomyces xantholiticus* G33, on the other hand, produced the

least quantity at 7.44 μg. mL^{-1}. These findings are marginally lower than those found in our study.

3.3. Polyphasic characterization of isolate AW22

3.3.1. Morphological and physiological characterization

The cultural aspects of AW22 are noted in Table 12, in Appendix A (Figure S2) and Appendix B (Table S1). Isolate AW 22 displayed typical morphological features of *Streptomyces* genus (van der Aart et *al*., 2019) and exhibited abundant growth on ISP2, ISP3, ISP5, ISP6 and ISP7, and various pigments were observed on integral test media. Soluble and diffusible pigments from dark brown to navy blue, reddish-orange, and pink to purple were observed on ISP2, ISP3 and ISP5. However, AW 22 growth was moderate on ISP1, good on ISP4 and ISP9, with light blue pigment observed exclusively on ISP1. Colony diameter and phenotype of aerial mycelium vary from one medium to another, with an abundant coloured sporulation rate observed on all tested media.

Table 13 records the primary physiological and biochemical attributes of AW 22, as these evaluations are critical assets for classifying and identifying Actinobacteria. A pH ranging between 5-11 enabled the isolate to grow.

No growth at pH13, with optimum growth at pH comprised between 6.8-9.2. Besides, AW22 displayed up to 7% NaCl tolerance. AW 22 was able to grow at temperatures ranging between 15- 40 °C. However, no bacterial development was noticed at 4°C or 45°C. The optimum temperature was revealed to be 28°C. After correlating the physiological and biochemical traits of isolate AW 22 to those of model organisms belonging to the genus, the strain was categorized as a *Streptomyces*.

3.3.2. Enzyme production and biochemical characteristics

Phenotyping for enzyme production from AW 22 under *in vitro* conditions showed that most results were positive (Table 13).

Isolate AW 22 showed significant proteolytic activity and produced cellulase, amylase, lipase, lipoproteinase, and esterase. The isolate AW 22 exhibited nitrate reduction and positive responses to the catalase and urease tests. Amylases, the thermostable enzymes, efficiently degrade organic matter and hasten the composting process (Turan et *al*., 2017). Through the

cleavage of cell wall proteins, microbial proteases play a crucial part in the interactions between the various soil microbiomes (Stach et *al*., 2018; Vranova et *al*., 2013).

Table 12. Relative cultural characteristics of isolate AW 22 on nine *ISP* media.

Medium	Aerial mycelium	Spore color	Substrate mycelium	Soluble pigment	Colony size	Growth status
ISP1	White to light greyish blue	Light blue	Creamy white to light blue	Light blue to green	Small	+
ISP2	Light blue to light grey, then dark grey	Dark grey	Orange to intense brown	Brown, then Navy blue to purple	Big	+++
ISP3	Creamy white to Orange	Dark Grey	Orange, burgundy, then dark brown	Pink to light blue to reddish-purple	Medium	+++
ISP4	Salmon to reddish grey	Light Grey	Orange to red to grey	None	Medium	++
ISP5	Greyish white	Greyish white	Pink to dark red	Pink to purple	Small	+++
ISP6	Creamy to orange	Orange	Creamy white	None	Small	+++
ISP7	Reddish orange to yellow to grey	White to grey	Orange to red to brown	None	Small	+++
ISP9	Creamy white to grey	White to grey	Creamy white	None	Small	++

Note: "+++" indicates abundant growth, "++" indicates good growth, "+" indicates moderate growth, "-" indicates no growth.

Table 13. Enzymatic profile, biochemical and physiological characteristics of isolate AW 22.

Physiological tests	Result
Temperature	
4°C	-
15°C	+
20°C	++
25°C	+++
30°C	+++
35°C	+
40°C	+/-
45°C	-
Tolerance to	
Tellurite 0.5%	+
Sodium Azide 0.1%	-
Phenol 0.2%	+
Growth at	
pH 5.6	+++
pH 6.8	+++
pH 9.2	+++
pH 11	+++
pH 13	-

Enzyme production	Result
Amylases	++
Cellulases	++
Lignin oxidases	UD
Xylanases	UD
Urease	+
Chitinases	+
Catalase	+
Protease (Caseinase)	+
Gelatinase	-
Lipase	-
Lipoproteinases	++
Lecithinase	++
Esterase	+
Twain 20	+
Twain 80	+
HCN	+
Ammonia	+

Biochemical tests	Result
Toletrance to [NaCl]	
1%	+++
2%	+++
2.5%	+++
3%	+++
4%	+++
5%	+++
6%	++
7%	+
8%	+
9%	+
10%	-
Simmons Citrate	+
Peptonisation of SM	+
Nitrate reductase	++

Note: In the Enzymes test items, the "+" means positive, "-" indicates negative, and "UD" indicates undetectable activity. In the other tests, "+++" indicates growth very good, "++" indicates growth good, "+" indicates growth is moderate, "-" indicates no growth.

Lipases are extensively prevalent amongst microbes with substantial industrial value since triglycerides' hydrolysis considerably contributes to the composting of sewage sludge (Pascoal et *al*., 2018). Catalase preserves cells from reactive oxygen species oxidative damages by catalyzing the destruction of hydrogen peroxide into H_2O and O_2. This feature gives the isolate remarkable resilience to several external mechanical and chemical limitations (Mushtaq et *al*., 2019). Furthermore, biosynthesis of the chitinolytic enzymes was detectable in the CCA medium.

Actinobacteria isolates's enzymatic activities exert a direct phytostimulation and biocontrol of pathogens, thus preventing plant pathologies. Nonetheless, degradation of lignin and xylan was undetectable in this isolate, despite the moderate growth of this isolate on their respective test media. Hydrolytic enzymes are essential for improving soil fertility and characteristics. Soil enzymes degrade complex polysaccharides and proteins into simpler molecules (Turan et *al*., 2017). These enzymes are biotechnologically interesting and of significant commercial value (Islam et *al*., 2015; Reetha et *al*., 2014).

The chitinolytic activity of *Streptomyces* AW 22 may be implicated in the fungal cell wall digestion. The chitinases synthesis effectively inhibits fungal growth. The aptitude of AW22 to secrete chitinases suggests its implication in the biocontrol of fungal phytopathogens and nutrient competition (Gherbawy et *al*., 2012). However, different mechanisms, like antibiosis, hyperparasitism, proteolytic and lipolytic enzymes (Xu et *al*., 2017), are less studied. Nonetheless, these mechanisms are also involved in the antagonism of plant-associated fungi.

3.3.3. Plant sugar and nitrogen utilization profile

Physiologically, most reported Actinobacteria isolates utilised different carbohydrates as the carbon source. This characteristic is pivotal in the Actinobacteria taxonomic analysis (Pridham and Gottlieb, 1948). For instance, isolate AW 22 efficiently assimilated D-glucose, D-Xylose, D-Galactose, D- Mannose, Maltose, α-Lactose, D-fructose, D- Ribose, L-Rhamnose, Inositol and D-Mannitol.

The isolate could utilize a low proportion of L-Arabinose, Melibiose, Sucrose and Sorbitol as carbon sources. However, AW 22 was unable to metabolise Raffinose. The results indicate broad carbon assimilation from various vegetal substrates and SCG (Table 14).

AW 22 showed an extensive plant sugar utilization profile and a wide array of hydrolytic enzymes. This multi-functionality of *Streptomyces* isolate may be accredited to their large

genome and epigenetic factors, such as the location and the high contents of organic matter in wheat fields.

Table 14. Carbon and nitrogen source utilization profile of AW 22.

Carbon test items	**Result**	**Carbon test items**	**Result**	**Nitrogen test items**	**Result**
L-Rhamnose	+++	D-Mannitol	+++	Glycine	+
Sucrose	++	D-Xylose	+++	Tyrosine	+++
D-Glucose	+++	L-Arabinose	+	L-Asparagine	++
Maltose	+++	Raffinose	-	Proline	++
D- Ribose	+++	D- Mannose	++	Casein	++
Melibiose	++	D- Galactose	+++	L-Methionine	+
Lactose anhydrous	+++	Starch	+++	α- Lactose	+++
Inositol	+++	D-Fructose	+++	Sorbitol	++

Note: "+++" indicates growth in carbon or nitrogen source is excellent, "++" indicates growth in carbon or nitrogen source is good in general, "+" indicates growth in carbon or nitrogen source is weak, "-" indicates no growth in carbon or nitrogen sources.

3.3.4. Molecular identification and phylogenetic analysis

The AW22-related 16S rDNA sequence was deposited in the NCBI GenBank under the accession ID OP176004. Taxonomical analyses derived from 16S rRNA gene sequencing of AW22, the higher IAA producer, were compared with 98–99,99% similar sequences retrieved from the GenBank database. Sequence alignment confirmed that the isolate belongs to the order Streptomycetales and the genus *Streptomyces*. 16S rRNA locus similarity calculations, based on neighbor-joining analysis, specified that the neighboring relatives for strain AW22 were: *S. rubrogriseus* (KX431235) and *S. fradiae* (AB184063) with similarity values of 99.22 %, and *Streptomyces violaceoruber* (MH155969) and *Streptomyces lividans* (KY767029) with 99.04% similarity.

The phylogenetic tree constructed with the neighbor-joining method and Tamura-Nei model is shown in Figure 6. Strain AW22 formed an independent clade with *S. anthocyanicus* KU973991 separated from *S. rubrogriseus* CS3KG4LA166 (OM971238).

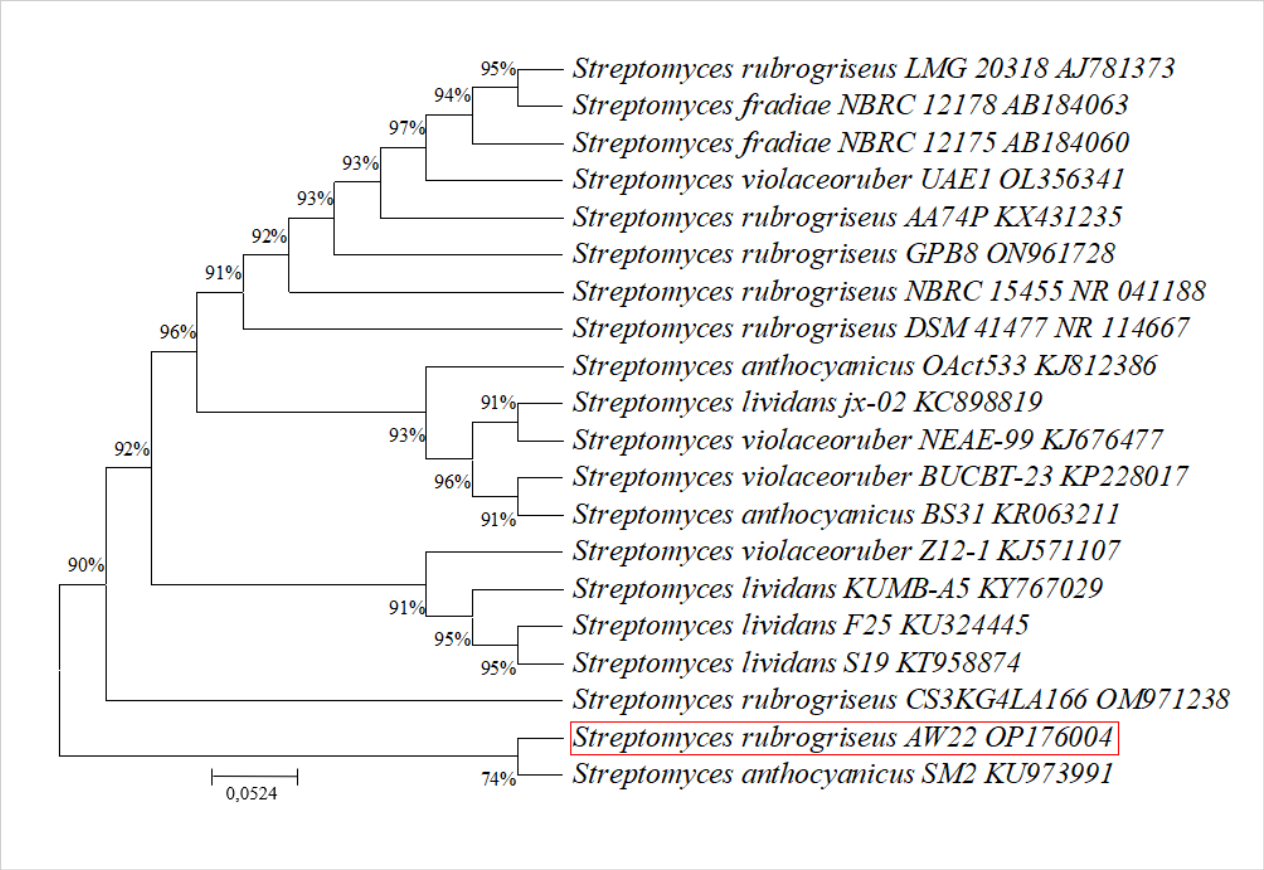


Figure 6. Phylogenetic tree of *S. rubrogriseus* AW22 and related strains.

Neighbor-joining-based tree displaying the taxonomic position of AW 22 compared to its interrelated *Streptomyces* species. The records at nodes indicate the percentage of replicate trees where associated taxonomic units clustered via the bootstrap test relying on 1000 replicates, with collapsed bootstrap replicates when values < 50%. The *p*-distance served to compute developmental distances representing the units of the number of base differences per site. Less than 50% of placement gaps and alignment openings, incomplete data or ambiguous bases were permitted at any position. Subsequently, positions with < 50% site coverage were eliminated. The scale bar illustrates 0.0524 substitutions per position of nucleotide.

3.4. TLC and HPLC analysis of putative IAA

The TLC plates were examined under UV light at 245 nm to visualize the spots developed separately. The results demonstrated that authentic IAA and putative IAA fractions extracted with EA from AW 22 filtrate exhibited similar retention factor (R_f) values of 0.69.

Moreover, the HPLC profile of the authentic IAA peaked at a retention time of 3.508 min, and putative IAA recovered from AW 22 showed up as a prominent area peak at a comparable

retention time of 3.711 min with 0.761 mg/mL, confirming that strain AW 22 produced IAA. These results correspond with previous studies (Myo et *al*., 2019).

3.5. Time course of IAA and biomass production from strain AW 22

Evolution in AW22's biomass and IAA production over ten days of incubation are represented in Figure 7.

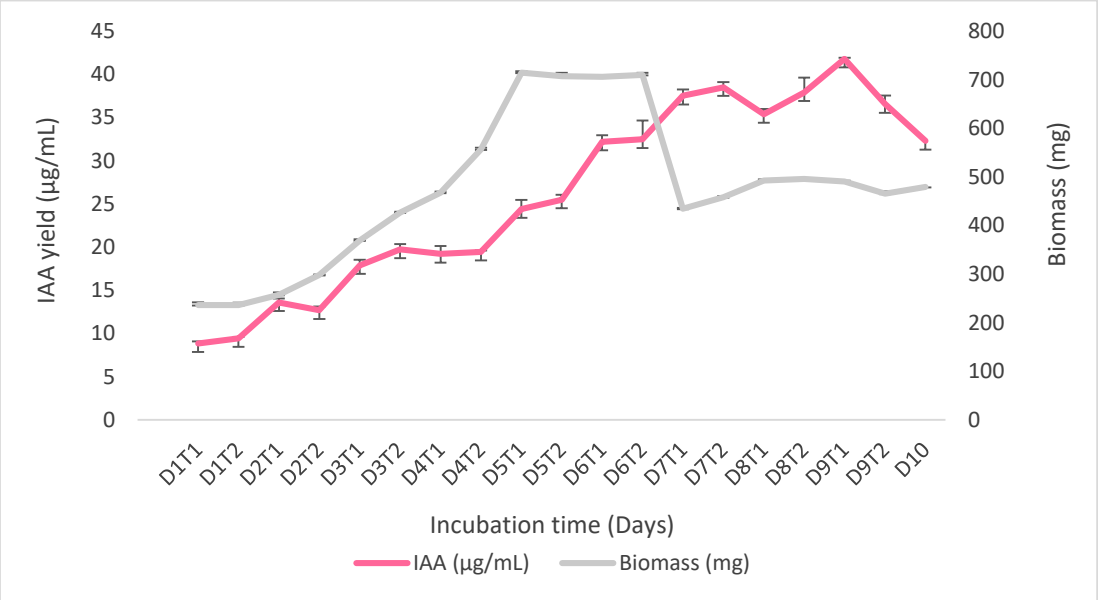


Figure 7. Time course for IAA and Biomass production under standard conditions.

Under optimal conditions, two growth phases of the growth cycle can be identified, a long phase and a short phase. The strain's growth rate peaked at 714.06±3.64 mg of dry weight on the fifth day of incubation and then stabilized for two days. Thus, the cell dry weight decreased by the seventh day to reach 434.53±1.40 mg. However, the biomass remained stable until the last day of incubation, where the germination of new spores may explain this phenomenon.

Furthermore, the IAA synthesis time course of AW 22 was studied over ten days. L-Trp was supplemented with 0.2% (w/v) at an early stage of cell growth. On the first day of incubation, AW22 yielded only 8.84±1.18 µg. mL^{-1} of IAA followed by a gradual enhancement in IAA secretion parallelly with cell growth over the first seven incubation days to attain 37.52±2.18 µg. mL^{-1} to reach its maximum yield of 41.79±1.73 µg. mL^{-1} by the day 9.

The growth of *Streptomyces rubrogriseus* AW22 was almost identical whether L-Trp was present or absent in the medium. Meanwhile, only the cultures fed with L-Trp exhibited increased IAA content. The comparison of the evolution of biomass and IAA production

indicates that this strain's synthesis of secondary metabolites was closely proportional to cell proliferation.

3.6. Influence of carbon sources on IAA production

Previous tests showed that GYM broth containing 0.2% (w/v) L-tryptophan yielded a maximum IAA of 41.79±1.73 µg. mL^{-1} under ideal media and growing conditions. According to Figure 8, the generation of IAA from *S. rubrogriseus* AW 22 in the presence of 0.2% L-tryptophan was evaluated using various carbon sources. Thus, SCG extract and Glucose (positive control) yielded the highest output, with significant differences according to the Tukey test at $p < 0.05$.

Compared to the positive control, the strain recorded medium output on corn starch, CCB, and oat. Even though CMC and WS are both complicated carbon sources, IAA synthesis was neglected on CMC and absent on WS. These findings point to the significance of carbon sources and L-Trp as a precursor in IAA synthesis. As a result, SCG was selected for future optimization research. The high proportion of sugars present in SCG, notably mannose, galactose, glucose, and arabinose, could explain the isolate's ability to develop on an SCG extract broth. Further, the IAA production of this isolate on SCG extract has been described for the first time in this report.

As a natural, safe, cost-effective agricultural product, cost-effective IAA production has been white biotechnology's significant priority. Thus, IAA-producing *Streptomyces* strains have been exploited using diverse agro-wastes to lower the charges associated with IAA production while keeping the same level of bioactivity. Scaling up the bioprocess, on the other hand, may necessitate the identification of optimal growth parameters.

Other investigations have reported IAA synthesis from different carbon sources. Actinobacteria, for instance, have been shown to degrade lignocellulose and create IAA. When starch and KNO_3 were present, *Streptomyces fradiae* NKZ-259 yielded 42.345 µg. mL^{-1} of IAA (Myo et *al*., 2019). Another study found significant levels of IAA (148 µg. mL^{-1}) generated by *Saccharothrix texasensis* MB15 on wheat waste, including cellulose and hemicellulose-rich leaves and roots (Benadjila et *al*., 2022). A recent study found similar IAA levels using raw feedstocks as economical alternatives for analytical-grade medium components (Bunsangiam et *al*., 2021). However, only our prior study reported on the production of IAA on SCG or CCB.

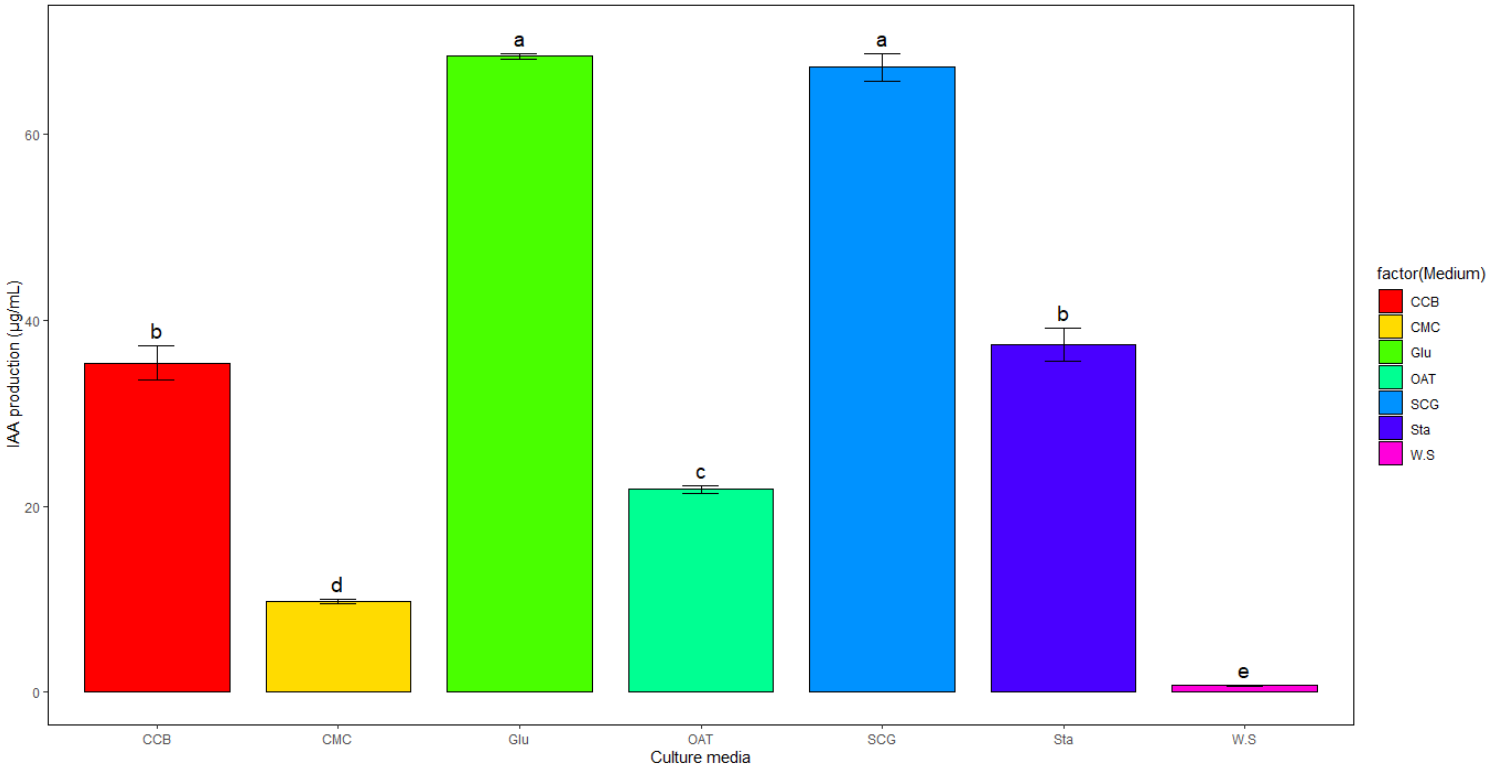


Figure 8. Effect of the variation of carbon sources on IAA production (µg. mL^{-1}). One-way ANOVA was used to compare means. Each assay was done three times with three biological replicates, and values represent their means ± standard deviation. Values followed by the same letter are not statistically different according to Tukey post hoc test ($p < 0.05$) (Rstudio). The labels in the x axis stands for: CCB: Collected Carob Bean; CMC: Carboxymethyl cellulose; GLU: Glucose; SCG: Spent Coffee Grounds; OAT: Oatmeal; Sta: Starch; W.S: Wheat straw.

3.7. Effect of substrate concentration on IAA production

Our previous experiments indicated that GYM broth containing 0.2% L-tryptophan resulted in the maximum IAA yield of 41.79±1.73 µg. mL^{-1} under optimal media and culture conditions. SCG was chosen as an alternative economical fermentation substrate to replace laboratory-grade carbon sources to achieve low-cost IAA synthesis (Tran et *al*., 2023). Subsequently, we briefly assessed the ability of strain AW 22 to produce IAA on a MM containing different carbon source concentrations. Fermentation performed with crude SCG and CBP extracts obtained with the hydrothermal method showed greater IAA yield after the same incubation period (data shown in Figure 9).

Nonetheless, depending on the strain, various carbon sources affect IAA production differently. In some cases, different bacteria have other preferences for using sugars, which can also impact auxin production through bacterial growth (Mohite, 2013; Sridevi et *al*., 2008).

The optimal IAA yield was detected at 50% CBP and 50% SCG, with respectively, 82.3 ±2.18 µg. mL^{-1} and 81.5 ±1.47 µg. mL^{-1}, being 10-fold higher than the negative control and about 2-

fold more elevated than the positive control. Nevertheless, no significant difference was observed between both IAA productivity on these two components when they were amended to the medium at a concentration of 50%.

The greater the concentration of SCG and CBP, the greater the IAA yield from strain AW 22. From the experimental data, we postulate a favourable impact of gradient concentration of CBP and SCG as the only carbon suppliers on IAA synthesis.

The glucose concentration had a smaller effect on IAA production than SCG and CBP. Conversely, IAA production was minimal in the medium containing SCG 10% with 8.4±0.14 µg. $mL^{-1}$and reaching only 17.5±0.14 µg. mL^{-1}, 27.3±0.22 µg. mL^{-1}, 23.3±0.17 µg. mL^{-1} with SCG 20 %, 30%, CBP 10 %, respectively.

Moderate IAA concentrations were obtained in medium containing Glucose 0.2%, SCG 40% and CBP 20% with respectively, 30.0±0.08 µg. mL^{-1}, 39.8±0.22 µg. mL^{-1} and 48.2±0.17 µg. mL^{-1}. Therefore, 30-50% SCG and CBP concentrations were selected for IAA production by strain AW 22.

Additionally, the production of IAA was significantly impacted by the precursor L-Trp, as indicated by the increased IAA level in the positive control consisting of Glucose 0.5% containing L-Trp compared with the negative control devoid of L-Trp. IAA content augmented from 8.7±0.30 to 41.4±0.96 µg. mL^{-1} in the broth containing 0.2% L-Trp.

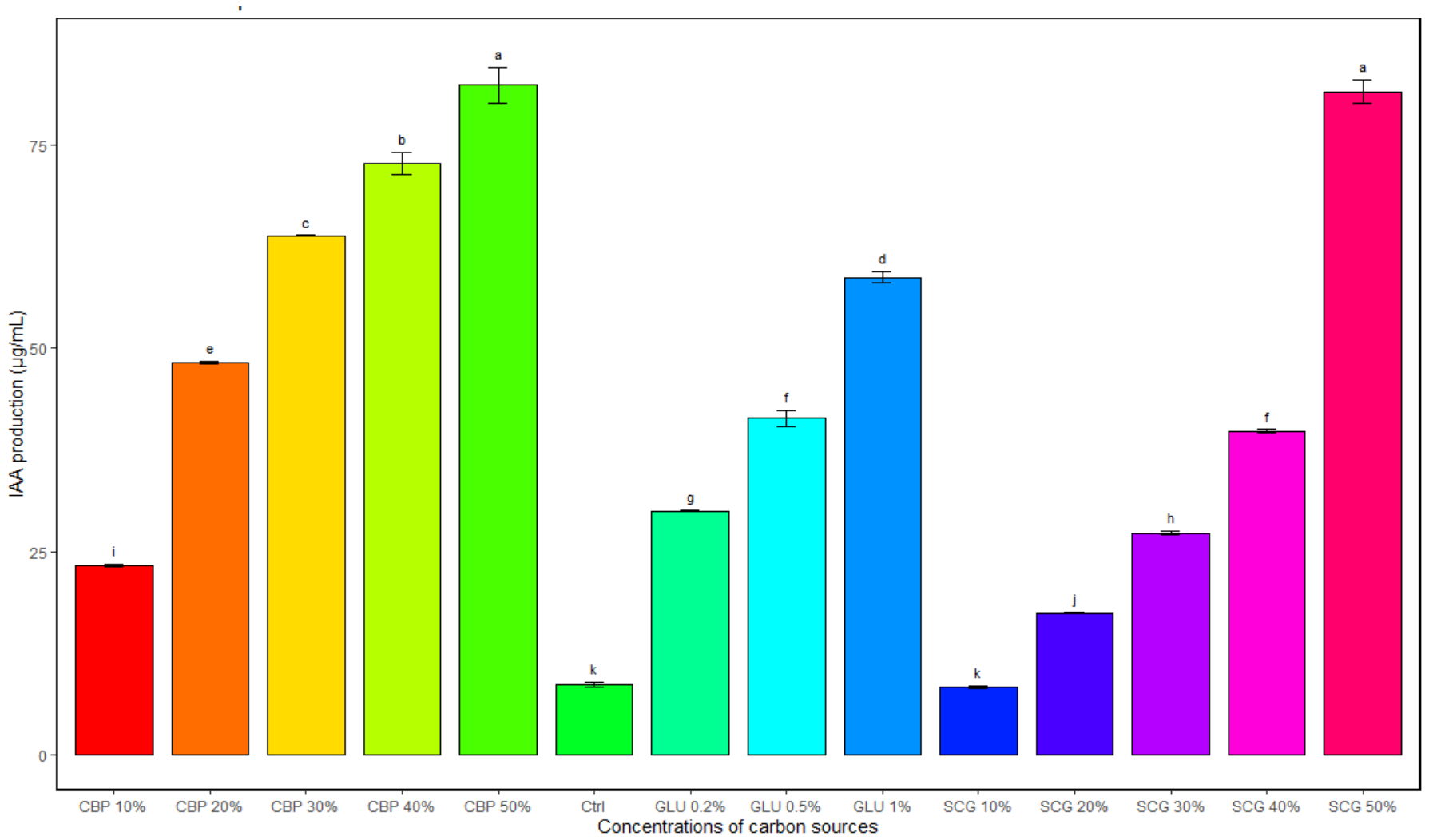


Figure 9. Effect of substrate concentrations on IAA production (µg. mL^{-1}). One-way ANOVA was used to compare means. Each assay was done three times with three biological replicates, and values represent their means ± standard deviation. Values followed by the same letter are not statistically different according to Tukey post hoc test ($p < 0.05$) (Rstudio).

These findings suggest that this strain may use both L-Trp dependant and independent pathways to synthesize Auxin in the culture medium. The low amount of IAA produced in the minimal medium from which carbon and nitrogen supplies were omitted demonstrates illustrates how these macronutrients affect the formation of IAA in AW22.

The increase in the carbon source (CBP, SCG or glucose) is followed by an increase in IAA productivity. These results advocate the critical role of carbon source concentration and L-Trp serving as a precursor in IAA production. Moreover, it highlights the positive correlation between carbon source concertation and IAA yield. This influence may not be directly related to IAA production but indirectly by stimulating bacterial growth suggested earlier from the kinetic of IAA production and biomass production.

Therefore, carbon sources (CBP and SCG) and L-Trp concentrations were optimized using the following statistical approaches to develop a low-cost medium. A recent study by Chaudhary et *al*. (2021) reported 18.74 mg/L mixing corn flour and soybean meal by *Kosakonia pseudosacchari* TCPS-4.

3.8. Influential factors selected by PBD

The PBD strategy investigated the fermentation parameters most significantly affecting IAA generation. The previous experiments identified four potentially important variables (SCG, CBP, tryptophan and incubation time) and were subject to the statistical screen using the PBD methodology.

Figure 10 illustrates the IAA yield from the PBD trials and the Table 15 records the statistical metrics of the PBD approach and factor effects on Y, the response value (IAA production in µg. mL $^{-1}$).

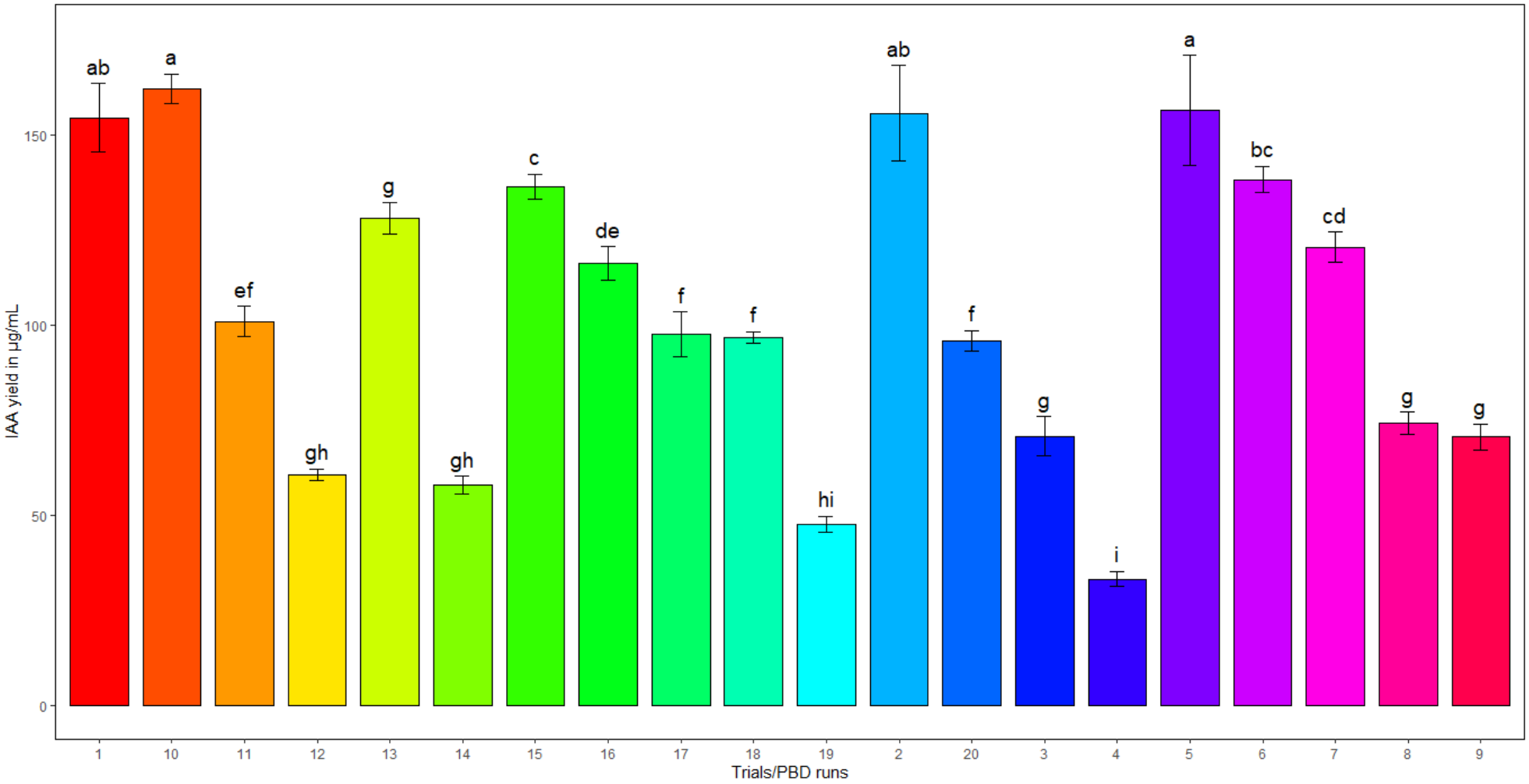


Figure 10. PBD trials related IAA production (µg. mL^{-1}). One-way ANOVA was used to compare means. Each assay was done three times with three biological replicates, and values represent their means ± standard deviation. Values followed by the same letter are not statistically different according to Tukey post hoc test ($p < 0.05$) (Rstudio).

Table 15. ANOVA analysis and coded coefficients of the tested factors for IAA yield.

Terms	Fd	SS	SM	Effect	Coef	SE Coef	T-value	F-value	*p*-Value
Model	14	28449.8	2032.1					6.78	0.022
Linear	14	28449.8	2032.1					6.78	0.022
Constant					103.67	3.87	26.78	0.00	0.000
X1: CBP (%)	1	0.4	0.4	-0.29	-0.14	3.87	-0.04	8.85	0.972
X2: SCG (%)	1	2651.2	2651.2	23.03	11.51	3.87	2.97	0.04	**0.031**
X3: Starch	1	13.1	13.1	-1.62	-0.81	3.87	-0.21	1.70	0.843
X4: Tryptone (g/L)	1	509.6	509.6	10.10	5.05	3.87	1.30	0.01	0.249
X5: Yeast E (g/L)	1	3.3	3.3	-0.81	-0.41	3.87	-0.11	41.16	0.920
X6: L-Trp (%)	1	12332.9	12332.9	49.66	24.83	3.87	6.42	0.22	**0.001**
X7 : NaCl (g/L)	1	67.3	67.3	3.67	1.83	3.87	0.47	0.55	0.656
X8: K2HPO4 (g/L)	1	164.0	164.0	-5.73	-2.86	3.87	-0.74	0.30	0.493
X9: MgSO4 (g/L)	1	90.4	90.4	4.25	2.13	3.87	0.55	1.94	0.606
X10 : CaCO3 (g/L)	1	580.6	580.6	-10.78	-5.39	3.87	-1.39	3.57	0.223
X11: Incub. Time (Days)	1	1068.3	1068.3	14.62	7.31	3.87	1.89	20.32	0.118
X12: T°	1	6088.2	6088.2	34.89	17.45	3.87	4.51	16.20	**0.006**
X13: pH	1	4854.4	4854.4	31.16	15.58	3.87	4.03	0.09	**0.010**
X14: Inoc. amount %	1	26.0	26.0	2.28	1.14	3.87	0.29	6.78	0.780
Error	5	1498.2	299.6						
Total	19	29948.0							

Figure 10 shows that factor combination 10 exhibited optimum IAA production values, reaching 161.95±3.96 µg. mL^{-1}. Nonetheless, strain AW22 produced 33.26±2.01 µg. mL^{-1} from medium composition 4.

According to one-way ANOVA, SCG, tryptophan, pH, and temperature, all had significant *p*-values (0.031, 0.001, 0.010, and 0.006, respectively). Based on this analysis, four of the fourteen variables significantly impact the IAA yield and have been recognized as essential for the RSM approach.

Temperature physiologically affects fermentation and ATP control (Yan et *al*., 2018). Due to their slow growth, Actinobacteria may be affected by high or low temperatures (Kanimozhi et

al., 2017; Sohn et *al*., 2023). This change, in turn, modulates the regulatory metabolic pathways and the cell wall composition, resulting in a diversity of metabolic responses and the production of a diverse range of products (Talukdar et *al*., 2016).

Different IAA biogenesis mechanisms evolve in microbiomes and their hosts (Di et *al*., 2016) identified unique IAA production routes in multiple species. In contrast to dietary effects, L-Trp plays an essential role in practically all bacterial strains that generate IAA. L-Trp is converted to indole-3-acetamide by tryptophan monooxygenase before being converted to IAA by indole acetamide hydrolase. The *iaaM* gene encodes tryptophan monooxygenase, whereas indole acetamide hydrolase is encoded by the *iaaH* gene (Casanova et *al*., 2005; Park et *al*., 2021). The researchers discovered three new L-Trp -dependent pathways. According to reports, plants can maintain a baseline level of auxin via L-Trp -independent mechanisms (Ribnicky et *al*., 2002).

L-tryptophan is required for bacteria to generate IAA, but the optimal concentration and optimum productivity vary depending on the species. Nonetheless, studies on these bacterial IAA biosynthetic pathways are limited. Furthermore, researchers still need to fully explain the Trp-independent route in plants (Di et *al*., 2016).
Although this, the *p*-values for CBP, Starch, Tryptone, Yeast E, NaCl, K_2HPO_4, $MgSO_4$, $CaCO_3$, Incubation time and Inoculation amount % were > 0,05, indicating that none of these factors was significant. Subsequently, in the following RSM experiment, these components were kept at their central (zero) level, and not excluded from the culture medium.

The Pareto chart (Figure 11) summarizes the elements that have the most significant influence on IAA productivity (Hymavathi et *al*., 2010).

The *t*-value reveals each factor's positive and negative effects (Talhi et *al*., 2022). A positive value indicates that the factor positively impacts IAA yield; if the value is negative, the opposite is true. Table 15 demonstrates that SCG, Tryptone, L-Trp, NaCl, $MgS0_4$, Inc. Time, T°, pH, and Inoculation amount had positive effects on the IAA production, while the CBP, Starch, Yeast E, K_2HPO_4, $CaCO_3$ had adverse effects.

Organic and inorganic nitrogen sources also impact IAA productivity by bacteria differently. According to Chandra et *al*. (2018), combining dextrose and beef extract as carbon and nitrogen sources was optimal for higher IAA productivity. Nonetheless, organic and inorganic nitrogen sources had no significant effect on IAA production from strain AW 22.

The variance of the actual response was explained accordingly with the decision coefficient R^2 values to verify the accuracy and fitness of the model. The model's ability to explain the variation in dependent variables reduces as the R-squared value decreases. In our case, R^2 values could account for 95% of the variation in the data (the model could not explain only 5% of the variation), indicating the suitability of the analysis and prediction of changes in IAA generation during fermentation.

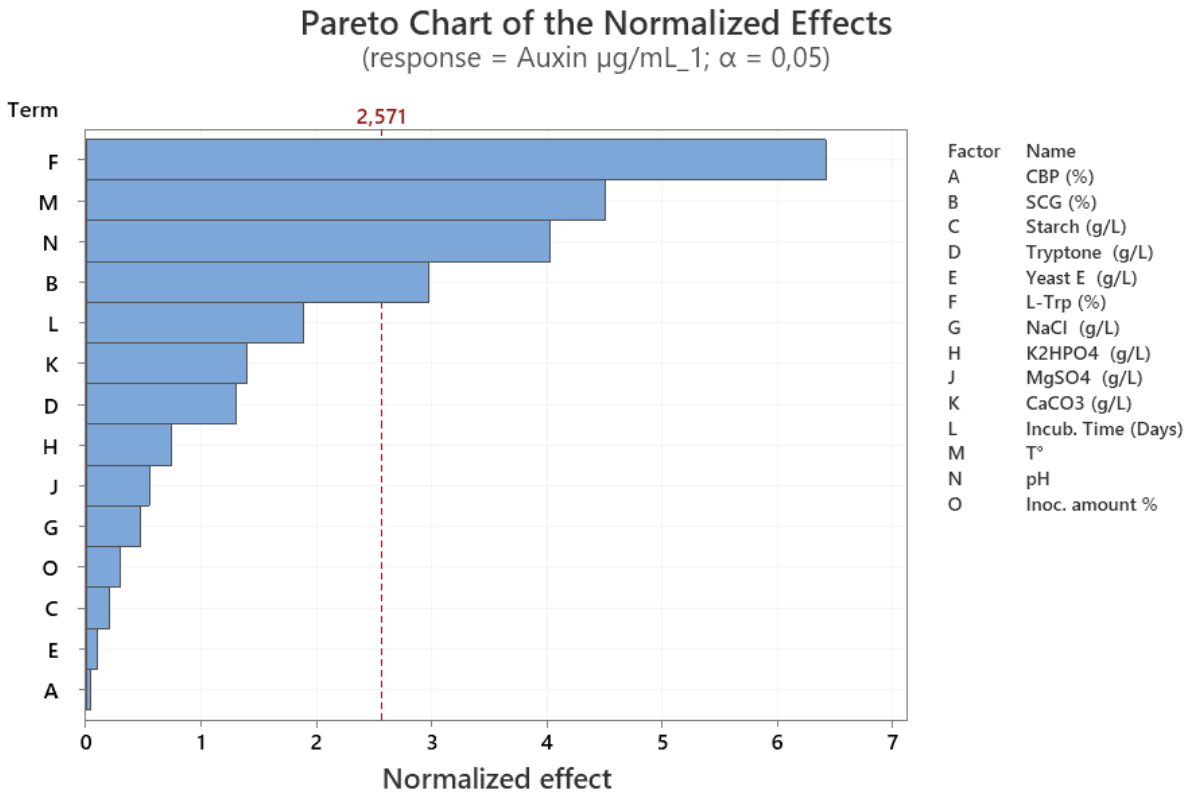


Figure 11. Pareto chart describing the normalized effects for IAA production.

3.9. Model elaborated by RSM-BBD

The microbial fermentation process is complex, nonlinear, and unstructured. The yields of specific compounds can be affected by slight changes in the fermentation media composition and the operating culture parameters, altering the strain's metabolic profile (Kaur et *al.*, 2014).

Ideal fermentation conditions can be challenging to determine, requiring experimental designs. RSM helps improve the culture medium composition and operating conditions, enhances IAA productivity and participates in the search for natural physiologically active substitutes for chemical agro-actives (Arul Jose and Jebakumar, 2014; Mazarei et *al.*, 2017).

The primary function of the Box-Behnken experimental design from RSM is to forecast which suitable quadratic model will better elucidate the correlation between inputs and outputs. BBD was carried out to optimise further the concentration of the four factors defined as significant using PBD analysis at three levels to identify the best fermentation conditions for the low-cost process. Figure 12 shows the IAA yield obtained from BBD trials.

In this design, run 12 comprising in (w/v) L-Trp, 0.6% and SCG, 30% with T°, 30.5 and pH 8.05 was ideal for IAA secretion, peaking to 184.36±7.85 µg. mL^{-1}. Nonetheless, the least IAA amount of 18.56±1.26 µg. mL^{-1} was noticed in run 11 containing in (w/v) L-Trp, 0.2% and SCG, 40% with T°, 35 and pH 8.05, which is about 10-fold.

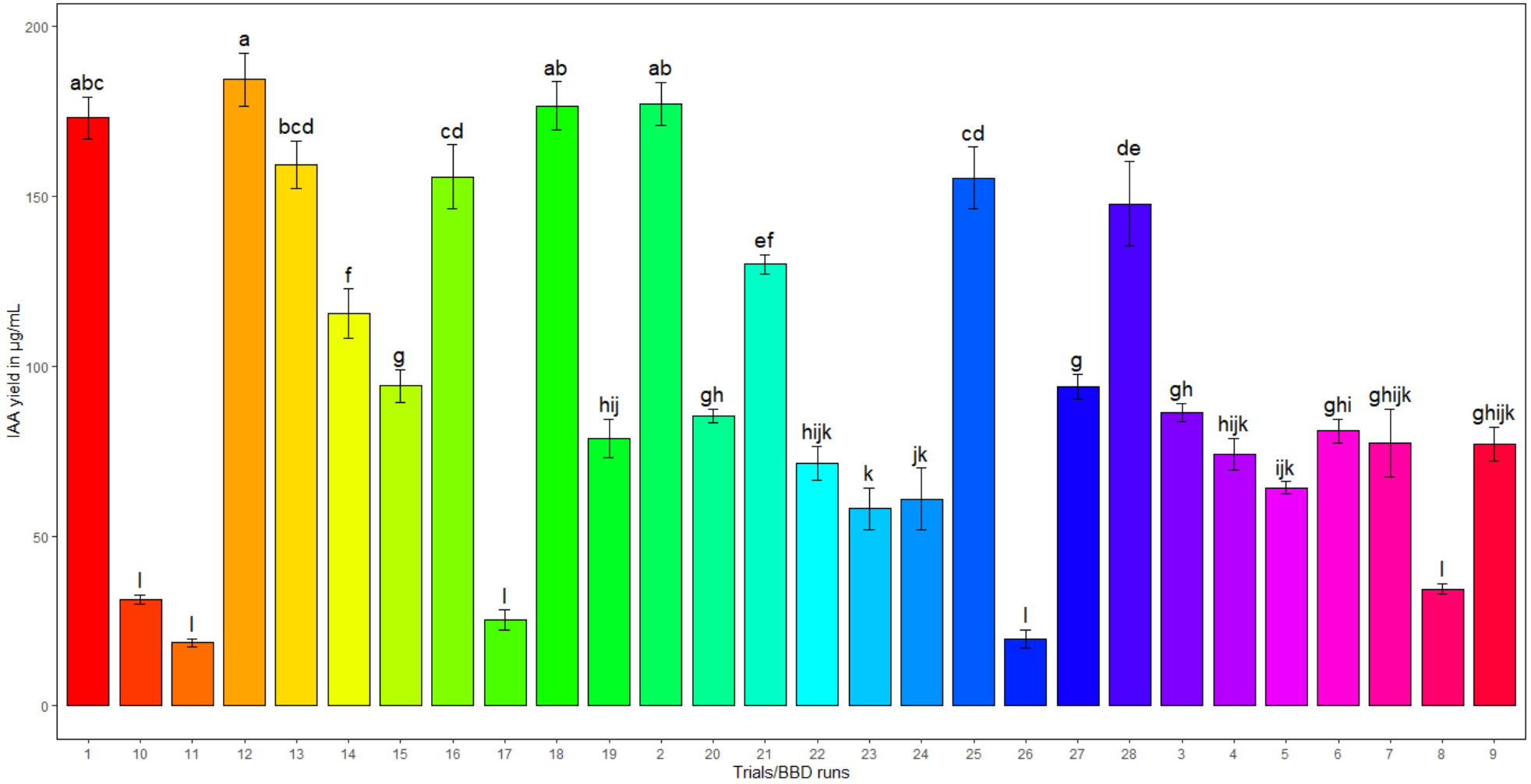


Figure 12. IAA production according to BBD trials (µg. mL^{-1}). One-way ANOVA was used to compare means. Each assay was done three times with three biological replicates, and values represent their means ± standard deviation. Values followed by the same letter are not statistically different according to Tukey post hoc test ($p < 0.05$) (Rstudio).

3.10. Postulated model and statistical validation

The statistical metrics of factor significance, the coded coefficients of variables and other model details obtained from ANOVA are summarized in Table 16. In contrast, Table 7 lists the actual and predicted response values for IAA productivity.

A *p*-value of less than 0.05 reveals the significance of the model. Furthermore, the probability value also shows that the model suits and fits the experimental data. Nevertheless, the low model F-value of 2.99 suggests a low model accuracy.

3.11. Factors effects and fitted model

From these data, the model terms L-Trp and pH were significant (p <0.05), while values greater than 0.1000 were insignificant. The linear effects were substantial, as revealed by the monomial coefficients L-Trp and pH having a *p*-value less than 0.05. The other terms' *p*-value was greater than 0.05, indicating a negligible linear impact. The lack of a significant interaction between L-Trp and pH shows no interaction between the two variables.

In our investigation, the *Fo* of 0.29 is inferior to *Fcritic* (0.05, 10.3) = 8.79. Moreover, an F-value and a *p*-value of the lack of fit of the response function are respectively 0.29 and 0.938, suggesting the model fitting was not satisfying. Therefore, we cannot settle that the model adequately fits the data (Montgomery, 2019).

An ANN-GA modeling based on nonlinear regression will be implemented to understand the data. The determination coefficient R^2, which had a value of 76.28% and indicated that the model did not account for 23.72% of the total variation in IAA production, was used to evaluate the significance and correctness of the model developed in this study. The adj R^2 value of 50.73% indicates low reliability between the experimental output values and those predicted by the model.

These results further demonstrated the model's low accuracy, suggesting that the equation used to create the model must suitably reflect the test value. As a result, the regression model was assumed to not adequately and effectively analyze and predict the IAA generation of *S. rubrogriseus* AW 22.

Table 16. Statistical metrics of the tested factors on IAA production for BBD experiment.

Terms	**Fd**	**SS**	**SM**	**Coef**	**SE Coef**	**T-value**	**F-value**	***p*-Value**
Model	14	55808.6	3986.3				2.99	0.028
Linear	4	42806.9	10701.7				8.02	0.002
Constant				120.3	18.3	6.58	0.000	0.000
L-Trp	1	30105.5	30105.5	50.1	10.5	4.75	0.000	**0.000**
T°	1	2309.5	2309.5	-13.9	10.5	-1.32	0.211	0.211
pH	1	7042.6	7042.6	24.2	10.5	2.30	0.039	**0.039**
SCG	1	3349.3	3349.3	-16.7	10.5	-1.58	0.137	0.137
Squares	4	6422.4	1605.6				1.20	0.356
L-Trp*L-Trp	1	4081.5	4081.5	-26.1	14.9	-1.75	3.06	0.104
T°*T°	1	3188.5	3188.5	-23.1	14.9	-1.55	2.39	0.146
pH*pH	1	149.8	149.8	-5.0	14.9	-0.33	0.11	0.743
SCG*SCG	1	6.6	6.6	-1.0	14.9	-0.07	0.00	0.945
Interactions	6	6579.3	1096.6				0.82	0.573
L-Trp*T°	1	901.7	901.7	-15.0	18.3	-0.82	0.68	0.426
L-Trp*pH	1	1084.1	1084.1	16.5	18.3	0.90	0.81	0.384
L-Trp*SCG	1	2314.4	2314.4	-24.1	18.3	-1.32	1.73	0.211
T°*pH	1	0.4	0.4	-0.3	18.3	-0.02	0.00	0.987
T°*SCG	1	1117.5	1117.5	16.7	18.3	0.91	0.84	0.377
pH*SCG	1	1161.2	1161.2	17.0	18.3	0.93	0.87	0.368
Error	13	17357.1	1335.2					
Lack of fit	10	8597.1	859.7				0.29	0.938
Pure error	3	8760.1	2920.0					
Total	27	73165.8						

Model	**S**	**R^2**	**R^2 (adj)**	**R^2 (pred)**
	36.5399	76.28%	50.73%	11.03%

The regression was conducted to fit the response function to the empirical data. Subsequently, the second-order polynomial regression equation analyzing the regression model and describing the predicted response (Y_{pred}=IAA in µg. mL^{-1}) generated from RSM is represented as follows (Eq. 14).

IAA (µg. mL^{-1}) = 120.3 + 50.1 L-Trp – 13.9 T° + 24.2 pH – 16.7 SCG – 26.1 L-Trp*L Trp- 23.1 T°*T° - 5.0 pH*pH – 1.0 SCG*SCG – 15.0 L-Trp*T° + 16.5 L-Trp*pH – 24.1 L-Trp*SCG – 0.3 T°*pH + 16.7 T°*SCG + 17.0 pH*SCG (Eq. 14)

3.12. Contour plots

The contour plots (2D) in Figure 13 designate the graphical representation of the correlations between the significant process factors, optimal values and the specific output variability (Baş and Boya, 2007). These graphics help understand and describe the two variables' combined effect on IAA production by AW 22.

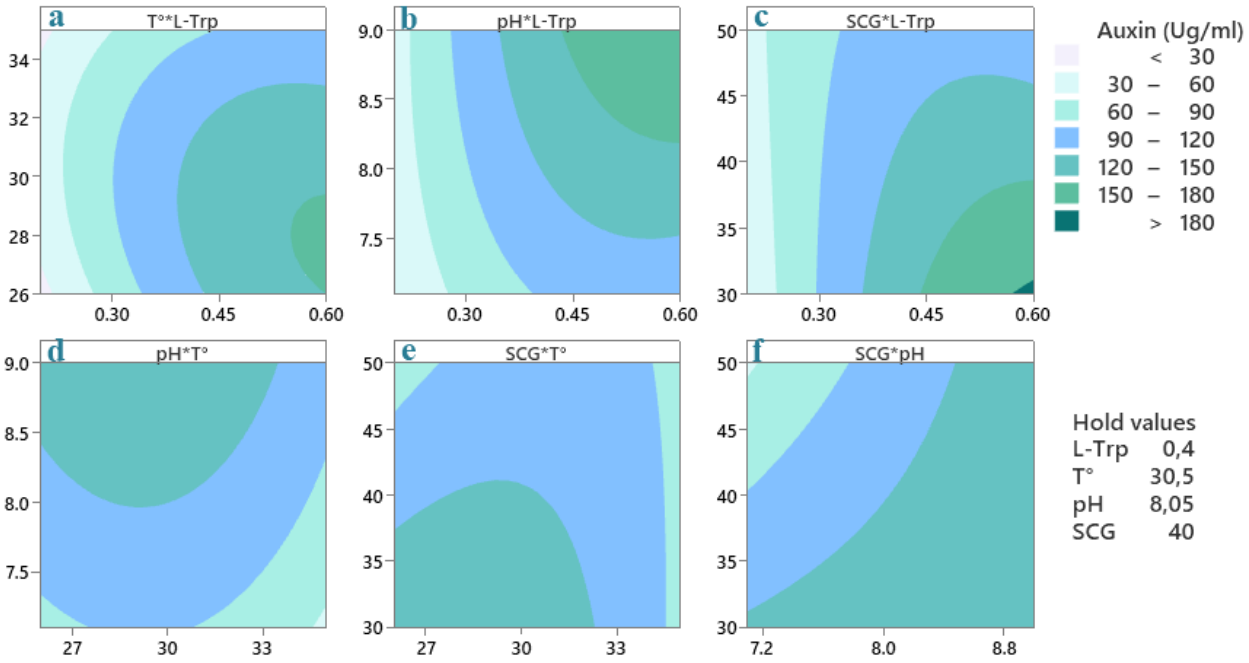


Figure 13. Contour plots IAA production. Plots (a-f) represents IAA production responses implicating binary independent variables as the coordinates.

According to the contour plot forms, it is possible to instantly verify the significance of the interaction, which may be high if the contour plot is elliptical and saddle or, on the contrary, low if it represents a circular shape (Berkani et *al*., 2019). At the same time, the remaining pair of factors were kept at their centre point, thus efficiently determining the maximum response value under the influence of the operating inputs. The elliptical contour plots describe the significant impact of interactions between (T°, L-Trp), (pH, L-Trp) and (SCG, L-Trp). The maximum IAA yield was achieved at low temperatures, and high L-Trp with pH and SCG were fixed at the zero level, as shown in Figure 13 (a). However, Figure 13 (b) explains a maximum

production at alkaline pH and high L-Trp concentration. Moreover, when SCG concentration was low and accompanied by a high concentration of the precursor L-Trp, IAA reached its maximum level, as shown in Figure 13 (c). Elliptical interactions were also noticed for (pH, T°), (SCG, T°), represented respectively in Figure 13 (d), (e), inform that low T° with alkaline pH and low T° with low SCG concentration respectively can lead to elevated IAA concentrations. For SCG and pH, represented in Figure 13 (f), contour lines were rounded, suggesting the absence of significance.

3.13. Data visualization and Correlations between variables

The relationships between four significant factors (SCG, T°, L-Trp and pH) and the ideal values for each variable impacting IAA output were also examined using a Matrix plot and a Scatterplot Matrix represented in Figure 14 (a,b).

The matrix plot (Figure 14a) represents the data clustering and distribution of the output variable Y=IAA (µg. mL^{-1}) and displays a visual representation of the relationship between four input variables (SCG, T°, L-Trp and pH) and the output. However, the scatterplot matrix (Figure 14b) displays different correlations between inputs and outputs and depicts pairwise dependencies between attributes.

The matrix (Figure 14a) shows plots exhibiting the relationships for all pairs of variables. From the matrix, some correlations may be observed between the pairs of variables. These types of correlations include linear (positive or negative), curved (quadratic or cubic), or no relationship at all.

The scatterplot (Figure 14b) suggests a significant positive correlation exists between L-Trp and IAA, where most data were clustered around the maxima. Moreover, the scatterplot suggests a moderate positive correlation between pH and IAA. Higher temperatures had a negative effect on IAA production. In contrast, moderate and medium temperatures around 26°C had a positive effect. However, no significant effect was noted for SCG on the variation of IAA production displayed by a more homogenous distribution of the data points on the interval range of values, suggesting a random or noisy influence.

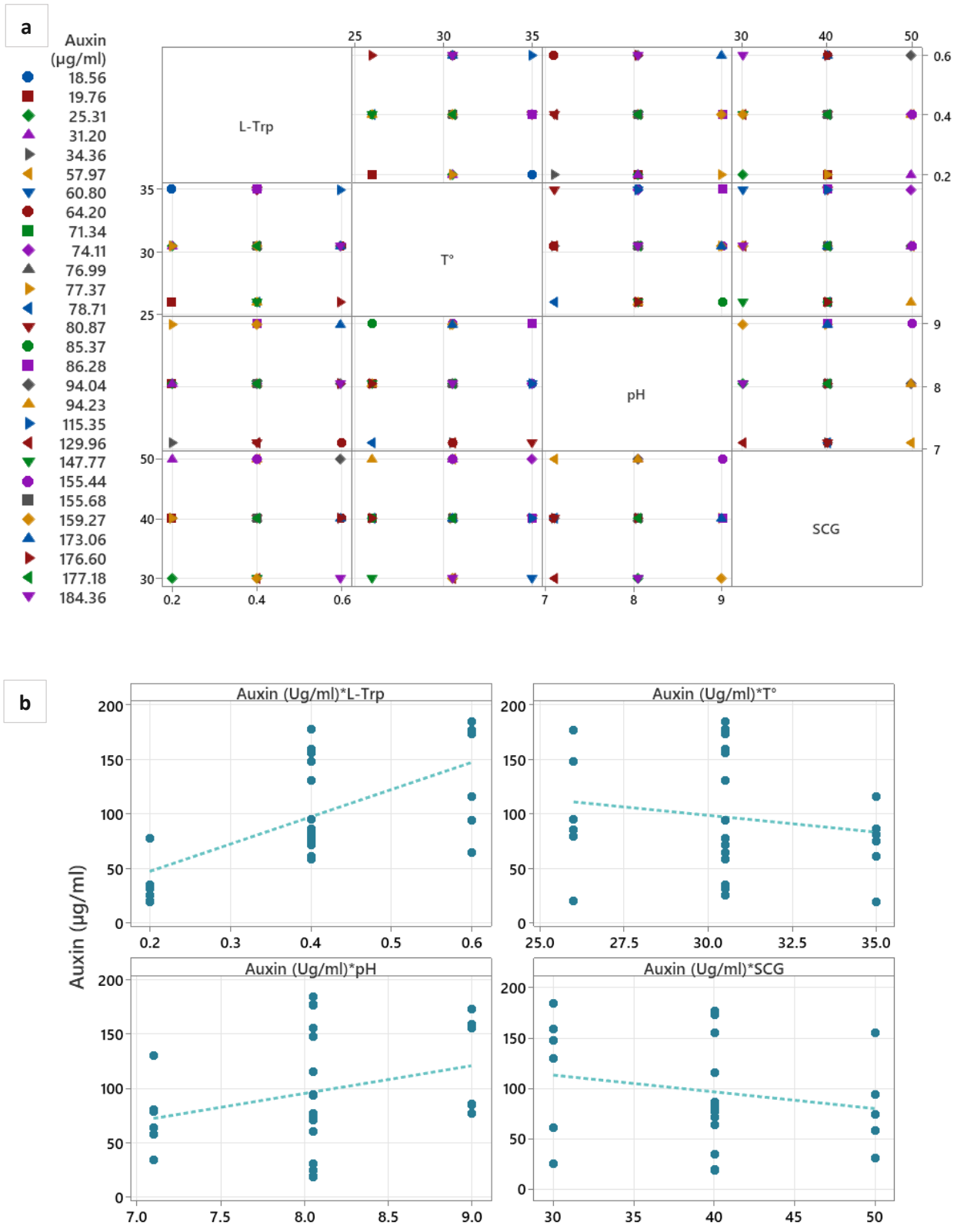


Figure 14. Data distribution and visualization via (a) Matrix Plot of input variables and (b) Scatterplot of Auxin (µg. mL^{-1}) vs L-Trp, T°, pH, SCG.

3.14. Process modeling using ANN-GA

For modeling the IAA production process, (1–10) hidden neurons were tested to select the optimal network topology according to the MSE value/number of neurons relationship. The one hidden layer standard multilayer feed-forward network has been considered a universal approximator (Lin et *al*., 2021; Zhang and Pan, 2014). However, this study configures the model with two hidden layers.

Figure 15 (a,b) displays the performance of the ANN model at the last stage of the training phase. According to Figure 15 (a,b), the training converged after 129 epochs with the lowest mean square error. Thus, upon iterative training of the ANN, the model achieved a maximum R-value of 0.999 (Figure 15a) along with a minimum MSE value of 1.86×10^{-5} (Figure 15b) at 129 epochs for 6 neurons in the hidden layer with tangent-sigmoidal transfer function.

Therefore, the best network architecture of 4-6-1 is used for process optimization, representing 4 inputs in the first layer, 6 neurons in the hidden layer, and one output in the last layer. The R^2 value close to 1 and a low MSE value indicate that the performance of the developed model was satisfying and suitably fits the IAA experimental values.

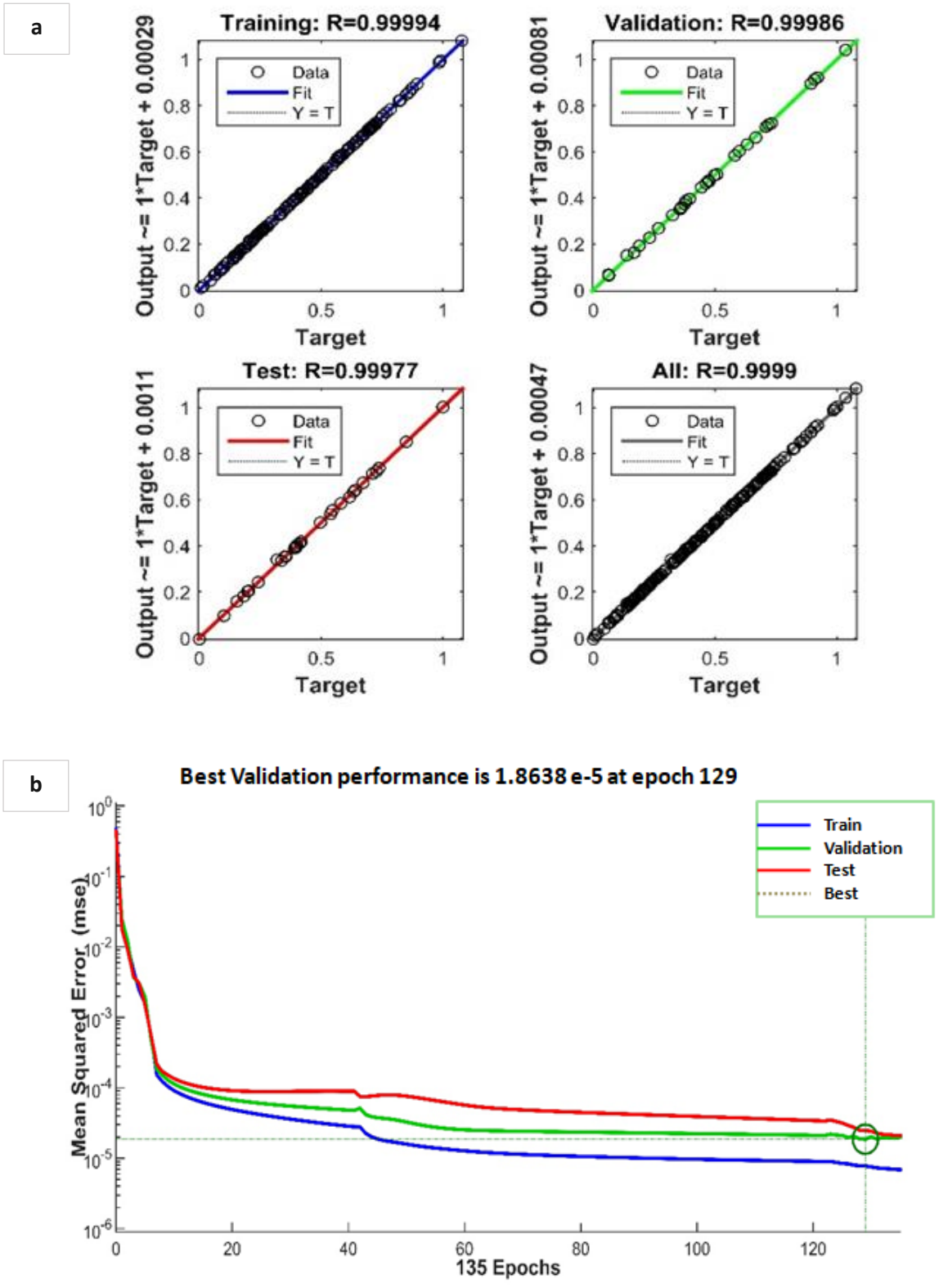


Figure 15. Convergence efficacy of ANN model during training stage. (a) Regression plot illustrating the correlation between predicted and experimental output values; (b) Performance of the ANN model at the training stage.

3.15. GA-assisted optimization

The GA algorithm was used to optimize the ANN model and find the stationary points of the operating conditions that would provide the maximum IAA yield. Starting with a population of random regimes, the GA technique optimized temperature, initial pH, initial concentration of L-Trp, and SCG as input parameters. The optimum points for the process variables were chosen between the lower and upper ranges shown in Table 7. Likewise, Table 17 summarizes the data, demonstrating that the maximum IAA level was 226.04 µg. mL^{-1}under the optimal circumstances of the four variables.

3.16. Experimental validation of the models

Predicted optimum levels of inputs, adjusted target output values, and actual response values for RSM-BBD and ANN-GA postulated models are illustrated in Table 17.

Table 17. Factors configuration with the predicted and experimental response values.

Factors	**Actual value of predicted optimum**	**Predicted max. Y value (µg. mL^{-1})**	**Desirability**	**Expreimental Y value (µg. mL^{-1})**
		RSM-BBD		
L-Trp	0.572	184.363	1	183.45±0.18
T°	31.25			
pH	9			
SCG	30			
		ANN-GA		
L-Trp	0.6	226.04		188.290±0.38
T°	25.8			
pH	9			
SCG	30			

The accuracy of both proposed models in predicting maximum output data was verified. For occurrence, triplicate experiment sets were run, with the results of RSM-assisted optimization utilizing the desirability function and the ANN-GA-predicted optimal levels of process parameters. The experimental output values were then compared to the simulated responses predicted by the RSM-BBD and ANN-GA models.

According to Table 17, the experimentally recorded IAA was 183.45±0.18 µg. mL^{-1}, close to the RSM-BBD model's predicted value (184.363 µg. mL^{-1}). For the ANN-GA, the observed IAA concentration obtained from validation experiments (188.290±0.38 µg. mL^{-1}) closely matched the expected value (226.04 µg. mL^{-1}) with a slight difference. These results suggest

the adequacy and validity of the model. However, the optimums obtained from ANN-GA permitted an even higher IAA yield than with RSM-BBD-based modeling.

Moreover, strain AW22 produced substantially more IAA while utilizing the improved medium. Enhancement in IAA productivity was up to 4.55-fold and 4.46-fold with ANN-GA and RSM, respectively, compared with the one obtained using the unoptimized medium. This difference between predicted and experimental values may be due to the genotyping source and some epigenetic factors, such as medium characteristics. This is the maximum yield strain AW22 can achieve on an SCG medium.

The ANN-GA presents higher prediction accuracy regarding IAA response prediction, the higher R^2 and the lower MSE compared to RSM, regarding the neglected possibility of the model getting into overfitting or underfitting after optimization. This performance is attributed to the overall ability of ANN-GA to analyze the nonlinear behaviour of the system. At the same time, the response surface model is limited by second-order polynomial regression. Therefore, these findings confirm the suitability of the ANN-GA-assisted modeling as an alternative to RSM-based models in predicting microbial metabolic profiles, such as IAA.

Conclusion and Perspectives

Conclusion and perspectives

Summary of key results

Evidence is growing on auxin's role in mediating abiotic stress tolerance in plants and physiological responses. The capacity of root-associated Actinobacteria to produce auxin/IAA, mainly native rhizospheric *Streptomyces* strains, has been studied. However, no reports on aquatic strains' auxin production ability have yet been described. Likewise, as plant inoculants, Actinobacteria may play a significant role and conquer their host rhizosphere. These strains constitute singular metabolic engineering targets as an alternative to synthetic auxin for the onset of growth stimulation and the induction of host tolerance to abiotic stresses.

This study aimed at isolating Actinobacteria strains with a growth promotion ability and the biocontrol potential of *Fusarium culmorum*, the wheat root rot-causing fungi from terrestrial and aquatic Algerian ecosystems, i.e. the wheat rhizosphere in the Tiffeche region (Souk-Ahras) and the aquatic sediments of Lake Oubeira (El Taref).

This report is the first to investigate and explore the IAA production potential of *Streptomyces rubrogriseus* AW 22 isolated for the first time from wheat rhizosphere. In addition, AW 22 displayed relevant enzymatic activities, *in vitro* biocontrol potential against *F. culmorum* and distinctive pigments on different culture media.

Furthermore, this study describes the valorization of coffee waste (SCGs) for *in vitro* and *silico cost-effective* medium engineering with machine learning tools like RSM-BBD and ANN-GA. These novel advanced approaches aimed at predicting the ideal operating conditions for maximizing organic IAA yield while decreasing process costs, processing time, and the number of experiments using SCG hydrothermal extract as a low-cost substrate. Our results show the higher accuracy of the ANN-GA-based model in comparison with RSM. The complexity and nonlinearity of microbial development and metabolism could cause such a contrast.

Interestingly, *S. rubrogriseus* AW 22's multifunctionality offers new opportunities for agricultural management strategies as an organic plant stimulant and its industrial-scale production.

Ongoing works and prospects

The main perspectives of the current work that needs to be regarded in our future research should focus on:

1- Identifying the IAA biosynthetic pathway in *S. rubrogriseus* AW 22.
2- Elaborating a metabolic profile to study *S. rubrogriseus* AW 22's improved plant nutrient uptake, such as phosphate solubilization, and assess soil-borne pathogens' *in vivo* biocontrol potential. This inquiry provides fascinating insights into how this bioprocess might be oriented to produce *Streptomyces*-originated IAA on an industrial scale for phytostimulant production for business users
3- Formulating an agroactive/biocontrol product based on the biomass of the strain constituting the active principle with an extended shelf life. Indeed, organic products can reduce environmental contamination and raise revenue while minimizing chemical fertilizer production costs and related threats.

References

References

Abbasi, S., Safaie, N., Sadeghi, A., and Shamsbakhsh, M. (2019). *Streptomyces* Strains Induce Resistance to *Fusarium oxysporum* f. Sp. *Lycopersici* Race 3 in Tomato through Different Molecular Mechanisms. *Frontiers in Microbiology*, *10*(JUL), 1505. https://doi.org/10.3389/fmicb.2019.01505

AbdElgawad, H., Saleh, A. M., Al Jaouni, S., Selim, S., Hassan, M. O., Wadaan, M. A. M., Shuikan, A. M., Mohamed, H. S., and Hozzein, W. N. (2019). Utilization of actinobacteria to enhance the production and quality of date palm (*Phoenix dactylifera* L.) fruits in a semi-arid environment. *Science of the Total Environment*, *665*, 690–697. https://doi.org/10.1016/J.SCITOTENV.2019.02.140

Abdelmoteleb, A., Troncoso-Rojas, R., Gonzalez-Soto, T., and González-Mendoza, D. (2017). Antifungical activity of autochthonous *Bacillus subtilis* isolated from prosopis juliflora against phytopathogenic fungi. *Mycobiology*, *45*(4), 385–391. https://doi.org/10.5941/MYCO.2017.45.4.385

Abrudan, M. I., Smakman, F., Grimbergen, A. J., Westhoff, S., Miller, E. L., Van Wezel, G. P., and Rozen, D. E. (2015). Socially mediated induction and suppression of antibiosis during bacterial coexistence. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(35), 11054–11059. https://doi.org/10.1073/PNAS.1504076112

Agarwal, S., Tyagi, I., Gupta, V. K., Ghaedi, M., Masoomzade, M., Ghaedi, A. M., and Mirtamizdoust, B. (2016). Kinetics and thermodynamics of methyl orange adsorption from aqueous solutions - Artificial neural network-particle swarm optimization modeling. In *Journal of Molecular Liquids* (Vol. 218, Issue 218, pp. 354–362). Elsevier B.V. https://doi.org/10.1016/j.molliq.2016.02.048

Aghaeinejad-Meybodi, A., Ebadi, A., Shafiei, S., Khataee, A., and Kiadehi, A. D. (2019). Degradation of Fluoxetine using catalytic ozonation in aqueous media in the presence of nano-Γ-alumina catalyst: Experimental, modeling and optimization study. *Separation and Purification Technology*, *211*, 551–563. https://doi.org/10.1016/j.seppur.2018.10.020

Ahirwar, S., Soni, H., Prajapati, B. P., and Kango, N. (2017). Isolation and screening of thermophilic and thermotolerant fungi for production of hemicellulases from heated environments. *Mycology*, *8*(3), 125–134. https://doi.org/10.1080/21501203.2017.1337657

Ahmad, M., Zahir, Z. A., Khalid, M., Nazli, F., and Arshad, M. (2013). Efficacy of *Rhizobium* and *Pseudomonas* strains to improve physiology, ionic balance and quality of mung bean under salt-affected conditions on farmer's fields. *Plant Physiology and Biochemistry*, *63*, 170–176. https://doi.org/10.1016/j.plaphy.2012.11.024

Al-Kharashi, A., and Skitmore, M. (2009). Causes of delays in Saudi Arabian public sector construction projects. *Construction Management and Economics*, *27*(1), 3–23. https://doi.org/10.1080/01446190802541457

Al Farraj, D. A., Varghese, R., Vágvölgyi, C., Soliman Elshikh, M., Alokda, A. M., and Hossam Mahmoud, A. (2020). Antibiotics production in optimized culture condition using low cost substrates from *Streptomyces* sp. AS4 isolated from mangrove soil sediment. *Journal of King Saud University - Science*, *32*(2), 1528–1535. https://doi.org/10.1016/j.jksus.2019.12.008

Aleboyeh, A., Kasiri, M. B., Olya, M. E., and Aleboyeh, H. (2008). Prediction of azo dye decolorization by UV/H2O2 using artificial neural networks. *Dyes and Pigments*, *77*(2), 288–294. https://doi.org/10.1016/J.DYEPIG.2007.05.014

Ali, A., Kurnia, N., Ulfah, A. A. N., Damayanti, P., Rante, H., and Jumadi, O. (2021). Diversity of Endophytic Actinomycetes Producing Indole-3-Acetic Acid and *in vitro* Evaluation of Plant Growth-promoting Activity on *Brassica oleracea* L. *Pertanika Journal of Tropical Agricultural Science*, *44*(2), 275–292. https://doi.org/10.47836/PJTAS.44.2.02

Ali, M. H., and Abd, A. M. (2021). Using of an Artificial Neural Networks with Particle Swarm Optimization (ANN-PSO) Model in Prediction of Cost and Delay in Construction Projects. *Diyala Journal of Engineering Sciences*, *14*(3), 78–93. https://doi.org/10.24237/djes.2021.14307

Allali, K., Goudjal, Y., Zamoum, M., Bouznada, K., Sabaou, N., and Zitouni, A. (2019). *Nocardiopsis dassonvillei* strain MB22 from the Algerian Sahara promotes wheat seedlings growth and potentially controls the common root rot pathogen Bipolaris sorokiniana. *Journal of Plant Pathology*, *101*(4), 1115–1125. https://doi.org/10.1007/s42161-019-00347-x

Alloun, W., Berkani, M., Benaissa, A., Shavandi, A., Gares, M., Danesh, C., Lakhdari, D., Ghfar, A. A., and Chaouche, N. K. (2023). Waste valorization as low-cost media engineering for auxin production from the newly isolated *Streptomyces rubrogriseus* AW22: Model development. *Chemosphere*, *326*, 138394. https://doi.org/10.1016/j.chemosphere.2023.138394

Arul Jose, P., and Jebakumar, S. R. D. (2014). Successive Nonstatistical and Statistical Approaches for the Improved Antibiotic Activity of Rare Actinomycete *Nonomuraea* sp. JAJ18. *BioMed Research International*, *2014*. https://doi.org/10.1155/2014/906097

Aryal, S., Neupane, L., Adhikari, R., Regmi, B., Koirala, N., and Joshi, D. R. (2020). Novel *Streptomyces* Sp. Reported in 2018: A Meta-Analysis. *Anti-Infective Agents*, *19*(5), 3–14. https://doi.org/10.2174/2211352518666200423083354

Asaf, S., Khan, M. A., Khan, A. L., Waqas, M., Shahzad, R., Kim, A. Y., Kang, S. M., and Lee, I. J. (2017). Bacterial endophytes from arid land plants regulate endogenous hormone content and promote growth in crop plants: An example of *Sphingomonas* sp. and *Serratia marcescens*. *Journal of Plant Interactions*, *12*(1), 31–38. https://doi.org/10.1080/17429145.2016.1274060

Azizi, S., Awad, M. M., and Ahmadloo, E. (2016). Prediction of water holdup in vertical and inclined oil-water two-phase flow using artificial neural network. *International Journal of Multiphase Flow*, *80*, 181–187. https://doi.org/10.1016/j.ijmultiphaseflow.2015.12.010

Badri, D. V., Chaparro, J. M., Zhang, R., Shen, Q., and Vivanco, J. M. (2013). Application of natural blends of phytochemicals derived from the root exudates of *Arabidopsis* to the soil reveal that phenolic-related compounds predominantly modulate the soil microbiome. *Journal of Biological Chemistry*, *288*(7), 4502–4512. https://doi.org/10.1074/JBC.M112.433300

Badri, D. V., Quintana, N., El Kassis, E. G., Kim, H. K., Choi, Y. H., Sugiyama, A., Verpoorte, R., Martinoia, E., Manter, D. K., and Vivanco, J. M. (2009). An ABC transporter mutation alters root exudation of phytochemicals that provoke an overhaul of natural soil microbiota. *Plant Physiology*, *151*(4), 2006–2017. https://doi.org/10.1104/PP.109.147462

Bagheri, M., Mirbagheri, S. A., Bagheri, Z., and Kamarkhani, A. M. (2015). Modeling and optimization of activated sludge bulking for a real wastewater treatment plant using hybrid artificial neural networks-genetic algorithm approach. *Process Safety and Environmental Protection*, *95*, 12–25. https://doi.org/10.1016/j.psep.2015.02.008

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., and Vivanco, J. M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology*, *57*, 233–266. https://doi.org/10.1146/ANNUREV.ARPLANT.57.032905.105159

Baoxin, Z., Xiangjing, W., and Wensheng, X. (2011). Optimization of fermentation medium for enhanced production of milbemycin by a mutant of *Streptomyces bingchenggensis* BC-X-1 using response surface methodology. *African Journal of Biotechnology*, *10*(37), 7225–7235. https://doi.org/10.5897/AJB11.077

Baş, D., and Boya, I. (2007). Modeling and optimization I: usability of response surface methodology. *J Food Eng*, *78*(3), 836–845. https://doi.org/10.1016/j.jfoodeng.2005.11.024

Bashir, W., Anwar, S., Zhao, Q., Hussain, I., and Xie, F. (2019). Interactive effect of drought and cadmium stress on soybean root morphology and gene expression. *Ecotoxicology and Environmental Safety*, *175*, 90–101. https://doi.org/10.1016/J.ECOENV.2019.03.042

Belimov, A. A., Puhalsky, I. V., Safronova, V. I., Shaposhnikov, A. I., Vishnyakova, M. A., Semenova, E. V., Zinovkina, N. Y., Makarova, N. M., Wenzel, W., and Tikhonovich, I. A. (2015). Role of Plant Genotype and Soil Conditions in Symbiotic Plant-Microbe Interactions for Adaptation of Plants to Cadmium-Polluted Soils. *Water, Air, and Soil Pollution*, *226*(8), 1–15. https://doi.org/10.1007/s11270-015-2537-9

Benadjila, A., Zamoum, M., Aouar, L., Zitouni, A., and Goudjal, Y. (2022). Optimization of cultural conditions using response surface methodology and modeling of indole-3-acetic acid production by *Saccharothrix texasensis* MB15. *Biocatalysis and Agricultural Biotechnology*, *39*, 102271. https://doi.org/10.1016/j.bcab.2021.102271

Bérdy, J. (2012). Thoughts and facts about antibiotics: Where we are now and where we are heading. In *Journal of Antibiotics* (Vol. 65, Issue 8, pp. 385–395). Nature Publishing Group. https://doi.org/10.1038/ja.2012.27

Berendsen, R. L., Pieterse, C. M. J., and Bakker, P. A. H. M. (2012). The rhizosphere microbiome and plant health. *Trends in Plant Science*, *17*(8), 478–486. https://doi.org/10.1016/J.TPLANTS.2012.04.001

Berg, G., Zachow, C., Müller, H., Philipps, J., and Tilcher, R. (2013). Next-Generation Bio-Products Sowing the Seeds of Success for Sustainable Agriculture. *Agronomy*, *3*(4), 648–656. https://doi.org/10.3390/agronomy3040648

Berkani, M., Bouhelassa, M., and Bouchareb, M. K. (2019). Implementation of a venturi photocatalytic reactor: Optimization of photodecolorization of an industrial azo dye. *Arabian Journal of Chemistry*, *12*(8), 3054–3063. https://doi.org/10.1016/j.arabjc.2015.07.004

Bhatia, N., Bozorg, B., Larsson, A., Ohno, C., Jönsson, H., and Heisler, M. G. (2016). Auxin Acts through MONOPTEROS to Regulate Plant Cell Polarity and Pattern Phyllotaxis. *Current Biology*, *26*(23), 3202–3208. https://doi.org/10.1016/J.CUB.2016.09.044/ATTACHMENT/9912084F-4362-47B9-

B6A4-0EF030B97EB9/MMC5.MP4

Bhattacharya, S., Dineshkumar, R., Dhanarajan, G., Sen, R., and Mishra, S. (2017). Improvement of ε-polylysine production by marine bacterium *Bacillus licheniformis* using artificial neural network modeling and particle swarm optimization technique. *Biochemical Engineering Journal*, *126*, 8–15. https://doi.org/10.1016/j.bej.2017.06.020

Bhattacharyya, P. N., and Jha, D. K. (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World J. Microbiol. Biotechnol.*, *28*, 1327–1350.

Bhoi, A., Palladino, F., and Fabrizio, P. (2021). Auxin confers protection against ER stress in Caenorhabditis elegans. *Biology Open*, *10*(2). https://doi.org/10.1242/BIO.057992

Bielach, A., Hrtyan, M., and Tognetti, V. B. (2017). Plants under stress: Involvement of auxin and cytokinin. In *International Journal of Molecular Sciences* (Vol. 18, Issue 7, p. 1427). Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/ijms18071427

Blakeslee, J. J., Spatola Rossi, T., and Kriechbaumer, V. (2019). Auxin biosynthesis: spatial regulation and adaptation to stress. *Journal of Experimental Botany*, *70*(19), 5041–5049. https://doi.org/10.1093/JXB/ERZ283

Bodenhausen, N., Horton, M. W., and Bergelson, J. (2013). Bacterial Communities Associated with the Leaves and the Roots of *Arabidopsis thaliana*. *PLoS ONE*, *8*(2). https://doi.org/10.1371/JOURNAL.PONE.0056329

Bonaldi, M., Chen, X., Kunova, A., Pizzatti, C., Saracchi, M., and Cortesi, P. (2015). Colonization of lettuce rhizosphere and roots by tagged *Streptomyces*. *Frontiers in Microbiology*, *6*(FEB). https://doi.org/10.3389/fmicb.2015.00025

Borah, A., and Thakur, D. (2020). Phylogenetic and Functional Characterization of Culturable Endophytic Actinobacteria Associated With *Camellia* spp. for Growth Promotion in Commercial Tea Cultivars. *Frontiers in Microbiology*, *11*, 318. https://doi.org/10.3389/fmicb.2020.00318

Botas Muñoz, A. M. (2013). *Regulación del metabolismo en Streptomyces: Control por ArgR*. Doctoral dissertation, Universidad de León.

Boubekri, K., Soumare, A., Mardad, I., Lyamlouli, K., Hafidi, M., Ouhdouch, Y., and Kouisni, L. (2021). The Screening of Potassium- and Phosphate-Solubilizing Actinobacteria and the Assessment of Their Ability to Promote Wheat Growth Parameters. *Microorganisms 2021, Vol. 9, Page 470*, *9*(3), 470. https://doi.org/10.3390/MICROORGANISMS9030470

Bouzroud, S., Gouiaa, S., Hu, N., Bernadac, A., Mila, I., Bendaou, N., Smouni, A. A., Bouzayen, M., and Zouine, M. (2018). Auxin response factors (ARFs) are potential mediators of auxin action in tomato response to biotic and abiotic stress (*Solanum lycopersicum*). *PLoS ONE*, *13*(2). https://doi.org/10.1371/journal.pone.0193517

Bressan, M., Roncato, M. A., Bellvert, F., Comte, G., Haichar, F. E. Z., Achouak, W., and Berge, O. (2009). Exogenous glucosinolate produced by *Arabidopsis thaliana* has an impact on microbes in the rhizosphere and plant roots. *ISME Journal*, *3*(11), 1243–1257. https://doi.org/10.1038/ISMEJ.2009.68

Brumos, J., Robles, L. M., Yun, J., Vu, T. C., Jackson, S., Alonso, J. M., and Stepanova, A. N. (2018). Local auxin biosynthesis is a key regulator of plant development. *Developmental Cell*, *47*(3), 306–318.

Bulak, P., Walkiewicz, A., and Brzezińska, M. (2014). Plant growth regulators-assisted phytoextraction. *Biologia Plantarum*, *58*(1), 1–8.

Bulgarelli, D., Rott, M., Schlaeppi, K., Ver Loren van Themaat, E., Ahmadinejad, N., Assenza, F., Rauf, P., Huettel, B., Reinhardt, R., Schmelzer, E., Peplies, J., Gloeckner, F. O., Amann, R., Eickhorst, T., and Schulze-Lefert, P. (2012). Revealing structure and assembly cues for *Arabidopsis* root-inhabiting bacterial microbiota. *Nature*, *488*(7409), 91–95. https://doi.org/10.1038/NATURE11336

Bull, A. T. (2010). Actinobacteria of the Extremobiosphere. In K. Horikoshi, G. Antranikian, A. T. Bull, F. T. Robb, and K. O. Stetter (Eds.), *Extremophiles Handbook* (pp. 1203–1240). Springer. https://kar.kent.ac.uk/28052/

Bunsangiam, S., Thongpae, N., Limtong, S., and Srisuk, N. (2021). Large scale production of indole-3-acetic acid and evaluation of the inhibitory effect of indole-3-acetic acid on weed growth. *Scientific Reports*, *11*(1), 1–13. https://doi.org/10.1038/s41598-021-92305-w

Bush, M. J., Bibb, M. J., Chandra, G., Findlay, K. C., and Buttner, M. J. (2013). Genes required for aerial growth, cell division, and chromosome segregation are targets of whia before sporulation in *Streptomyces venezuelae*. *MBio*, *4*(5). https://doi.org/10.1128/MBIO.00684-13

Cappuccino, J. C., and Sherman, N. (1992). *Cappuccino: Microbiology: a laboratory manual/James... - Google Scholar*. 3rd Edn. New York, NY: Benjamin-Cummings Publishing Company. https://scholar.google.com/scholar_lookup?title=Microbiology%3A+A+Laboratory+Manuelandauthor=Cappuccino+J.++C.andauthor=and+Sherman+N.andpublication_year=1992andpages=125-179

Carrión, V. J., Perez-Jaramillo, J., Cordovez, V., Tracanna, V., De Hollander, M., Ruiz-Buck, D., Mendes, L. W., van Ijcken, W. F. J., Gomez-Exposito, R., Elsayed, S. S., Mohanraju, P., Arifah, A., van der Oost, J., Paulson, J. N., Mendes, R., van Wezel, G. P., Medema, M. H., and Raaijmakers, J. M. (2019). Pathogen-induced activation of disease-suppressive functions in the endophytic root microbiome. *Science*, *366*(6465), 606–612. https://doi.org/10.1126/science.aaw9285

Carvalhais, L. C., Dennis, P. G., Badri, D. V., Kidd, B. N., Vivanco, J. M., and Schenk, P. M. (2015). Linking Jasmonic acid signaling, root exudates, and rhizosphere microbiomes. *Molecular Plant-Microbe Interactions*, *28*(9), 1049–1058. https://doi.org/10.1094/MPMI-01-15-0016-R

Casanova, E., Trillas, M. I., Moysset, L., and Vainstein, A. (2005). Influence of rol genes in floriculture. *Biotechnology Advances*, *23*(1), 3–39. https://doi.org/10.1016/J.BIOTECHADV.2004.06.002

Çelekli, A., Bozkurt, H., and Geyik, F. (2013). Use of artificial neural networks and genetic algorithms for prediction of sorption of an azo-metal complex dye onto lentil straw. *Bioresource Technology*, *129*, 396–401. https://doi.org/10.1016/j.biortech.2012.11.085

Cha, J. Y., and al., et. (2016). Microbial and biochemical basis of a Fusarium wilt-suppressive soil. *ISME J.*, *10*, 119–129.

Challis, G. L., and Hopwood, D. A. (2003). Synergy and contingency as driving forces for the evolution of multiple secondary metabolite production by *Streptomyces* species. *Proceedings of the National Academy of Sciences of the United States of America*,

100(24), 14555–14561. https://doi.org/10.1073/PNAS.1934677100

Chandra, S., Askari, K., and Kumari, M. (2018). Optimization of indole acetic acid production by isolated bacteria from *Stevia rebaudiana* rhizosphere and its effects on plant growth. *Journal of Genetic Engineering and Biotechnology*, *16*(2), 581–586. https://doi.org/10.1016/j.jgeb.2018.09.001

Chaudhary, T., Yadav, D., Chhabra, D., Gera, R., and Shukla, P. (2021). Low - cost media engineering for phosphate and IAA production by *Kosakonia pseudosacchari* TCPS - 4 using Multi - objective Genetic Algorithm (MOGA) statistical tool. *3 Biotech*, *11*(4), 1–11. https://doi.org/10.1007/s13205-021-02690-2

Chen, X., Marszałkowska, M., and Reinhold-Hurek, B. (2020). Jasmonic Acid, Not Salicyclic Acid Restricts Endophytic Root Colonization of Rice. *Frontiers in Plant Science*, *10*. https://doi.org/10.3389/fpls.2019.01758

Chen, Z., Agnew, J. L., Cohen, J. D., He, P., Shan, L., Sheen, J., and Kunkel, B. N. (2007). *Pseudomonas syringae* type III effector AvrRpt2 alters *Arabidopsis thaliana* auxin physiology. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(50), 20131–20136. https://doi.org/10.1073/PNAS.0704901104

Chou, J. S., Cheng, M. Y., and Wu, Y. W. (2013). Improving classification accuracy of project dispute resolution using hybrid artificial intelligence and support vector machine models. *Expert Systems with Applications*, *40*(6), 2263–2274. https://doi.org/10.1016/j.eswa.2012.10.036

Choudhury, F. K., Rivero, R. M., Blumwald, E., and Mittler, R. (2017). Reactive oxygen species, abiotic stress and stress combination. *Plant Journal*, *90*(5), 856–867. https://doi.org/10.1111/tpj.13299

Chukwuneme, C. F., Babalola, O. O., Kutu, F. R., and Ojuederie, O. B. (2020). Characterization of actinomycetes isolates for plant growth promoting traits and their effects on drought tolerance in maize. *Journal of Plant Interactions*, *15*(1), 93–105. https://doi.org/10.1080/17429145.2020.1752833

Compant, S., Clément, C., and Sessitsch, A. (2010). Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry*, *42*(5), 669–678. https://doi.org/10.1016/j.soilbio.2009.11.024

Compant, S., Samad, A., Faist, H., and Sessitsch, A. (2019). A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. *Journal of Advanced Research*, *19*(April), 29–37. https://doi.org/10.1016/j.jare.2019.03.004

Conn, V. M., Walker, A. R., and Franco, C. M. M. (2008). Endophytic actinobacteria induce defense pathways in *Arabidopsis thaliana*. *Molecular Plant-Microbe Interactions*, *21*(2), 208–218. https://doi.org/10.1094/MPMI-21-2-0208

Cordovez, V., Carrion, V. J., Etalo, D. W., Mumm, R., Zhu, H., van Wezel, G. P., and Raaijmakers, J. M. (2015). Diversity and functions of volatile organic compounds produced by *Streptomyces* from a disease-suppressive soil. *Frontiers in Microbiology*, *6*(OCT), 1081. https://doi.org/10.3389/fmicb.2015.01081

De Oliveira, J., Rodrigues, C., Vandenberghe, L. P. S., Câmara, M. C., Libardi, N., and Soccol, C. R. (2017). Gibberellic Acid Production by Different Fermentation Systems Using

Citric Pulp as Substrate/Support. *BioMed Research International*, *2017*. https://doi.org/10.1155/2017/5191046

De Vries, F. T., Griffiths, R. I., Knight, C. G., Nicolitch, O., and Williams, A. (2020). Harnessing rhizosphere microbiomes for drought-resilient crop production. *Science*, *368*(6488), 270–274. https://doi.org/10.1126/SCIENCE.AAZ5192

del Río, L. A., Corpas, F. J., López-Huertas, E., and Palma, J. M. (2018). Plant superoxide dismutases: Function under abiotic stress conditions. *Antioxidants and Antioxidant Enzymes in Higher Plants*, 1–26. https://doi.org/10.1007/978-3-319-75088-0_1/COVER

Desai, K. M., Survase, S. A., Saudagar, P. S., Lele, S. S., and Singhal, R. S. (2008). Comparison of artificial neural network (ANN) and response surface methodology (RSM) in fermentation media optimization: Case study of fermentative production of scleroglucan. *Biochemical Engineering Journal*, *3*(41), 266–273. https://doi.org/10.1016/J.BEJ.2008.05.009

Dhanarajan, G., Mandal, M., and Sen, R. (2014). Regular Article. *Biochemical Engineering Journal*, *C*(84), 59–65. https://doi.org/10.1016/J.BEJ.2014.01.002

Di, D. W., Zhang, C., Luo, P., An, C. W., and Guo, G. Q. (2016). The biosynthesis of auxin: how many paths truly lead to IAA? In *Plant Growth Regulation* (Vol. 78, Issue 3, pp. 275–285). Springer Netherlands. https://doi.org/10.1007/s10725-015-0103-5

Ding, X., Cao, Y., Huang, L., Zhao, J., Xu, C., Li, X., and Wang, S. (2008). Activation of the indole-3-acetic acid-amido synthetase GH3-8 suppresses expansin expression and promotes salicylate- and jasmonate-independent basal immunity in rice. *Plant Cell*, *20*(1), 228–240. https://doi.org/10.1105/TPC.107.055657

Ding, Z., Galván-Ampudia, C. S., Demarsy, E., Łangowski, Ł., Kleine-Vehn, J., Fan, Y., Morita, M. T., Tasaka, M., Fankhauser, C., Offringa, R., and Friml, J. (2011). Light-mediated polarization of the PIN3 auxin transporter for the phototropic response in Arabidopsis. *Nature Cell Biology 2011 13:4*, *13*(4), 447–452. https://doi.org/10.1038/ncb2208

Djebaili, R., Pellegrini, M., Smati, M., Del Gallo, M., and Kitouni, M. (2020). Actinomycete strains isolated from saline soils: Plant-growth-promoting traits and inoculation effects on *Solanum lycopersicum*. *Sustainability (Switzerland)*, *12*(11). https://doi.org/10.3390/su12114617

Dragoi, E. N., and Vasseghian, Y. (2021). Modeling of mass transfer in vacuum membrane distillation process for radioactive wastewater treatment using artificial neural networks. *Toxin Reviews*, *40*(4), 1526–1535. https://doi.org/10.1080/15569543.2020.1744659

Drake, J. E., Darby, B. A., Giasson, M. A., Kramer, M. A., Phillips, R. P., and Finzi, A. C. (2013). Stoichiometry constrains microbial response to root exudation–insights from a model and a field experiment in a temperate forest. *Biogeosciences*, *10*(2), 821–838. https://doi.org/10.5194/bg-10-821-2013

Duca, D. R., and Glick, B. R. (2020). Indole-3-acetic acid biosynthesis and its regulation in plant-associated bacteria. *Applied Microbiology and Biotechnology*, *104*(20), 8607–8619. https://doi.org/10.1007/s00253-020-10869-5

Edwards, C. (1993). Isolation properties and potential applications of thermophilic actinomycetes. *Applied Biochemistry and Biotechnology*, *42*, 161–179.

Esquivel, P., international, V. J.-F. research, and 2012, undefined. (2012). Functional properties of coffee and coffee by-products. *Elsevier*, *46*(2), 488–495. https://doi.org/10.1016/j.foodres.2011.05.028

Etchells, J. P., Smit, M. E., Gaudinier, A., Williams, C. J., and Brady, S. M. (2016). A brief history of the TDIF-PXY signalling module: Balancing meristem identity and differentiation during vascular development. In *New Phytologist* (Vol. 209, Issue 2, pp. 474–484). Blackwell Publishing Ltd. https://doi.org/10.1111/nph.13642

Fan, M., Hu, J., Cao, R., Ruan, W., and Wei, X. (2018). A review on experimental design for pollutants removal in water treatment with the aid of artificial intelligence. In *Chemosphere* (Vol. 200, pp. 330–343). Chemosphere. https://doi.org/10.1016/j.chemosphere.2018.02.111

Farda, B., Mattedi, A., Djebaili, R., Pace, L., Del Gallo, M., and Pellegrini, M. (2022). Microbial Community Investigation of Wild Brambles with Root Nodulation from a Calcareous Nitrogen-Deficient Soil. *Soil Systems*, *6*(4), 96. https://doi.org/10.3390/soilsystems6040096

Fässler, E., Evangelou, M. W., Robinson, B. H., and Schulin, R. (2010). Effects of indole-3-acetic acid (IAA) on sunflower growth and heavy metal uptake in combination with ethylene diamine disuccinic acid (EDDS). *Chemosphere*, *80*(8), 901–907. https://doi.org/10.1016/J.CHEMOSPHERE.2010.04.077

Fatmawati, U., Meryandini, A., Asih Nawangsih, A., and Tri Wahyudi, A. (2019). Screening and characterization of actinomycetes isolated from soybean rhizosphere for promoting plant growth. *Biodiversitas*, *20*(10), 2970–2977. https://doi.org/10.13057/biodiv/d201027

Fattorini, L., Ronzan, M., Piacentini, D., Della Rovere, F., De Virgilio, C., Sofo, A., Altamura, M. M., and Falasca, G. (2017). Cadmium and arsenic affect quiescent centre formation and maintenance in *Arabidopsis thaliana* post-embryonic roots disrupting auxin biosynthesis and transport. *Environmental and Experimental Botany*, *144*, 37–48. https://doi.org/10.1016/j.envexpbot.2017.10.005

Fay, P. A., Prober, S. M., Harpole, W. S., Knops, J. M. H., Bakker, J. D., Borer, E. T., Lind, E. M., MacDougall, A. S., Seabloom, E. W., Wragg, P. D., and Others. (2015). *Grassland productivity limited by multiple nutrients. Nat Plants 1: 15080* (p. 15080).

Feng, J., Zhang, J., Zhang, J., He, Y., Zhang, R., Chen, C., and Liu, G. (2017). Enhanced methane production of vinegar residue by response surface methodology (RSM). *AMB Express*, *7*(1), 89. http://www.ncbi.nlm.nih.gov/pubmed/28484998

Franche, C., Lindström, K., and Elmerich, C. (2009). Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. *Plant and Soil*, *321*, 35–59. https://doi.org/10.1007/s11104-008-9833-8

Frank, S. A. (1994). Spatial polymorphism of bacteriocins and other allelopathic traits. *Evolutionary Ecology*, *8*(4), 369–386. https://doi.org/10.1007/BF01238189

Galvan-Ampudia, C. S., Julkowska, M. M., Darwish, E., Gandullo, J., Korver, R. A., Brunoud, G., Haring, M. A., Munnik, T., Vernoux, T., and Testerink, C. (2013). Halotropism is a response of plant roots to avoid a saline environment. *Current Biology*, *23*(20), 2044–2050. https://doi.org/10.1016/J.CUB.2013.08.042

Gao, B., and Gupta, R. S. (2012). Phylogenetic Framework and Molecular Signatures for the

Main Clades of the Phylum Actinobacteria. *Microbiology and Molecular Biology Reviews*, *76*(1), 66–112. https://doi.org/10.1128/mmbr.05011-11

Ghaedi, A. M., and Vafaei, A. (2017). Applications of artificial neural networks for adsorption removal of dyes from aqueous solution: A review. *Advances in Colloid and Interface Science*, *245*, 20–39. https://doi.org/10.1016/J.CIS.2017.04.015

Gherbawy, Y., Elhariry, H., Altalhi, A., El-Deeb, B., and Khiralla, G. (2012). Molecular screening of *Streptomyces* isolates for antifungal activity and family 19 chitinase enzymes. *Journal of Microbiology*, *50*(3), 459–468. https://doi.org/10.1007/s12275-012-2095-4

Ghodhbane-Gtari, F., Nouioui, I., Hezbri, K., Lundstedt, E., D'Angelo, T., McNutt, Z., Laplaze, L., Gherbi, H., Vaissayre, V., Svistoonoff, S., Ahmed, H. ben, Boudabous, A., and Tisa, L. S. (2019). The plant-growth-promoting actinobacteria of the genus *Nocardia* induces root nodule formation in *Casuarina glauca*. *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, *112*(1), 75–90. https://doi.org/10.1007/s10482-018-1147-0

Ghosh, S., and Basu, P. S. (2006). Production and metabolism of indole acetic acid in roots and root nodules of *Phaseolus mungo*. *Microbiological Research*, *161*(4), 362–366. https://doi.org/10.1016/j.micres.2006.01.001

Girão, M., Ribeiro, I., Ribeiro, T., Azevedo, I. C., Pereira, F., Urbatzka, R., Leão, P. N., and Carvalho, M. F. (2019). Actinobacteria isolated from *Laminaria ochroleuca*: A source of new bioactive compounds. *Frontiers in Microbiology*, *10*(APR), 1–13. https://doi.org/10.3389/fmicb.2019.00683

Giri, R., and Sharma, R. K. (2020). Fungal pretreatment of lignocellulosic biomass for the production of plant hormone by *Pichia fermentans* under submerged conditions. *Bioresources and Bioprocessing*, *7*(1), 1–11. https://doi.org/10.1186/s40643-020-00319-5

Glick, B. R. (2012). Plant Growth-Promoting Bacteria: Mechanisms and Applications. *Scientifica*, *2012*, 1–15. https://doi.org/10.6064/2012/963401

Gómez-De La Cruz, F. J., Cruz-Peragón, F., Casanova-Peláez, P. J., and Palomar-Carnicero, J. M. (2015). A vital stage in the large-scale production of biofuels from spent coffee grounds: The drying kinetics. *Fuel Processing Technology*, *130*(C), 188–196. https://doi.org/10.1016/j.fuproc.2014.10.012

Gómez Ramírez, M., Rojas Avelizapa, L. I., Rojas Avelizapa, N. G., and Cruz Camarillo, R. (2004). Colloidal chitin stained with Remazol Brilliant Blue R®, a useful substrate to select chitinolytic microorganisms and to evaluate chitinases. *Journal of Microbiological Methods*, *56*(2), 213–219. https://doi.org/10.1016/J.MIMET.2003.10.011

Gonzalez-Franco, A. C., Deobald, L. A., Spivak, A., and Crawford, D. L. (2003). Actinobacterial chitinase-like enzymes: Profiles of rhizosphere versus non-rhizosphere isolates. *Canadian Journal of Microbiology*, *49*(11), 683–698. https://doi.org/10.1139/w03-089

Goodfellow, M., Kampfer, P., and Busse, H. J. B. (2012). Bergey's Manual® of Systematic Bacteriology. In *Bergey's Manual® of Systematic Bacteriology* (Volume 5). Springer New York. https://doi.org/10.1007/978-0-387-68233-4

Goudjal, Y., Toumatia, O., Sabaou, N., Barakate, M., Mathieu, F., and Zitouni, A. (2013). Endophytic actinomycetes from spontaneous plants of Algerian Sahara: Indole-3-acetic acid production and tomato plants growth promoting activity. *World Journal of Microbiology and Biotechnology*, *29*(10), 1821–1829. https://doi.org/10.1007/s11274-013-1344-y

Goudjal, Y., Toumatia, O., Yekkour, A., Sabaou, N., Mathieu, F., and Zitouni, A. (2014). Biocontrol of *Rhizoctonia solani* damping-off and promotion of tomato plant growth by endophytic actinomycetes isolated from native plants of Algerian Sahara. *Microbiological Research*, *169*(1), 59–65. https://doi.org/10.1016/j.micres.2013.06.014

Goudjal, Y., Zamoum, M., Meklat, A., Sabaou, N., Mathieu, F., and Zitouni, A. (2016). Plant-growth-promoting potential of endosymbiotic actinobacteria isolated from sand truffles (*Terfezia leonis* Tul.) of the Algerian Sahara. *Annals of Microbiology*, *66*(1), 91–100. https://doi.org/10.1007/s13213-015-1085-2

Grones, P., Abas, M., Hajný, J., Jones, A., Waidmann, S., Kleine-Vehn, J., and Friml, J. (2018). PID/WAG-mediated phosphorylation of the *Arabidopsis* PIN3 auxin transporter mediates polarity switches during gravitropism. *Scientific Reports*, *8*(1), 1–11. https://doi.org/10.1038/s41598-018-28188-1

Gupta, R. S., Chen, W. J., Adeolu, M., and Chai, Y. (2013). Molecular signatures for the class Coriobacteriia and its different clades; proposal for division of the class Coriobacteriia into the emended order Coriobacteriales, containing the emended family Coriobacteriaceae and Atopobiaceae fam. nov., and Eggerthe. *International Journal of Systematic and Evolutionary Microbiology*, *63*(PART9), 3379–3397. https://doi.org/10.1099/ijs.0.048371-0

Gupta, S., Kaushal, R., and Sood, G. (2018). Impact of Plant Growth–Promoting Rhizobacteria on Vegetable Crop Production. In *International Journal of Vegetable Science* (Vol. 24, Issue 3, pp. 289–300). Taylor and Francis Inc. https://doi.org/10.1080/19315260.2017.1407984

Habibi, S., Djedidi, S., Prongjunthuek, K., Mortuza, M. F., Ohkama-Ohtsu, N., Sekimoto, H., and Yokoyoma, T. (2014). Physiological and genetic characterization of rice nitrogen fixer PGPR isolated from rhizosphere soils of different crops. *Plant and Soil*, *379*(1–2), 51–66. https://doi.org/10.1007/s11104-014-2035-7

Haichar, F. E. Z., Marol, C., Berge, O., Rangel-Castro, J. I., Prosser, J. I., Balesdent, J., Heulin, T., and Achouak, W. (2008). Plant host habitat and root exudates shape soil bacterial community structure. *ISME Journal*, *2*(12), 1221–1230. https://doi.org/10.1038/ISMEJ.2008.80

Haichar, F. el Z., Roncato, M. A., and Achouak, W. (2012). Stable isotope probing of bacterial community structure and gene expression in the rhizosphere of *Arabidopsis thaliana*. *FEMS Microbiology Ecology*, *81*(2), 291–302. https://doi.org/10.1111/J.1574-6941.2012.01345.X

Hamedi, J., and Mohammadipanah, F. (2015). Biotechnological application and taxonomical distribution of plant growth promoting actinobacteria. *J. Ind. Microbiol. Biotechnol.*, *42*, 157–171.

Han, E. H., Petrella, D. P., and Blakeslee, J. J. (2017). "Bending" models of halotropism: Incorporating protein phosphatase 2A, ABCB transporters, and auxin metabolism. In

Journal of Experimental Botany (Vol. 68, Issue 12, pp. 3071–3089). J Exp Bot. https://doi.org/10.1093/jxb/erx127

Hao, Z., Yang, B., and Jahng, D. (2018). Spent coffee ground as a new bulking agent for accelerated biodrying of dewatered sludge. *Water Research*, *138*, 250–263. https://doi.org/10.1016/J.WATRES.2018.03.049

Hardgrove, S. J., and Livesley, S. J. (2016). Applying spent coffee grounds directly to urban agriculture soils greatly reduces plant growth. *Urban Forestry and Urban Greening*, *18*, 1–8. https://doi.org/10.1016/j.ufug.2016.02.015

Hasan, M. K., Ahammed, G. J., Sun, S., Li, M., Yin, H., and Zhou, J. (2019). Melatonin Inhibits Cadmium Translocation and Enhances Plant Tolerance by Regulating Sulfur Uptake and Assimilation in *Solanum lycopersicum* L. *Journal of Agricultural and Food Chemistry*, *67*(38), 10563–10576. https://doi.org/10.1021/acs.jafc.9b02404

Hasanuzzaman, M., Borhannuddin Bhuyan, M. H. M., Anee, T. I., Parvin, K., Nahar, K., Al Mahmud, J., and Fujita, M. (2019). Regulation of Ascorbate-Glutathione Pathway in Mitigating Oxidative Damage in Plants under Abiotic Stress. *Antioxidants (Basel, Switzerland)*, *8*(9). https://doi.org/10.3390/ANTIOX8090384

Hengst, C. D. de., Tran, N. T., Bibb, M. J., Chandra, G., Leskiw, B. K., and Buttner, M. J. (2010). Genes essential for morphological development and antibiotic production in *Streptomyces coelicolor* are targets of BldD during vegetative growth. *Molecular Microbiology*, *78*(2), 361–379. https://doi.org/10.1111/J.1365-2958.2010.07338.X

Hopwood, D. A. (2019). Highlights of *Streptomyces* genetics. In *Heredity* (Vol. 123, Issue 1, pp. 23–32). Nature Publishing Group. https://doi.org/10.1038/s41437-019-0196-0

Hu, Y. F., Zhou, G., Na, X. F., Yang, L., Nan, W. Bin, Liu, X., Zhang, Y. Q., Li, J. L., and Bi, Y. R. (2013). Cadmium interferes with maintenance of auxin homeostasis in *Arabidopsis* seedlings. *Journal of Plant Physiology*, *170*(11), 965–975. https://doi.org/10.1016/J.JPLPH.2013.02.008

Huang, J., Mei, L. H., and Xia, J. (2007). Application of artificial neural network coupling particle swarm optimization algorithm to biocatalytic production of GABA. *Biotechnology and Bioengineering*, *96*(5), 924–931. https://doi.org/10.1002/bit.21162

Huang, W., Liu, X., Zhou, X., Wang, X., Liu, X., and Liu, H. (2020). Calcium Signaling Is Suppressed in *Magnaporthe oryzae* Conidia by *Bacillus cereus* HS24. *Phytopathology*, *110*(2), 309—316. https://doi.org/10.1094/phyto-08-18-0311-r

Huang, X. F., Chaparro, J. M., Reardon, K. F., Zhang, R., Shen, Q., and Vivanco, J. M. (2014). Rhizosphere interactions: Root exudates, microbes, and microbial communities1. *Botany*, *92*(4), 267–275. https://doi.org/10.1139/CJB-2013-0225

Hymavathi, M., Sathish, T., Brahmaiah, P., and Prakasham, R. S. (2010). Impact of carbon and nitrogen sources on L-Asparaginase production by isolated *Bacillus circulans* (MTCC 8574): Application of saturated plackett-burman design. *Chemical and Biochemical Engineering Quarterly*, *24*(4), 473–480. https://hrcak.srce.hr/clanak/93944

Iqbal, M., and Ashraf, M. (2013). Gibberellic acid mediated induction of salt tolerance in wheat plants: Growth, ionic partitioning, photosynthesis, yield and hormonal homeostasis. *Environmental and Experimental Botany*, *86*, 76–85. https://doi.org/10.1016/J.ENVEXPBOT.2010.06.002

Islam, E., Khan, M. T., and Irem, S. (2015). Biochemical mechanisms of signaling: Perspectives in plants under arsenic stress. *Ecotoxicology and Environmental Safety*, *114*, 126–133. https://doi.org/10.1016/J.ECOENV.2015.01.017

Jasso-Salcedo, A. B., Hoppe, S., Pla, F., Escobar-Barrios, V. A., Camargo, M.-C., and Meimaroglou, D. (2017). Modeling and optimization of a photocatalytic process: Degradation of endocrine disruptor compounds by Ag/ZnO. *Chemical Engineering Research and Design*, *128*, 174–191. https://doi.org/10.1016/j.cherd.2017.10.012

Jiang, B., Zhang, F., Sun, Y., Zhou, X., Dong, J., and Zhang, L. (2014). Modeling and optimization for curing of polymer flooding using an artificial neural network and a genetic algorithm. *Journal of the Taiwan Institute of Chemical Engineers*, *45*(5), 2217–2224. https://doi.org/10.1016/J.JTICE.2014.03.020

Jog, R., Nareshkumar, G., and Rajkumar, S. (2012). Plant growth promoting potential and soil enzyme production of the most abundant *Streptomyces* spp. from wheat rhizosphere. *Journal of Applied Microbiology*, *113*(5), 1154–1164. https://doi.org/10.1111/j.1365-2672.2012.05417.x

Jog, R., Nareshkumar, G., and Rajkumar, S. (2016). Enhancing soil health and plant growth promotion by actinomycetes. In *Plant Growth Promoting Actinobacteria: A New Avenue for Enhancing the Productivity and Soil Fertility of Grain Legumes* (pp. 33–45). Springer Singapore. https://doi.org/10.1007/978-981-10-0707-1_3

Jogawat, A., Yadav, B., Chhaya, Lakra, N., Singh, A. K., and Narayan, O. P. (2021). Crosstalk between phytohormones and secondary metabolites in the drought stress tolerance of crop plants: A review. *Physiologia Plantarum*, *172*(2), 1106–1132. https://doi.org/10.1111/PPL.13328

Jones, S. E., and Elliot, M. A. (2017). *Streptomyces* Exploration: Competition, Volatile Communication and New Bacterial Behaviours. *Trends in Microbiology*, *25*(7), 522–531. https://doi.org/10.1016/J.TIM.2017.02.001

Jung, J. H., and Park, C. M. (2011). Auxin modulation of salt stress signaling in Arabidopsis seed germination. *Plant Signaling and Behavior*, *6*(8), 1198–1200. https://doi.org/10.4161/PSB.6.8.15792

Kaltenpoth, M. (2009). Actinobacteria as mutualists: general healthcare for insects? *Trends in Microbiology*, *17*(12), 529–535. https://doi.org/10.1016/J.TIM.2009.09.006

Kanimozhi, J., Moorthy, I. G., Sivashankar, R., and Sivasubramanian, V. (2017). Optimization of dextran production by *Weissella cibaria* NITCSK4 using Response Surface Methodology-Genetic Algorithm based technology. *Carbohydrate Polymers*, *174*, 103–110. https://doi.org/10.1016/J.CARBPOL.2017.06.021

Kaur, H., Arora, D. S., and Sharma, V. (2014). Isolation, purification, and characterization of antimicrobial compound 6-[1,2-dimethyl-6-(2-methyl-allyloxy)-hexyl]-3-(2-methoxy-phenyl)-chromen-4-one from *Penicillium* sp. HT-28. *Applied Biochemistry and Biotechnology*, *173*(8), 1963–1976. https://doi.org/10.1007/S12010-014-0979-Y

Kaur, R., and Kaur, S. (2021). Plant growth-promoting potential of '*Myroides gitamensis*' isolated from virgin soils of Punjab. *Archives of Microbiology*, *203*(5), 2551–2561. https://doi.org/10.1007/s00203-021-02231-8

Kavitha, G., Kurinjimalar, C., Sivakumar, K., Kaarthik, M., Aravind, R., Palani, P., and

Rengasamy, R. (2016). Optimization of polyhydroxybutyrate production utilizing waste water as nutrient source by *Botryococcus braunii* Kütz using response surface methodology. *International Journal of Biological Macromolecules*, *93*, 534–542. https://doi.org/10.1016/J.IJBIOMAC.2016.09.019

Kazan, K. (2013). Auxin and the integration of environmental signals into plant root development. *Annals of Botany*, *112*(9), 1655–1665. https://doi.org/10.1093/AOB/MCT229

Kemung, H. M., Tan, L. T. H., Khan, T. M., Chan, K. G., Pusparajah, P., Goh, B. H., and Lee, L. H. (2018). *Streptomyces*as a prominent resource of future anti-MRSA drugs. In *Frontiers in Microbiology* (Vol. 9, p. 2221). Frontiers Media S.A. https://doi.org/10.3389/fmicb.2018.02221

Keswani, C., Singh, S. P., Cueto, L., García-Estrada, C., Mezaache-Aichour, S., Glare, T. R., Borriss, R., Singh, S. P., Blázquez, M. A., and Sansinenea, E. (2020). Auxins of microbial origin and their use in agriculture. In *Applied Microbiology and Biotechnology* (Vol. 104, Issue 20, pp. 8549–8565). Appl Microbiol Biotechnol. https://doi.org/10.1007/s00253-020-10890-8

Khalid, A., Latif1, M. A., and Adnan, M. (2017). AN APPROACH TO ESTIMATE THE DURATION OF SOFTWARE PROJECT THROUGH MACHINE LEARNING TECHNIQUES. *Gomal University Journal of Research*, *33*(1), 1–13. http://www.gujr.com.pk/index.php/GUJR/article/view/81

Khamna, S., Yokota, A., Peberdy, J. F., and Lumyong, S. (2010). Indole-3-acetic acid production by *Streptomyces* sp. isolated from some Thai medicinal plant rhizosphere soils. *EurAsian Journal of Biosciences*, 23–32. https://doi.org/10.5053/EJOBIOS.2010.4.0.4

Khoi Nghia, N. (2017). Isolation and Characterization of Indole Acetic Acid Producing Halophilic Bacteria from Salt Affected Soil of Rice–Shrimp Farming System in the Mekong Delta, Vietnam. *Agriculture, Forestry and Fisheries*, *6*(3), 69. https://doi.org/10.11648/j.aff.20170603.11

Khosravi-Darani, K., and Zoghi, A. (2008). Comparison of pretreatment strategies of sugarcane baggase: Experimental design for citric acid production. *Bioresource Technology*, *99*(15), 6986–6993. https://doi.org/10.1016/j.biortech.2008.01.024

Kinkel, L. L., Schlatter, D. C., Xiao, K., and Baines, A. D. (2014). Sympatric inhibition and niche differentiation suggest alternative coevolutionary trajectories among Streptomycetes. *ISME Journal*, *8*(2), 249–256. https://doi.org/10.1038/ismej.2013.175

Kinoshita, N., Wang, H., Kasahara, H., Liu, J., MacPherson, C., Machida, Y., Kamiya, Y., Hannah, M. A., and Chuaa, N. H. (2012). IAA-Ala Resistant3, an evolutionarily conserved target of miR167, mediates *Arabidopsis* root architecture changes during high osmotic stress. *Plant Cell*, *24*(9), 3590–3602. https://doi.org/10.1105/TPC.112.097006

Korenblum, E., Dong, Y., Szymanski, J., Panda, S., Jozwiak, A., Massalha, H., Meir, S., Rogachev, I., and Aharoni, A. (2020). Rhizosphere microbiome mediates systemic root metabolite exudation by root-to-root signaling. *Proceedings of the National Academy of Sciences*, *117*(7).

Korver, R. A., Koevoets, I. T., and Testerink, C. (2018). Out of Shape During Stress: A Key Role for Auxin. *Trends in Plant Science*, *23*(9), 783. https://doi.org/10.1016/J.TPLANTS.2018.05.011

Kucho, K. I., Hay, A. E., and Normand, P. (2010). The determinants of the actinorhizal symbiosis. *Microbes and Environments*, *25*(4), 241–252. https://doi.org/10.1264/JSME2.ME10143

Kudjordjie, E. N., Sapkota, R., Steffensen, S. K., Fomsgaard, I. S., and Nicolaisen, M. (2019). Maize synthesized benzoxazinoids affect the host associated microbiome. *Microbiome*, *7*(1), 1–17. https://doi.org/10.1186/S40168-019-0677-7/FIGURES/7

Kumar, S., Stecher, G., and Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, *33*(7), 1870–1874. https://doi.org/10.1093/MOLBEV/MSW054

Kumar Sharma, R. (2018). Production of Secondary Metabolites in Plants under Abiotic Stress: An Overview. *Significances of Bioengineering and Biosciences*, *2*(4). https://doi.org/10.31031/sbb.2018.02.000545

Kunova, A., Bonaldi, M., Saracchi, M., Pizzatti, C., Chen, X., and Cortesi, P. (2016). Selection of *Streptomyces* against soil borne fungal pathogens by a standardized dual culture assay and evaluation of their effects on seed germination and plant growth. *BMC Microbiology*, *16*(1), 1–11.

Kurosawa, K., and al., et. (2008). Rhodostreptomycins, antibiotics biosynthesized following horizontal gene transfer from *Streptomyces* padanus to *Rhodococcus fascians*. *J. Am. Chem. Soc.*, *130*, 1126–1127.

Kusuma, A. B., Nouioui, I., Klenk, H. P., and Goodfellow, M. (2020). *Streptomyces* harenosi sp. nov., a home for a gifted strain isolated from Indonesian sand dune soil. *International Journal of Systematic and Evolutionary Microbiology*, *70*(9), 4874–4882. https://doi.org/10.1099/IJSEM.0.004346/CITE/REFWORKS

Lahiri, S. K., and Ghanta, K. C. (2008). Artificial neural network model with parameter tuning assisted by differential evolution technique: Study of pressure drop of slurry flow in pipeline. *Chemical Product and Process Modeling*, *3*(1), 103–117. https://doi.org/10.2202/1934-2659.1255

Landi, S., Paradisone, V., and Esposito, S. (2020). Molecular Investigation of Metalloid Stress Response in Higher Plants. *Metalloids in Plants*, 213–230. https://doi.org/10.1002/9781119487210.CH11

Łangowski, Ł., Wabnik, K., Li, H., Vanneste, S., Naramoto, S., Tanaka, H., and Friml, J. (2016). Cellular mechanisms for cargo delivery and polarity maintenance at different polar domains in plant cells. *Cell Discovery*, *2*(1), 1–18.

Lanka, S., and Latha, J. N. L. (2015). Response Surface Methodology as a Statistical Tool for Fermentation Media Optimization in Lipase Production by Palm Oil Mill Effluent (POME) Isolate *Emericella Nidulans* NFCCI 3643. *International Journal of Innovative Research in Science, Engineering and Technology (An ISO*, *3297*, 2535–2545. https://doi.org/10.15680/IJIRSET.2015.0404060

Lasudee, K., Tokuyama, S., Lumyong, S., and Pathom-Aree, W. (2018). Actinobacteria Associated with arbuscular mycorrhizal *Funneliformis mosseae* spores, taxonomic characterization and their beneficial traits to plants: Evidence obtained from mung bean (Vigna radiata) and Thai Jasmine Rice (*Oryza sativa*). *Frontiers in Microbiology*, *9*(JUN). https://doi.org/10.3389/FMICB.2018.01247

Law, J. W. F., Ser, H. L., Khan, T. M., Chuah, L. H., Pusparajah, P., Chan, K. G., Goh, B. H., and Lee, L. H. (2017). The potential of *Streptomyces* as biocontrol agents against the rice blast fungus, *Magnaporthe oryzae* (*Pyricularia oryzae*). *Frontiers in Microbiology*, *8*(JAN). https://doi.org/10.3389/FMICB.2017.00003

Le, K. D., Yu, N. H., Park, A. R., Park, D. J., Kim, C. J., and Kim, J. C. (2022). *Streptomyces* sp. AN090126 as a Biocontrol Agent against Bacterial and Fungal Plant Diseases. *Microorganisms*, *10*(4), 791. https://doi.org/10.3390/microorganisms10040791

Lebeis, S. L., and al., et. (2015). Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science*, *349*, 860–864.

Lebrazi, S., Fadil, M., Chraibi, M., and Fikri-Benbrahim, K. (2020). Screening and optimization of indole-3-acetic acid production by *Rhizobium* sp. strain using response surface methodology. *Journal of Genetic Engineering and Biotechnology*, *18*(1). https://doi.org/10.1186/S43141-020-00035-9/FIGURES/5

Lee, H., Kim, H., Park, J. M., Cho, H. S., and Jeon, J. H. (2020). PIN-mediated polar auxin transport facilitates root− obstacle avoidance. *New Phytologist*, *225*(3), 1285–1296.

Lessa, E. F., Nunes, M. L., and Fajardo, A. R. (2018). Chitosan/waste coffee-grounds composite: An efficient and eco-friendly adsorbent for removal of pharmaceutical contaminants from water. *Carbohydrate Polymers*, *189*, 257–266. https://doi.org/10.1016/J.CARBPOL.2018.02.018

Li, X., Li, Y., Mai, J., Tao, L., Qu, M., Liu, J., Shen, R., Xu, G., Feng, Y., Xiao, H., Wu, L., Shi, L., Guo, S., Liang, J., Zhu, Y., He, Y., Baluška, F., Shabala, S., and Yu, M. (2018). Boron alleviates aluminum toxicity by promoting root alkalization in transition zone via polar auxin transport. *Plant Physiology*, *177*(3), 1254–1266. https://doi.org/10.1104/pp.18.00188

Liebthal, M., Maynard, D., and Dietz, K. J. (2018). Proxiredoxins and Redox Signaling in Plants. In *Antioxidants and Redox Signaling* (Vol. 28, Issue 7, pp. 609–624). Antioxid Redox Signal. https://doi.org/10.1089/ars.2017.7164

Lin, Q., Luo, A., Zhang, Y., Wang, Y., Liang, Z., and Yuan, P. (2021). Employing Artificial Neural Networks to Predict the Performance of Domestic Sewage Treatment Terminals in the Rural Region. *Mathematical Problems in Engineering*, *2021*. https://doi.org/10.1155/2021/5264531

López, M. E., Rene, E. R., Boger, Z., Veiga, M. C., and Kennes, C. (2017). Modelling the removal of volatile pollutants under transient conditions in a two-stage bioreactor using artificial neural networks. *Journal of Hazardous Materials*, *324*(Pt A), 100–109. https://doi.org/10.1016/J.JHAZMAT.2016.03.018

Lorck, H. (1948). Production of Hydrocyanic Acid by Bacteria. *Physiologia Plantarum*, *1*(2), 142–146. https://doi.org/10.1111/j.1399-3054.1948.tb07118.x

Loria, R., Kers, J., and Joshi, M. (2006). Evolution of plant pathogenicity in *Streptomyces*. *Annual Review of Phytopathology*, *44*, 469–487. https://doi.org/10.1146/ANNUREV.PHYTO.44.032905.091147

Ludwig, W., Euzéby, J., Schumann, P., Busse, H.-J., Trujillo, M. E., Kämpfer, P., and Whitman, W. B. (2012). Road map of the phylum Actinobacteria. In *Bergey's Manual® of Systematic Bacteriology* (pp. 1–28). Springer, New York, NY.

https://doi.org/10.1007/978-0-387-68233-4_1

Lundberg, D. S., and al., et. (2012). Defining the core *Arabidopsis thaliana* root microbiome. *Nature*, *488*, 86–90.

Luo, Y., Wei, Y., Sun, S., Wang, J., Wang, W., Han, D., Shao, H., Jia, H., and Fu, Y. (2019). Selenium modulates the level of auxin to alleviate the toxicity of cadmium in Tobacco. *International Journal of Molecular Sciences*, *20*(15). https://doi.org/10.3390/ijms20153772

Luziatelli, F., Melini, F., Bonini, P., Melini, V., Cirino, V., and Ruzzi, M. (2021). Production of indole auxins by *Enterobacter* sp. Strain p-36 under submerged conditions. *Fermentation*, *7*(3), 138. https://doi.org/10.3390/fermentation7030138

Maggini, V., Mengoni, A., Bogani, P., Firenzuoli, F., and Fani, R. (2020). Promoting Model Systems of Microbiota–Medicinal Plant Interactions. *Trends in Plant Science*, *25*(3), 223–225. https://doi.org/10.1016/J.TPLANTS.2019.12.013

Mahalingam, R. (2015). Combined stresses in plants: Physiological, molecular, and biochemical aspects. In *Combined Stresses in Plants: Physiological, Molecular, and Biochemical Aspects*. Springer International Publishing. https://doi.org/10.1007/978-3-319-07899-1

Maji, K., Pratihar, D. K., and Nath, A. K. (2014). Laser forming of a dome shaped surface: Experimental investigations, statistical analysis and neural network modeling. *Optics and Lasers in Engineering*, *53*, 31–42. https://doi.org/10.1016/j.optlaseng.2013.08.014

Malheiros, R. S. P., Costa, L. C., Ávila, R. T., Pimenta, T. M., Teixeira, L. S., Brito, F. A. L., Zsögön, A., Araújo, W. L., and Ribeiro, D. M. (2019). Selenium downregulates auxin and ethylene biosynthesis in rice seedlings to modify primary metabolism and root architecture. *Planta*, *250*(1), 333–345. https://doi.org/10.1007/s00425-019-03175-6

Mandal, S. M., Mondal, K. C., Dey, S., and Pati, B. R. (2007). Optimization of Cultural and Nutritional Conditions for Indole 3-acetic Acid (IAA) Production by a *Rhizobium* sp. Isolated from Root Nodules of Vigna mungo (L.) Hepper. *Research Journal of Microbiology*, *2*(3), 239–246. https://doi.org/10.3923/jm.2007.239.246

Marasco, R., Fusi, M., Rolli, E., Ettoumi, B., Tambone, F., Borin, S., Ouzari, H. I., Boudabous, A., Sorlini, C., Cherif, A., Adani, F., and Daffonchio, D. (2021). Aridity modulates belowground bacterial community dynamics in olive tree. *Environmental Microbiology*, *23*(10), 6275–6291. https://doi.org/10.1111/1462-2920.15764

Mastan, A., Bharadwaj, R., Kushwaha, R. K., and Vivek Babu, C. S. (2019). Functional Fungal Endophytes in *Coleus forskohlii* Regulate Labdane Diterpene Biosynthesis for Elevated Forskolin Accumulation in Roots. *Microbial Ecology*, *78*(4), 914–926. https://doi.org/10.1007/s00248-019-01376-w

Mazarei, F., Jooyandeh, H., Noshad, M., and Hojjati, M. (2017). Polysaccharide of caper (Capparis spinosa L.) Leaf: Extraction optimization, antioxidant potential and antimicrobial activity. *International Journal of Biological Macromolecules*, *95*, 224–231. https://doi.org/10.1016/J.IJBIOMAC.2016.11.049

Mendes, R., Kruijt, M., De Bruijn, I., Dekkers, E., Van Der Voort, M., Schneider, J. H. M., Piceno, Y. M., DeSantis, T. Z., Andersen, G. L., Bakker, P. A. H. M., and Raaijmakers, J. M. (2011). Deciphering the rhizosphere microbiome for disease-suppressive bacteria.

Science, *332*(6033), 1097–1100. https://doi.org/10.1126/SCIENCE.1203980

Mickelbart, M. V., and al., et. (2015). Genetic mechanisms of abiotic stress tolerance that translate to crop yield stability. *Nat. Rev. Genet.*, *16*, 237–251.

Moghri, M., Dragoi, E. N., Salehabadi, A., Shukla, D. K., and Vasseghian, Y. (2017). Effect of various formulation ingredients on thermal characteristics of PVC/clay nanocomposite foams: Experimental and modeling. *E-Polymers*, *17*(2), 119–128. https://doi.org/10.1515/epoly-2016-0151

Mohite, B. (2013). Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. *Journal of Soil Science and Plant Nutrition*, *13*(3), 638–649. https://doi.org/10.4067/S0718-95162013005000051

Montealegre, J. R., Reyes, R., Pérez, L. M., Herrera, R., Silva, P., and Besoain, X. (2003). Selection of bioantagonistic bacteria to be used in biological control of *Rhizoctonia solani* in tomato. *Electronic Journal of Biotechnology*, *6*(2), 38–50. https://doi.org/10.2225/vol6-issue2-fulltext-8

Montgomery, D. C. (2019). Design and Analysis of Experiments, 10th Edition | Wiley. In *Wiley*. https://www.wiley.com/en-us/Design+and+Analysis+of+Experiments%2C+10th+Edition-p-9781119492443

Mourabet, M., Rhilassi, A. El, Bennani-Ziatni, M., and Taitai, A. (2014). Comparative Study of Artificial Neural Network and Response Surface Methodology for Modelling and Optimization the Adsorption Capacity of Fluoride onto Apatitic Tricalcium Phosphate. *Universal Journal of Applied Mathematics*, *2*(2), 84–91. https://doi.org/10.13189/ujam.2014.020202

Mroue, S., Simeunovic, A., and Robert, H. S. (2018). Auxin production as an integrator of environmental cues for developmental growth regulation. In *Journal of Experimental Botany* (Vol. 69, Issue 2, pp. 201–212). Oxford Academic. https://doi.org/10.1093/jxb/erx259

Müller-Moulé, P., Nozue, K., Pytlak, M. L., Palmer, C. M., Covington, M. F., Wallace, A. D., Harmer, S. L., and Maloof, J. N. (2016). YUCCA auxin biosynthetic genes are required for Arabidopsis shade avoidance. *PeerJ*, *2016*(10), e2574. https://doi.org/10.7717/PEERJ.2574/FIG-6

Murthy, N., and Bleakley, B. (2012). Simplified Method of Preparing Colloidal Chitin Used For Screening of Chitinase- Producing Microorganisms. *The Internet Journal of Microbiology*, *10*(1), 1–5. https://doi.org/10.5580/2e93

Mushtaq, S., Shafiq, M., Ashraf, T., Haider, M. S., Ashfaq, M., and Ali, M. (2019). Characterization of plant growth promoting activities of bacterial endophytes and their antibacterial potential isolated from citrus. *Journal of Animal and Plant Sciences*, *29*(4), 978–991. http://www.thejaps.org.pk/docs/v-29-04/10.pdf

Mushtaq, Z. (2021). PGPR: present role, mechanism of action and future prospects along bottlenecks in commercialization. *EQA - International Journal of Environmental Quality*, *41*, 9–15. https://doi.org/10.6092/ISSN.2281-4485/11103

Mutturi, S., Sahai, V., Sharma, S., and Bisaria, V. S. (2016). Strategies for High-Density Cultivation of Bio-inoculants in Submerged Culture with Special Reference to Pseudomonads. *Microbial Inoculants in Sustainable Agricultural Productivity: Vol. 1:*

Research Perspectives, 181–196. https://doi.org/10.1007/978-81-322-2647-5_10

Myo, E. M., Ge, B., Ma, J., Cui, H., Liu, B., Shi, L., Jiang, M., and Zhang, K. (2019). Indole-3-acetic acid production by *Streptomyces fradiae* NKZ-259 and its formulation to enhance plant growth. *BMC Microbiology 2019 19:1*, *19*(1), 1–14. https://doi.org/10.1186/S12866-019-1528-1/TABLES/4

Nadeem, S. M., Ahmad, M., Zahir, Z. A., Javaid, A., and Ashraf, M. (2014). The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. *Biotechnology Advances*, *32*(2), 429–448. https://doi.org/10.1016/j.biotechadv.2013.12.005

Nafis, A., Raklami, A., Bechtaoui, N., Khalloufi, F. El, Alaoui, A. El, Glick, B. R., Hafidi, M., Kouisni, L., Ouhdouch, Y., and Hassani, L. (2019). Actinobacteria from extreme niches in morocco and their plant growth-promoting potentials. *Diversity*, *11*(8), 1–15. https://doi.org/10.3390/D11080139

Nah, H. J., Park, J., Choi, S., and Kim, E. S. (2021). WblA, a global regulator of antibiotic biosynthesis in *Streptomyces*. *Journal of Industrial Microbiology and Biotechnology*, *48*(3–4), 7. https://doi.org/10.1093/jimb/kuab007

Nakurte, I., Keisa, A., and Rostoks, N. (2012). Development and validation of a reversed-phase liquid chromatography method for the simultaneous determination of indole-3-acetic acid, indole-3-pyruvic acid, and abscisic acid in Barley (*Hordeum vulgare* L.). *Journal of Analytical Methods in Chemistry*, *1*(1). https://doi.org/10.1155/2012/103575

Narayana, K. J., Peddikotla, P., Krishna, P. S. J., Yenamandra, V., and Muvva, V. (2009). Indole-3-acetic acid production by *Streptomyces albidoflavus*. *J Biol Res*, *11*, 49–55.

Navarro, L., Dunoyer, P., Jay, F., Arnold, B., Dharmasiri, N., Estelle, M., Voinnet, O., and Jones, J. D. G. (2006). A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. *Science*, *312*(5772), 436–439. https://doi.org/10.1126/SCIENCE.1126088

Nguyen, H., Bui, X. N., Bui, H. B., and Mai, N. L. (2021). Correction to: A comparative study of artificial neural networks in predicting blast-induced air-blast overpressure at Deo Nai open-pit coal mine, Vietnam (Neural Computing and Applications, (2020), 32, 8, (3939-3955), 10.1007/s00521-018-3717-5). In *Neural Computing and Applications* (Vol. 33, Issue 16, p. 10615). Springer Science and Business Media Deutschland GmbH. https://doi.org/10.1007/s00521-021-05773-6

Nicault, M., Zaiter, A., Dumarcay, S., Chaimbault, P., Gelhaye, E., Leblond, P., and Bontemps, C. (2021). Elicitation of antimicrobial active compounds by *Streptomyces*-fungus co-cultures. *Microorganisms*, *9*(1), 1–13. https://doi.org/10.3390/microorganisms9010178

Nimnoi, P., Pongsilp, N., and Lumyong, S. (2010). Endophytic actinomycetes isolated from Aquilaria crassna Pierre ex Lec and screening of plant growth promoters production. *World Journal of Microbiology and Biotechnology*, *26*(2), 193–203.

Nodzyński, T., Vanneste, S., Zwiewka, M., Pernisová, M., Hejátko, J., and Friml, J. (2016). Enquiry into the topology of plasma membrane-localized PIN auxin transport components. *Molecular Plant*, *9*(11), 1504–1519.

Norris, P. R. (2012). Class II. Acidimicrobiia class. nov. *Bergey's Manual of Systematic Bacteriology*, 1968.

Nouioui, I., Carro, L., García-López, M., Meier-Kolthoff, J. P., Woyke, T., Kyrpides, N. C., Pukall, R., Klenk, H. P., Goodfellow, M., and Göker, M. (2018). Genome-based taxonomic classification of the phylum actinobacteria. *Frontiers in Microbiology*, *9*, 2007. https://doi.org/10.3389/fmicb.2018.02007

Nutaratat, P., Amsri, W., Srisuk, N., Arunrattiyakorn, P., and Limtong, S. (2015). Indole-3-acetic acid production by newly isolated red yeast *Rhodosporidium paludigenum*. *Journal of General and Applied Microbiology*, *61*(1), 1–9. https://doi.org/10.2323/jgam.61.1

Ogbe, A. A., Finnie, J. F., and Van Staden, J. (2020). The role of endophytes in secondary metabolites accumulation in medicinal plants under abiotic stress. *South African Journal of Botany*, *134*, 126–134. https://doi.org/10.1016/j.sajb.2020.06.023

Olanrewaju, O. S., and Babalola, O. O. (2019). *Streptomyces*: implications and interactions in plant growth promotion. *Applied Microbiology and Biotechnology*, *103*(3), 1179–1188. https://doi.org/10.1007/S00253-018-09577-Y/TABLES/1

Oleńska, E., Małek, W., Wójcik, M., Swiecicka, I., Thijs, S., and Vangronsveld, J. (2020). Beneficial features of plant growth-promoting rhizobacteria for improving plant growth and health in challenging conditions: A methodical review. In *Science of the Total Environment* (Vol. 743). Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2020.140682

Ostrowski, M., Ciarkowska, A., and Jakubowska, A. (2016). The auxin conjugate indole-3-acetyl-aspartate affects responses to cadmium and salt stress in *Pisum sativum* L. *Journal of Plant Physiology*, *191*, 63–72. https://doi.org/10.1016/j.jplph.2015.11.012

Palaniyandi, S. A., Damodharan, K., Yang, S. H., and Suh, J. W. (2014). *Streptomyces* sp. strain PGPA39 alleviates salt stress and promotes growth of "Micro Tom" tomato plants. *Journal of Applied Microbiology*, *117*(3), 766–773. https://doi.org/10.1111/jam.12563

Palaniyandi, S. A., Yang, S. H., Cheng, J. H., Meng, L., and Suh, J. W. (2011). Biological control of anthracnose (*Colletotrichum gloeosporioides*) in yam by *Streptomyces* sp.MJM5763. *Journal of Applied Microbiology*, *111*(2), 443–455. https://doi.org/10.1111/j.1365-2672.2011.05048.x

Pandey, D. S., Das, S., Pan, I., Leahy, J. J., and Kwapinski, W. (2016). Artificial neural network based modelling approach for municipal solid waste gasification in a fluidized bed reactor. *Waste Management*, *58*, 202–213. https://doi.org/10.1016/j.wasman.2016.08.023

Pang, Z., Chen, J., Wang, T., Gao, C., Li, Z., Guo, L., Xu, J., and Cheng, Y. (2021). Linking Plant Secondary Metabolites and Plant Microbiomes: A Review. *Frontiers in Plant Science*, *12*. https://doi.org/10.3389/FPLS.2021.621276/REFERENCE

Park, J., Lee, Y., Martinoia, E., and Geisler, M. (2017). Plant hormone transporters: what we know and what we would like to know. *BMC Biology*, *15*(1), 1–15.

Park, S., Kim, A. L., Hong, Y. K., Shin, J. H., and Joo, S. H. (2021). A highly efficient auxin-producing bacterial strain and its effect on plant growth. *Journal of Genetic Engineering and Biotechnology*, *19*(1). https://doi.org/10.1186/s43141-021-00252-w

Parte, A. C., Carbasse, J. S., Meier-Kolthoff, J. P., Reimer, L. C., and Göker, M. (2020). List of prokaryotic names with standing in nomenclature (LPSN) moves to the DSMZ. *International Journal of Systematic and Evolutionary Microbiology*, *70*(11), 5607–5612. https://doi.org/10.1099/ijsem.0.004332

Pascale, A., Pascale, A., Proietti, S., Pantelides, I. S., and Stringlis, I. A. (2020). *Modulation of the Root Microbiome by Plant Molecules : The Basis for Targeted Disease Suppression and Plant Growth Promotion Modulation of the Root Microbiome by Plant Molecules : The Basis for Targeted Disease Suppression and Plant Growth Promotion*. *January*. https://doi.org/10.3389/fpls.2019.01741

Pascoal, A., Estevinho, L. M., Martins, I. M., and Choupina, A. B. (2018). REVIEW: Novel sources and functions of microbial lipases and their role in the infection mechanisms. In *Physiological and Molecular Plant Pathology* (Vol. 104, pp. 119–126). Academic Press. https://doi.org/10.1016/j.pmpp.2018.08.003

Pasin, L. A. A. P., Abreu, M. S. de, and Souza, I. P. (2011). Influence of the fungi population on the physicochemical and chemical composition of coffee (*Coffea arabica* L.). *Food Science and Technology (Campinas)*, *31*(3), 681–687. https://doi.org/10.1590/s0101-20612011000300020

Passari, A. K., Chandra, P., Zothanpuia, Mishra, V. K., Leo, V. V., Gupta, V. K., Kumar, B., and Singh, B. P. (2016). Detection of biosynthetic gene and phytohormone production by endophytic actinobacteria associated with *Solanum lycopersicum* and their plant-growth-promoting effect. *Research in Microbiology*, *167*(8), 692–705. https://doi.org/10.1016/j.resmic.2016.07.001

Passari, A. K., Mishra, V. K., Gupta, V. K., Yadav, M. K., Saikia, R., and Singh, B. P. (2015). *In vitro* and in vivo plant growth promoting activities and DNA fingerprinting of antagonistic endophytic actinomycetes associates with medicinal plants. *PLoS ONE*, *10*(9). https://doi.org/10.1371/journal.pone.0139468

Passari, A. K., Mishra, V. K., Saikia, R., Gupta, V. K., and Singh, B. P. (2015). Isolation, abundance and phylogenetic affiliation of endophytic actinomycetes associated with medicinal plants and screening for their *in vitro* antimicrobial biosynthetic potential. *Frontiers in Microbiology*, *6*(APR), 273. https://doi.org/10.3389/fmicb.2015.00273

Passari, A. K., Mishra, V. K., Singh, G., Singh, P., Kumar, B., Gupta, V. K., Sharma, R. K., Saikia, R., Donovan, A. O., and Singh, B. P. (2017). Insights into the functionality of endophytic actinobacteria with a focus on their biosynthetic potential and secondary metabolites production. *Scientific Reports*, *7*(1), 1–17. https://doi.org/10.1038/s41598-017-12235-4

Passari, A. K., Upadhyaya, K., Singh, G., Abdel-Azeem, A. M., Thankappan, S., Uthandi, S., Hashem, A., Abd-Allah, E. F., Malik, J. A., As, A., Gupta, V. K., Ranjan, S., and Singh, B. P. (2019). Enhancement of disease resistance, growth potential, and photosynthesis in tomato (*Solanum lycopersicum*) by inoculation with an endophytic actinobacterium, *Streptomyces* thermocarboxydus strain BPSAC147. *PLoS ONE*, *14*(7), e0219014. https://doi.org/10.1371/journal.pone.0219014

Patten, C. L., Blakney, A. J. C., and Coulson, T. J. D. (2013). Activity, distribution and function of indole-3-acetic acid biosynthetic pathways in bacteria. *Critical Reviews in Microbiology*, *39*(4), 395–415. https://doi.org/10.3109/1040841X.2012.716819

Pham, V. T. T., Nguyen, C. T., Dhakal, D., Nguyen, H. T., Kim, T. S., and Sohng, J. K. (2021). Recent advances in the heterologous biosynthesis of natural products from *Streptomyces*. In *Applied Sciences (Switzerland)* (Vol. 11, Issue 4, pp. 1–22). https://doi.org/10.3390/app11041851

Pieterse, C. M., and al., et. (2012). Hormonal modulation of plant immunity. *Annu. Rev. Cell Dev. Biol.*, *28*, 489–521.

Poh, P. E., Gouwanda, D., Mohan, Y., Gopalai, A. A., and Tan, H. M. (2016). Optimization of Wastewater Anaerobic Digestion Using Mechanistic and Meta-heuristic Methods: Current Limitations and Future Opportunities. *Water Conservation Science and Engineering 2016 1:1*, *1*(1), 1–20. https://doi.org/10.1007/S41101-016-0001-3

Pridham, T. G., and Gottlieb, D. (1948). The Utilization of Carbon Compounds by Some Actinomycetales as an Aid for Species Determination. *Journal of Bacteriology*, *56*(1), 107–114. https://doi.org/10.1128/jb.56.1.107-114.1948

Purama, R. K., and Goyal, A. (2008). Application of response surface methodology for maximizing dextransucrase production from *Leuconostoc mesenteroides* NRRL B-640 in a bioreactor. *Applied Biochemistry and Biotechnology*, *151*(2–3), 182–192. https://doi.org/10.1007/S12010-008-8165-8

Auxin response factors (ARFs) differentially regulate rice antiviral immune response against rice dwarf virus, 16 PLOS Pathogens e1009118 (2020). https://doi.org/10.1371/JOURNAL.PPAT.1009118

Qin, S., Feng, W. W., Zhang, Y. J., Wang, T. T., Xiong, Y. W., and Xing, K. (2018). Diversity of bacterial microbiota of coastal halophyte Limonium sinense and amelioration of salinity stress damage by symbiotic plant growthpromoting actinobacterium Glutamicibacter halophytocola KLBMP 5180. *Applied and Environmental Microbiology*, *84*(19), 1533–1551. https://doi.org/10.1128/AEM.01533-18

Qin, S., Wu, Z., Rasool, A., and Li, C. (2012). Synthesis and characterization of slow-release nitrogen fertilizer with water absorbency: Based on poly(acrylic acid-acrylic amide)/Na-bentonite. *Journal of Applied Polymer Science*, *126*(5), 1687–1697. https://doi.org/10.1002/app.37007

Qin, S., Xing, K., Jiang, J. H., Xu, L. H., and Li, W. J. (2011). Biodiversity, bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria. In *Applied Microbiology and Biotechnology* (Vol. 89, Issue 3, pp. 457–473). https://doi.org/10.1007/s00253-010-2923-6

Raheem, A., Shaposhnikov, A., Belimov, A. A., Dodd, I. C., and Ali, B. (2018). Auxin production by rhizobacteria was associated with improved yield of wheat (Triticum aestivum L.) under drought stress. *Archives of Agronomy and Soil Science*, *64*(4), 574–587. https://doi.org/10.1080/03650340.2017.1362105

Rajendra, M., Jena, P. C., and Raheman, H. (2009). Prediction of optimized pretreatment process parameters for biodiesel production using ANN and GA. *Fuel*, *88*(5), 868–875. https://doi.org/10.1016/J.FUEL.2008.12.008

Rajeswari, P., Jose, P. A., Amiya, R., and Jebakumar, S. R. D. (2014). Characterization of saltern based *Streptomyces* sp. and statistical media optimization for its improved antibacterial activity. *Frontiers in Microbiology*, *5*, 753. http://www.ncbi.nlm.nih.gov/pubmed/25653640

Rakusová, H., Han, H., Valošek, P., and Friml, J. (2019). Genetic screen for factors mediating PIN polarization in gravistimulated *Arabidopsis thaliana* hypocotyls. *The Plant Journal*, *98*(6), 1048–1059.

Rampey, R. A., LeClere, S., Kowalczyk, M., Ljung, K., Sandberg, G., and Bartel, B. (2004). A family of auxin-conjugate hydrolases that contributes to free indole-3-acetic acid levels during *Arabidopsis* germination. *Plant Physiology*, *135*(2), 978–988. https://doi.org/10.1104/PP.104.039677

Rashad, F. M., and al., et. (2015). Isolation and characterization of multifunctional *Streptomyces* species with antimicrobial, nematicidal and phytohormone activities from marine environments in Egypt. *Microbiol. Res.*, *175*, 34–47.

Ratcliff, W. C., and Denison, R. F. (2011). Alternative actions for antibiotics. In *Science* (Vol. 332, Issue 6029, pp. 547–548). American Association for the Advancement of Science. https://doi.org/10.1126/science.1205970

Reetha, S., Selvakumar, G., Bhuvaneswari, G., Thamizhiniyan, P., and Ravimycin, T. (2014). Screening of cellulase and pectinase by using *Pseudomonas fluorescens* and *Bacillus subtilis*. *International Letters of Natural Sciences*, *8*(2). https://agro.icm.edu.pl/agro/element/bwmeta1.element.agro-8e705d54-78b1-4e4d-ad75-41eeb6901d5d

Reinhold-Hurek, B., Bünger, W., Burbano, C. S., Sabale, M., and Hurek, T. (2015). Roots shaping their microbiome: global hotspots for microbial activity. *Annu Rev Phytopathol*, *53*, 403–424. https://doi.org/10.1146/annurev-phyto-082712-102342

Retnowati, D., Solihin, D. D., Ghulamahdi, M., and Lestari, Y. (2019). Characterization of sponge-associated actinobacteria with potential to promote plant growth on tidal swamps. *Journal of Biological Research - Bollettino Della Società Italiana Di Biologia Sperimentale*, *92*(2). https://doi.org/10.4081/jbr.2019.8191

Rey, T., and Dumas, B. (2017). Plenty Is No Plague: *Streptomyces* Symbiosis with Crops. *Trends in Plant Science*, *22*(1), 30–37. https://doi.org/10.1016/j.tplants.2016.10.008

Ribeiro, M. H. L., Silveira, D., Ebert, C., and Ferreira-Dias, S. (2003). Response surface modelling of the consumption of bitter compounds from orange juice by *Acinetobacter calcoaceticus*. *Journal of Molecular Catalysis B: Enzymatic*, *21*(1–2), 81–88. https://doi.org/10.1016/S1381-1177(02)00144-3

Ribnicky, D. M., Cohen, J. D., Hu, W. S., and Cooke, T. J. (2002). An auxin surge following fertilization in carrots: a mechanism for regulating plant totipotency. *Planta*, *214*(4), 505–509. https://doi.org/10.1007/S004250100639

Robert, H. S., Park, C., Gutièrrez, C. L., Wójcikowska, B., Pěnčík, A., Novák, O., Chen, J., Grunewald, W., Dresselhaus, T., and Friml, J. (2018). Maternal auxin supply contributes to early embryo patterning in Arabidopsis. *Nature Plants*, *4*(8), 548–553.

Rodrigues, A. A. (2018). Isolation and Screening for Multi-trait Plant Growth Promoting Actinobacteria From Organic Sugarcane Rhizosphere. *International Journal of Microbiology Research*, *ISSN*, 975–5276.

Romero, D., Traxler, M. F., López, D., and Kolter, R. (2011). Antibiotics as signal molecules. In *Chemical Reviews* (Vol. 111, Issue 9, pp. 5492–5505). https://doi.org/10.1021/cr2000509

Rosenblueth, M., and Martínez-Romero, E. (2006). Bacterial endophytes and their interactions with hosts. In *Molecular Plant-Microbe Interactions* (Vol. 19, Issue 8, pp. 827–837). https://doi.org/10.1094/MPMI-19-0827

Roy, S., Sengupta, S., Manna, S., and Das, P. (2018). Chemically reduced tea waste biochar and its application in treatment of fluoride containing wastewater: Batch and optimization using response surface methodology. *Process Safety and Environmental Protection*, *116*, 553–563. https://doi.org/10.1016/J.PSEP.2018.03.009

Rungin, S., Indananda, C., Suttiviriya, P., Kruasuwan, W., Jaemsaeng, R., and Thamchaipenet, A. (2012). Plant growth enhancing effects by a siderophore-producing endophytic streptomycete isolated from a Thai jasmine rice plant (*Oryza sativa* L. cv. KDML105). *Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology*, *102*(3), 463–472. https://doi.org/10.1007/s10482-012-9778-z

Ryan, R. P., Germaine, K., Franks, A., Ryan, D. J., and Dowling, D. N. (2008). Bacterial endophytes: Recent developments and applications. *FEMS Microbiology Letters*, *278*(1), 1–9. https://doi.org/10.1111/j.1574-6968.2007.00918.x

Sadeghi, A., Karimi, E., Dahaji, P. A., Javid, M. G., Dalvand, Y., and Askari, H. (2012). Plant growth promoting activity of an auxin and siderophore producing isolate of *Streptomyces* under saline soil conditions. *World Journal of Microbiology and Biotechnology*, *28*(4), 1503–1509. https://doi.org/10.1007/s11274-011-0952-7

Sahay, H., Yadav, A. N., Singh, A. K., Singh, S., Kaushik, R., and Saxena, A. K. (2017). Hot springs of Indian Himalayas: potential sources of microbial diversity and thermostable hydrolytic enzymes. *3 Biotech*, *7*(2), 1–11. https://doi.org/10.1007/s13205-017-0762-1

Saini, D., Yadav, D., Pabbi, S., Chhabra, D., and Shukla, P. (2020). Phycobiliproteins from *Anabaena variabilis* CCC421 and its production enhancement strategies using combinatory evolutionary algorithm approach. *Bioresource Technology*, *309*. https://doi.org/10.1016/j.biortech.2020.123347

Salehin, M., Li, B., Tang, M., Katz, E., Song, L., Ecker, J. R., Kliebenstein, D. J., and Estelle, M. (2019). Auxin-sensitive Aux/IAA proteins mediate drought tolerance in *Arabidopsis* by regulating glucosinolate levels. *Nature Communications*, *10*(1), 1–9. https://doi.org/10.1038/s41467-019-12002-1

Salem, M. A., Yoshida, T., Perez de Souza, L., Alseekh, S., Bajdzienko, K., Fernie, A. R., and Giavalisco, P. (2020). An improved extraction method enables the comprehensive analysis of lipids, proteins, metabolites and phytohormones from a single sample of leaf tissue under water-deficit stress. *Plant Journal*, *103*(4), 1614–1632. https://doi.org/10.1111/tpj.14800

Sasse, J., Martinoia, E., and Northen, T. (2018). Feed your friends: do plant exudates shape the root microbiome? *Trends Plant Sci*, *23*(1), 25–41. https://doi.org/10.1016/j.tplants.2017.09.003

Schmidt, J., Marques, M. R. G., Botti, S., and Marques, M. A. L. (2019). Recent advances and applications of machine learning in solid-state materials science. *Npj Computational Materials 2019 5:1*, *5*(1), 1–36. https://doi.org/10.1038/s41524-019-0221-0

Seipke, R. F., Kaltenpoth, M., and Hutchings, M. I. (2012). *Streptomyces* as symbionts: An emerging and widespread theme? In *FEMS Microbiology Reviews* (Vol. 36, Issue 4, pp. 862–876). Oxford Academic. https://doi.org/10.1111/j.1574-6976.2011.00313.x

Sellstedt, A., and Richau, K. H. (2013). Aspects of nitrogen-fixing actinobacteria, in particular free-living and symbiotic *Frankia*. In *FEMS Microbiology Letters* (Vol. 342, Issue 2, pp. 179–186). https://doi.org/10.1111/1574-6968.12116

Shahzad, R., Waqas, M., Khan, A. L., Asaf, S., Khan, M. A., Kang, S. M., Yun, B. W., and Lee, I. J. (2016). Seed-borne endophytic *Bacillus amyloliquefaciens* RWL-1 produces gibberellins and regulates endogenous phytohormones of *Oryza sativa*. *Plant Physiology and Biochemistry*, *106*, 236–243. https://doi.org/10.1016/j.plaphy.2016.05.006

Sharma, V., Kaur, R., and Salwan, R. (2021). *Streptomyces*: host for refactoring of diverse bioactive secondary metabolites. In *3 Biotech* (Vol. 11, Issue 7, p. 340). https://doi.org/10.1007/s13205-021-02872-y

Shirling, E. B., and Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *International Journal of Systematic Bacteriology*, *16*(3), 313–340. https://doi.org/10.1099/00207713-16-3-313

Siddikee, M. A., Chauhan, P. S., Anandham, R., Han, G.-H., Sa, T., and Others. (2010). Isolation, characterization, and use for plant growth promotion under salt stress, of ACC deaminase-producing halotolerant bacteria derived from coastal soil. *J Microbiol Biotechnol*, *20*(11), 1577–1584.

Singh, H., Bhat, J. A., Singh, V. P., Corpas, F. J., and Yadav, S. R. (2021). Auxin metabolic network regulates the plant response to metalloids stress. *Journal of Hazardous Materials*, *405*. https://doi.org/10.1016/j.jhazmat.2020.124250

Singh, N., and Rai, V. (2012). Optimization of cultural parameters for antifungal and antibacterial metabolite from microbial isolate; *Streptomyces rimosus* MTCC 10792 from soil of Chhattisgarh. *International Journal of Pharmacy and Pharmaceutical Sciences*, *4*(4), 94–101. https://www.researchgate.net/profile/Neha-Singh-67/publication/232725674_Optimization_of_cultural_parameters_for_antifungal_and_antibacterial_metabolite_from_microbial_isolate_*Streptomyces_rimosus*_MTCC_10792_from_soil_of_Chhattisgarh/links/0deec5240e7eb0d

Singh, R. N., Gaba, S., Yadav, A. N., Gaur, P., Gulati, S., Kaushik, R., and Saxena, A. K. (2016). First high quality draft genome sequence of a plant growth promoting and cold active enzyme producing psychrotrophic *Arthrobacter agilis* strain L77. *Standards in Genomic Sciences*, *11*(1), 1–9. https://doi.org/10.1186/s40793-016-0176-4

Singh, V., and Tripathi, C. K. M. (2008). Production and statistical optimization of a novel olivanic acid by *Streptomyces olivaceus* MTCC 6820. *Process Biochemistry*, *43*(11), 1313–1317. https://doi.org/10.1016/j.procbio.2008.07.015

Sivakala, K. K., Gutiérrez-García, K., Jose, P. A., Thinesh, T., Anandham, R., Barona-Gómez, F., and Sivakumar, N. (2021). Desert Environments Facilitate Unique Evolution of Biosynthetic Potential in *Streptomyces*. *Molecules 2021, Vol. 26, Page 588*, *26*(3), 588. https://doi.org/10.3390/MOLECULES26030588

Sivapathasekaran, C., Mukherjee, S., Ray, A., Gupta, A., and Sen, R. (2010). Artificial neural network modeling and genetic algorithm based medium optimization for the improved production of marine biosurfactant. *Bioresource Technology*, *101*(8), 2884–2887. https://doi.org/10.1016/j.biortech.2009.09.093

Slama, H. Ben, Cherif-Silini, H., Bouket, A. C., Qader, M., Silini, A., Yahiaoui, B., Alenezi, F. N., Luptakova, L., Triki, M. A., Vallat, A., Oszako, T., Rateb, M. E., and Belbahri, L. (2019). Screening for *Fusarium* antagonistic bacteria from contrasting niches designated the endophyte *Bacillus halotoleransas* plant warden against *Fusarium*. *Frontiers in Microbiology*, *10*(JAN), 1–24. https://doi.org/10.3389/fmicb.2018.03236

Smaali, A., Berkani, M., Merouane, F., Le, V. T., Vasseghian, Y., Rahim, N., and Kouachi, M. (2021). Photocatalytic-persulfate- oxidation for diclofenac removal from aqueous solutions: Modeling, optimization and biotoxicity test assessment. *Chemosphere*, *266*, 129158. https://doi.org/10.1016/j.chemosphere.2020.129158

Smati, M., and Kitouni, M. (2019). Diversity of actinobacteria in the marshes of Ezzemoul and Djendli in northeastern Algeria. *European Journal of Ecology*, *5*(2), 41–53. https://doi.org/10.2478/eje-2019-0009

Spaepen, S., Vanderleyden, J., and Remans, R. (2007). Indole-3-acetic acid in microbial and microorganism-plant signaling. In *FEMS Microbiology Reviews* (Vol. 31, Issue 4, pp. 425–448). https://doi.org/10.1111/j.1574-6976.2007.00072.x

Sridevi, M., Yadav, N. C. S., and Mallaiah, K. V. (2008). Production of indole acetic acid by *Rhizobium* isolates from *Crolataria* species. *Res J Microbiol*, *3*(4), 276–281. https://doi.org/10.3923/jm.2008.276.281

Stach, N., Kaszycki, P., Wladyka, B., and Dubin, G. (2018). Extracellular Proteases of Staphylococcus spp. *Pet-to-Man Travelling Staphylococci: A World in Progress*, 135–145. https://doi.org/10.1016/B978-0-12-813547-1.00011-X

Stringlis, I. A., Yu, K., Feussner, K., De Jonge, R., Van Bentum, S., Van Verk, M. C., Berendsen, R. L., Bakker, P. A. H. M., Feussner, I., and Pieterse, C. M. J. (2018). MYB72-dependent coumarin exudation shapes root microbiome assembly to promote plant health. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(22), E5213–E5222. https://doi.org/10.1073/pnas.1722335115

Suárez-Moreno, Z. R., Vinchira-Villarraga, D. M., Vergara-Morales, D. I., Castellanos, L., Ramos, F. A., Guarnaccia, C., Degrassi, G., Venturi, V., and Moreno-Sarmiento, N. (2019a). Plant-growth promotion and biocontrol properties of three *Streptomyces* spp. isolates to control bacterial rice pathogens. *Frontiers in Microbiology*, *10*(FEB), 290. https://doi.org/10.3389/fmicb.2019.00290

Suárez-Moreno, Z. R., Vinchira-Villarraga, D. M., Vergara-Morales, D. I., Castellanos, L., Ramos, F. A., Guarnaccia, C., Degrassi, G., Venturi, V., and Moreno-Sarmiento, N. (2019b). Plant-growth promotion and biocontrol properties of three *Streptomyces* spp. isolates to control bacterial rice pathogens. *Frontiers in Microbiology*, *10*(FEB), 1–17. https://doi.org/10.3389/fmicb.2019.00290

Subramaniam, G., Arumugam, S., and Rajendran, V. (2016). Plant growth promoting actinobacteria: A new avenue for enhancing the productivity and soil fertility of grain legumes. In *Plant Growth Promoting Actinobacteria: A New Avenue for Enhancing the Productivity and Soil Fertility of Grain Legumes*. Springer Singapore. https://doi.org/10.1007/978-981-10-0707-1

Sudha, S., and Masilamani, S. M. (2012). Characterization of cytotoxic compound from marine sediment derived actinomycete *Streptomyces avidinii* strain SU4. *Asian Pacific Journal of Tropical Biomedicine*, *2*(10), 770–773. https://doi.org/10.1016/S2221-1691(12)60227-5

Sun, D., Liu, C., Zhu, J., and Liu, W. (2017). Connecting metabolic pathways: Sigma factors in *Streptomyces* spp. *Frontiers in Microbiology*, *8*(DEC). https://doi.org/10.3389/FMICB.2017.02546

Sun, L., Qiu, F., Zhang, X., Dai, X., Dong, X., and Song, W. (2008). Endophytic bacterial

diversity in rice (*Oryza sativa* L.) roots estimated by 16S rDNA sequence analysis. *Microbial Ecology*, *55*(3), 415–424. https://doi.org/10.1007/S00248-007-9287-1

Suzuki, K. (2015). Rubrobacteria class. nov. *Bergey's Manual of Systematics of Archaea and Bacteria*, 1–4.

Suzuki, K., and Whitman, W. B. (2012). Class VI. *Thermoleophilia* class. nov. *Bergey's Manual of Systematic Bacteriology*, *5*, 2010–2028.

Swain, M. R., Naskar, S. K., and Ray, R. C. (2007). Indole-3-acetic acid production and effect on sprouting of yam (*Dioscorea rotundata* L.) minisetts by *Bacillus subtilis* isolated from culturable cowdung microflora. *Polish Journal of Microbiology*, *56*(2), 103–110.

Sytar, O., Kumari, P., Yadav, S., Brestic, M., and Rastogi, A. (2019). Phytohormone Priming: Regulator for Heavy Metal Stress in Plants. In *Journal of Plant Growth Regulation* (Vol. 38, Issue 2, pp. 739–752). Springer New York LLC. https://doi.org/10.1007/s00344-018-9886-8

Talhi, I., Dehimat, L., Jaouani, A., Cherfia, R., Berkani, M., Almomani, F., Vasseghian, Y., and Chaouche, N. K. (2022). Optimization of thermostable proteases production under agro-wastes solid-state fermentation by a new thermophilic *Mycothermus thermophilus* isolated from a hydrothermal spring Hammam Debagh, Algeria. *Chemosphere*, *286*(P1), 131479. https://doi.org/10.1016/j.chemosphere.2021.131479

Talukdar, S., Talukdar, M., Buragohain, M., Yadav, A., Yadav, R. N. S., and Bora, T. C. (2016). Enhanced candicidal compound production by a new soil isolate *Penicillium verruculosum* MKH7 under submerged fermentation. *BMC Microbiology*, *16*(1). https://doi.org/10.1186/s12866-016-0713-8

Teale, W. D., Paponov, I. A., and Palme, K. (2006). Auxin in action: signalling, transport and the control of plant growth and development. *Nature Reviews Molecular Cell Biology 2006 7:11*, *7*(11), 847–859. https://doi.org/10.1038/nrm2020

Thakur, D., Yadav, A., Gogoi, B. K., and Bora, T. C. (2007). Isolation and screening of *Streptomyces* in soil of protected forest areas from the states of Assam and Tripura, India, for antimicribial metabolites. *Journal de Mycologie Medicale*, *17*(4), 242–249. https://doi.org/10.1016/j.mycmed.2007.08.001

Theunis, M., Kobayashi, H., Broughton, W. J., and Prinsen, E. (2004). Flavonoids, NodD1, NodD2, and Nod-Box NB15 modulate expression of the y4wEFG locus that is required for indole-3-acetic acid synthesis in *Rhizobium* sp. strain NGR234. *Molecular Plant-Microbe Interactions*, *17*(10), 1153–1161. https://doi.org/10.1094/MPMI.2004.17.10.1153

Tian, T., Reverdy, A., She, Q., Sun, B., and Chai, Y. (2020). The role of rhizodeposits in shaping rhizomicrobiome. *Environmental Microbiology Reports*, *12*(2), 160–172. https://doi.org/10.1111/1758-2229.12816

Toumatia, O., Compant, S., Yekkour, A., Goudjal, Y., Sabaou, N., Mathieu, F., Sessitsch, A., and Zitouni, A. (2016). Biocontrol and plant growth promoting properties of *Streptomyces mutabilis* strain IA1 isolated from a Saharan soil on wheat seedlings and visualization of its niches of colonization. *South African Journal of Botany*, *105*, 234–239.

Tripathi, C. K. M., Khan, M., Praveen, V., Khan, S., and Srivastava, A. (2012). Enhanced antibiotic production by *Streptomyces sindenensis* using artificial neural networks

coupled with genetic algorithm and Nelder- Mead downhill simplex. *Journal of Microbiology and Biotechnology*, *22*(7), 939–946. https://doi.org/10.4014/jmb.1109.09018

Turan, M., Nikerel, E., Kaya, K., Kitir, N., Gunes, A., Mokhtari, N. E. P., Tüfenkçi, S., Karaman, M. R., and Çimrin, K. M. (2017). Enzyme Dynamic in Plant Nutrition Uptake and Plant Nutrition. In *Enzyme Inhibitors and Activators*. https://doi.org/10.5772/66938

Tyc, O., Song, C., Dickschat, J. S., Vos, M., and Garbeva, P. (2017). The Ecological Role of Volatile and Soluble Secondary Metabolites Produced by Soil Bacteria. In *Trends in Microbiology* (Vol. 25, Issue 4, pp. 280–292). https://doi.org/10.1016/j.tim.2016.12.002

Ulrich, L. E., and Zhulin, I. B. (2007). MiST: A microbial signal transduction database. *Nucleic Acids Research*, *35*(SUPPL. 1), 386–390. https://doi.org/10.1093/nar/gkl932

Upreti, K., and Sharma, M. (2016). *Role of plant growth regulators in abiotic stress tolerance* (pp. 19–46). Springer.

Vaish, S., Gupta, D., Mehrotra, R., Mehrotra, S., and Basantani, M. K. (2020). Glutathione S-transferase: a versatile protein family. *3 Biotech*, *10*(7), 321. https://doi.org/10.1007/s13205-020-02312-3

van der Aart, L. T., Nouioui, I., Kloosterman, A., Igual, J. M., Willemse, J., Goodfellow, M., and van Wezel, G. P. (2019). Polyphasic classification of the gifted natural product producer *Streptomyces roseifaciens* sp. Nov. *International Journal of Systematic and Evolutionary Microbiology*, *69*(4), 899–908. https://doi.org/10.1099/ijsem.0.003215

van der Meij, A., Worsley, S. F., Hutchings, M. I., and van Wezel, G. P. (2017). Chemical ecology of antibiotic production by actinomycetes. In *FEMS Microbiology Reviews* (Vol. 41, Issue 3, pp. 392–416). Oxford Academic. https://doi.org/10.1093/femsre/fux005

Vannier, N., Agler, M., and Hacquard, S. (2019). Microbiota-mediated disease resistance in plants. *PLOS Pathogens*, *15*(6), e1007740. https://doi.org/10.1371/JOURNAL.PPAT.1007740

Vasseghian, Y., Ahmadi, M., and Joshaghani, M. (2017). Ultrasound Assisted Ash and Sulphur Removal from Bitumen Using Column Flotation Technique: Experimental, RSM and ANN Methods in Modelling and Optimization of Process. *Iranian Journal of Science and Technology, Transaction A: Science*, *41*(4), 1149–1163. https://doi.org/10.1007/s40995-016-0068-x

Vasseghian, Y., Bahadori, A., Khataee, A., Dragoi, E. N., and Moradi, M. (2020). Modeling the Interfacial Tension of Water-Based Binary and Ternary Systems at High Pressures Using a Neuro-Evolutive Technique. *ACS Omega*, *5*(1), 781–790. https://doi.org/10.1021/acsomega.9b03518

Vasseghian, Y., and Dragoi, E.-N. (2018). Modeling and Optimization of Acid Blue 193 Removal by UV and Peroxydisulfate Process. *Journal of Environmental Engineering*, *144*(8), 06018003. https://doi.org/10.1061/(asce)ee.1943-7870.0001405

Ventura, M., Canchaya, C., Tauch, A., Chandra, G., Fitzgerald, G. F., Chater, K. F., and van Sinderen, D. (2007). Genomics of Actinobacteria : Tracing the Evolutionary History of an Ancient Phylum. *Microbiology and Molecular Biology Reviews*, *71*(3), 495–548. https://doi.org/https://doi.org/10.1128/mmbr.00005-07

Verma, P., Yadav, A. N., Khannam, K. S., Mishra, S., Kumar, S., Saxena, A. K., and Suman,

A. (2019). Appraisal of diversity and functional attributes of thermotolerant wheat associated bacteria from the peninsular zone of India. *Saudi Journal of Biological Sciences*, *26*(7), 1882–1895. https://doi.org/10.1016/J.SJBS.2016.01.042

Verma, V. C., Gond, S. K., Kumar, A., Mishra, A., Kharwar, R. N., and Gange, A. C. (2009). Endophytic actinomycetes from azadirachta indica A. Juss.: Isolation, diversity, and anti-microbial activity. *Microbial Ecology*, *57*(4), 749–756. https://doi.org/10.1007/s00248-008-9450-3

Viaene, T., Langendries, S., Beirinckx, S., Maes, M., and Goormachtig, S. (2016). *Streptomyces* as a plant's best friend? *FEMS Microbiology Ecology*, *92*(8). https://doi.org/10.1093/femsec/fiw119

Vives-Peris, V., de Ollas, C., Gómez-Cadenas, A., and Pérez-Clemente, R. M. (2019). Root exudates: from plant to rhizosphere and beyond. *Plant Cell Reports 2019 39:1*, *39*(1), 3–17. https://doi.org/10.1007/S00299-019-02447-5

Vranova, V., Rejsek, K., and Formanek, P. (2013). Proteolytic activity in soil: A review. In *Applied Soil Ecology* (Vol. 70, pp. 23–32). Elsevier. https://doi.org/10.1016/j.apsoil.2013.04.003

Wahyudi, A. T., Priyanto, J. A., Fijrina, H. N., Mariastuti, H. D., and Nawangsih, A. A. (2019). *Streptomyces* spp. From rhizosphere soil of maize with potential as plant growth promoter. *Biodiversitas*, *20*(9), 2547–2553. https://doi.org/10.13057/biodiv/d200916

Wang, R., Zhang, Y., Kieffer, M., Yu, H., Kepinski, S., and Estelle, M. (2016). HSP90 regulates temperature-dependent seedling growth in *Arabidopsis* by stabilizing the auxin co-receptor F-box protein TIR1. *Nature Communications*, *7*(1), 1–11. https://doi.org/10.1038/ncomms10269

Wang, S., Kwak, S. H., Zeng, Q., Ellis, B. E., Chen, X. Y., Schiefelbein, J., and Chen, J. G. (2007). TRICHOMELESS1 regulates trichome patterning by suppressing GLABRA1 in *Arabidopsis*. *Development*, *134*(21), 3873–3882. https://doi.org/10.1242/DEV.009597

Wang, Y., and al., et. (2009). Auxin redistribution modulates plastic development of root system architecture under salt stress in *Arabidopsis thaliana*. *J. Plant Physiol.*, *166*, 1637–1645.

Wanner, L. A. (2006). A survey of genetic variation in *Streptomyces* isolates causing potato common scab in the United States. *Phytopathology*, *96*(12), 1363–1371. https://doi.org/10.1094/PHYTO-96-1363

Ward, A. C., and Allenby, N. E. (2018). Genome mining for the search and discovery of bioactive compounds: The *Streptomyces* paradigm. In *FEMS Microbiology Letters* (Vol. 365, Issue 24, p. 240). Oxford Academic. https://doi.org/10.1093/femsle/fny240

Williams, S. T., Goodfellow, M., Wellington, E. M. H., Vickers, J. C., Alderson, G., Sneath, P. H., Sackin, M. J., and Mortimer, A. M. (1983). A probability matrix for identification of some streptomycetes. *Journal of General Microbiology*, *129*(6), 1815–1830. https://doi.org/10.1099/00221287-129-6-1815

Woodward, A. W., and Bartel, B. (2005). Auxin: Regulation, action, and interaction. *Annals of Botany*, *95*(5), 707–735. https://doi.org/10.1093/AOB/MCI083

Wu, C., Agrawal, D. C., Huang, W., Hsu, H., Yang, S., Huang, S., and Lin, Y. (2019). *Functionality Analysis of Spent Coffee Ground Extracts Obtained by the Hydrothermal*

Method. *2019*.

Xiang, W., Zhao, L., Xu, X., Qin, Y., and Yu, G. (2012). Mutual Information Flow between Beneficial Microorganisms and the Roots of Host Plants Determined the Bio-Functions of Biofertilizers. *American Journal of Plant Sciences*, *03*(08), 1115–1120. https://doi.org/10.4236/ajps.2012.38134

Xu, T., Li, Y., Zeng, X., Yang, X., Yang, Y., Yuan, S., Hu, X., Zeng, J., Wang, Z., Liu, Q., Liu, Y., Liao, H., Tong, C., Liu, X., and Zhu, Y. (2017). Isolation and evaluation of endophytic *Streptomyces endus* OsiSh-2 with potential application for biocontrol of rice blast disease. *Journal of the Science of Food and Agriculture*, *97*(4), 1149–1157. https://doi.org/10.1002/jsfa.7841

Xu, W., Jia, L., Shi, W., Liang, J., Zhou, F., Li, Q., and Zhang, J. (2013). Abscisic acid accumulation modulates auxin transport in the root tip to enhance proton secretion for maintaining root growth under moderate water stress. *New Phytologist*, *197*(1), 139–150. https://doi.org/10.1111/nph.12004

Yadav, B., Jogawat, A., Rahman, M. S., and Narayan, O. P. (2021). Secondary metabolites in the drought stress tolerance of crop plants: A review. In *Gene Reports* (Vol. 23, p. 101040). Elsevier. https://doi.org/10.1016/j.genrep.2021.101040

Yahya, H. S. M., Abbas, T., and Amin, N. A. S. (2020). Optimization of hydrogen production via toluene steam reforming over Ni–Co supported modified-activated carbon using ANN coupled GA and RSM. *International Journal of Hydrogen Energy*, *46*(48), 24632–24651. https://doi.org/10.1016/J.IJHYDENE.2020.05.033

Yan, T., Id, Y., Feng, R. J., Zhou, D. B., Pan, Y. Y., Chen, Y. F., Wang, F., Yin, L. Y., Zhang, Y. D., and Xie, J. H. (2018). Optimization of fermentation conditions through response surface methodology for enhanced antibacterial metabolite production by *Streptomyces* sp . 1-14 from cassava rhizosphere. *PLoS ONE*, 1–14.

Yetilmezsoy, K., and Demirel, S. (2008). Artificial neural network (ANN) approach for modeling of Pb(II) adsorption from aqueous solution by Antep pistachio (Pistacia Vera L.) shells. *Journal of Hazardous Materials*, *153*(3), 1288–1300. https://doi.org/10.1016/J.JHAZMAT.2007.09.092

Yuan, W., Suo, J., Shi, B., Zhou, C., Bai, B., Bian, H., Zhu, M., and Han, N. (2019). The barley miR393 has multiple roles in regulation of seedling growth, stomatal density, and drought stress tolerance. *Plant Physiology and Biochemistry*, *142*, 303–311. https://doi.org/10.1016/j.plaphy.2019.07.021

Yun, T. Y., Feng, R. J., Zhou, D. B., Pan, Y. Y., Chen, Y. F., Wang, F., Yin, L. Y., Zhang, Y. D., and Xie, J. H. (2018). Optimization of fermentation conditions through response surface methodology for enhanced antibacterial metabolite production by *Streptomyces* sp. 1-14 from cassava rhizosphere. *PLOS ONE*, *13*(11), e0206497. https://doi.org/10.1371/JOURNAL.PONE.0206497

Zamoum, M., Goudjal, Y., Sabaou, N., Barakate, M., Mathieu, F., and Zitouni, A. (2015). Biocontrol capacities and plant growth-promoting traits of endophytic actinobacteria isolated from native plants of Algerian sahara. *Journal of Plant Diseases and Protection*, *122*(5–6), 215–233. https://doi.org/10.1007/bf03356555

Zamoum, M., Goudjal, Y., Sabaou, N., Mathieu, F., and Zitouni, A. (2017). Development of formulations based on *Streptomyces rochei* strain PTL2 spores for biocontrol of

Rhizoctonia solani damping-off of tomato seedlings. *Biocontrol Science and Technology*, *27*(6), 723–738. https://doi.org/10.1080/09583157.2017.1334257

Zerrouk, I. Z., Rahmoune, B., Khelifi, L., Mounir, K., Baluska, F., and Ludwig-Müller, J. (2019). Algerian Sahara PGPR confers maize root tolerance to salt and aluminum toxicity via ACC deaminase and IAA. *Acta Physiologiae Plantarum*, *41*(6), 91. https://doi.org/10.1007/s11738-019-2881-2

Zhang, H., Wu, X., Li, G., and Qin, P. (2011). Interactions between arbuscular mycorrhizal fungi and phosphate-solubilizing fungus (*Mortierella* sp.) and their effects on *Kostelelzkya virginica* growth and enzyme activities of rhizosphere and bulk soils at different salinities. *Biology and Fertility of Soils*, *47*(5), 543. https://doi.org/10.1007/s00374-011-0563-3

Zhang, J., Liu, Y. X., Zhang, N., Hu, B., Jin, T., Xu, H., Qin, Y., Yan, P., Zhang, X., Guo, X., Hui, J., Cao, S., Wang, X., Wang, C., Wang, H., Qu, B., Fan, G., Yuan, L., Garrido-Oter, R., … Bai, Y. (2019). NRT1.1B is associated with root microbiota composition and nitrogen use in field-grown rice. *Nature Biotechnology*, *37*(6), 676–684. https://doi.org/10.1038/s41587-019-0104-4

Zhang, Y., Li, Y., Hassan, M. J., Li, Z., and Peng, Y. (2020). Indole-3-acetic acid improves drought tolerance of white clover via activating auxin, abscisic acid and jasmonic acid related genes and inhibiting senescence genes. *BMC Plant Biology*, *20*(1). https://doi.org/10.1186/s12870-020-02354-y

Zhang, Y., and Pan, B. (2014). Modeling batch and column phosphate removal by hydrated ferric oxide-based nanocomposite using response surface methodology and artificial neural network. *Chemical Engineering Journal*, *249*, 111–120. https://doi.org/10.1016/j.cej.2014.03.073

Zhao, J., Wang, X., Sun, W., Mou, Y., Peng, Y., and Zhou, L. (2013). Medium optimization for palmarumycin C13 production in liquid culture of endophytic fungus *Berkleasmium* sp. Dzf12 using response surface methodology. *Electronic Journal of Biotechnology*, *16*(6), 16. https://doi.org/10.2225/vol16-issue6-fulltext-10

Zhong, J., Zhang, X., Ren, Y., Yang, J., Tan, H., and Zhou, J. (2014). Optimization of *Bacillus subtilis* cell growth effecting jiean-peptide production in fed batch fermentation using central composite design. *Electronic Journal of Biotechnology*, *17*(3), 132–136. https://doi.org/10.1016/j.ejbt.2014.04.010

Zhou, J., Cheng, K., Huang, G., Chen, G., Zhou, S., Huang, Y., Zhang, J., Duan, H., and Fan, H. (2020). Effects of exogenous 3-indoleacetic acid and cadmium stress on the physiological and biochemical characteristics of *Cinnamomum camphora*. *Ecotoxicology and Environmental Safety*, *191*, 109998. https://doi.org/10.1016/j.ecoenv.2019.109998

Zhou, N., Zhao, S., and Tian, C. Y. (2017). Effect of halotolerant rhizobacteria isolated from halophytes on the growth of sugar beet (*Beta vulgaris* L.) under salt stress. In *FEMS Microbiology Letters* (Vol. 364, Issue 11, p. 91). Oxford Academic. https://doi.org/10.1093/femsle/fnx091

Zhou, X., Wang, J. T., Zhang, Z. F., Li, W., Chen, W., and Cai, L. (2020). Microbiota in the Rhizosphere and Seed of Rice From China, With Reference to Their Transmission and Biogeography. *Frontiers in Microbiology*, *11*(July), 1–13. https://doi.org/10.3389/fmicb.2020.00995

Zhu, X. F., Wang, Z. W., Dong, F., Lei, G. J., Shi, Y. Z., Li, G. X., and Zheng, S. J. (2013). Exogenous auxin alleviates cadmium toxicity in *Arabidopsis thaliana* by stimulating synthesis of hemicellulose 1 and increasing the cadmium fixation capacity of root cell walls. *Journal of Hazardous Materials*, *263*, 398–403. https://doi.org/10.1016/j.jhazmat.2013.09.018

Zúñiga, A., Poupin, M. J., Donoso, R., Ledger, T., Guiliani, N., Gutiérrez, R. A., and González, B. (2013). Quorum sensing and indole-3-acetic acid degradation play a role in colonization and plant growth promotion of *Arabidopsis thaliana* by burkholderia phytofirmans PsJN. *Molecular Plant-Microbe Interactions*, *26*(5), 546–553. https://doi.org/10.1094/MPMI-10-12-0241-R

Appendices

Appendix A: Supplementary figures

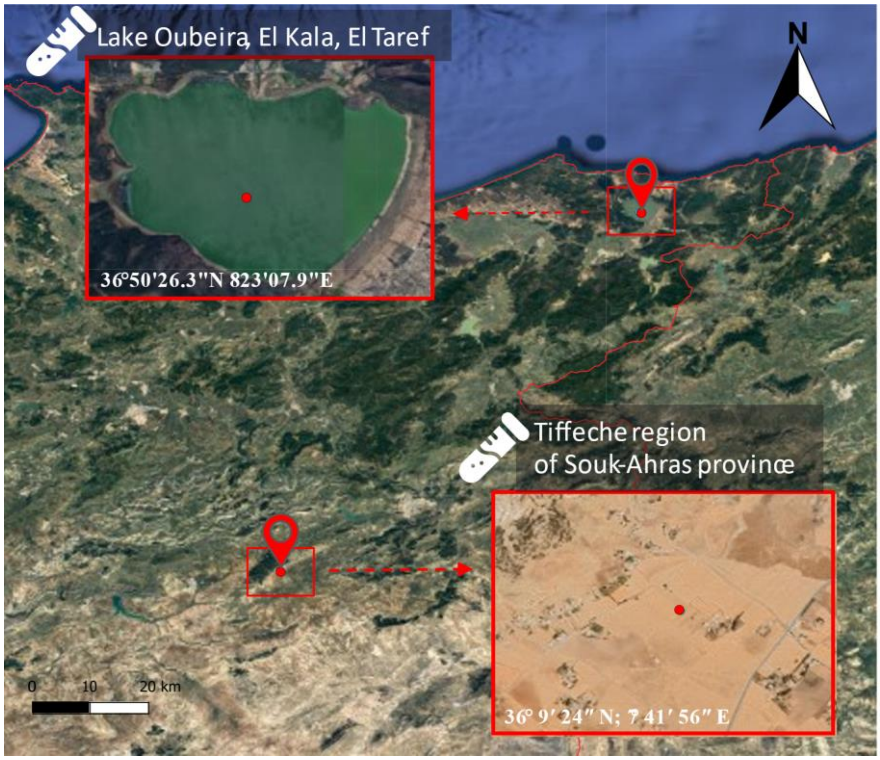


Figure S1. Sampling sites for the isolation of antagonist Actinobacteria strains (Google maps : https://www.google.com/maps).

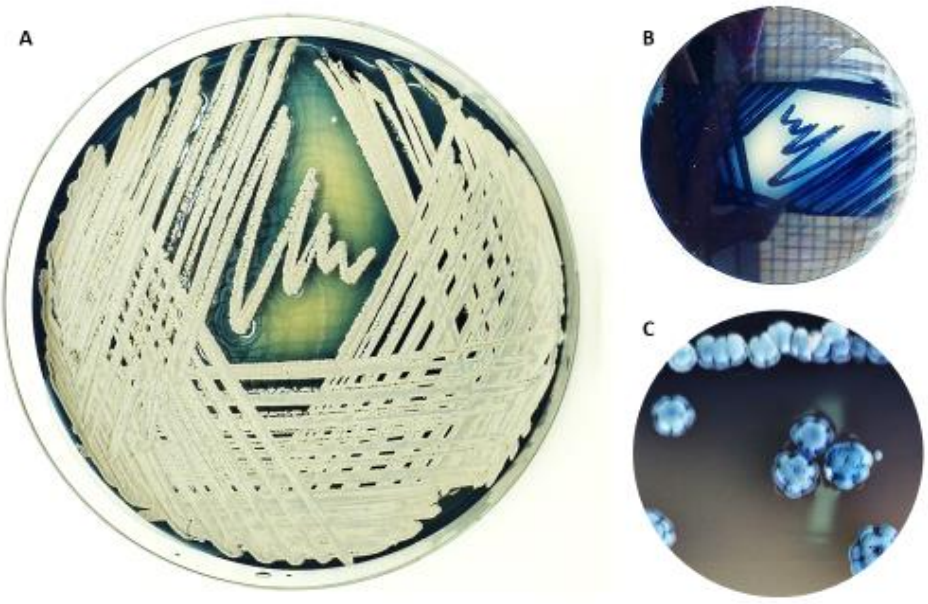
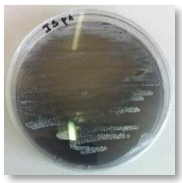
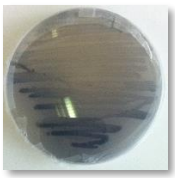
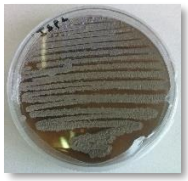
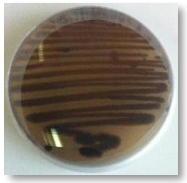
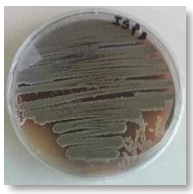
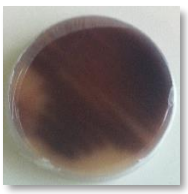
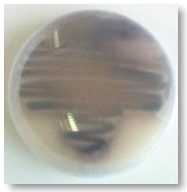
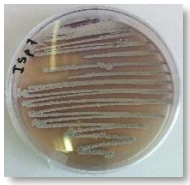
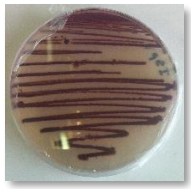
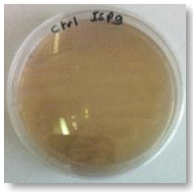
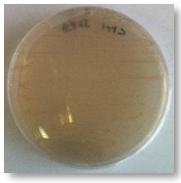
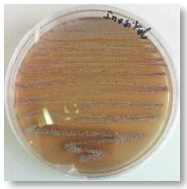
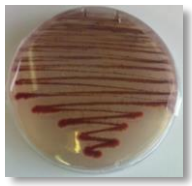
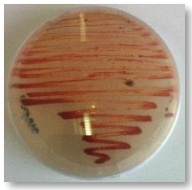
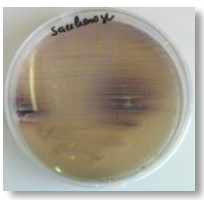
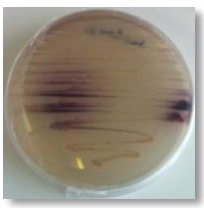


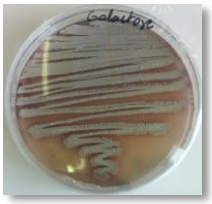
Figure S2. Morpho-cultural aspects of AW22 on GYM agar. Represented in this figure the front (A) and reverse (B) of the culture showing aerial and substrate mycelium, respectively, and the single colony shape of the isolate (C).

Appendix B: Supplementary tables

Table S1. Phenotype of AW22 on various culture media.

milieu	recto	verso
ISP1		
ISP2		
ISP3		
ISP4		
ISP5		
ISP6		
ISP7		

ISP9		
ISP9(Glucose)		
ISP9(Mannitol)		
ISP9(Inositol)		
ISP9(Fructose)		
Isp9(Lactose)		
ISP9(Rhamnose)		
ISP9(Saccharose)		

ISP9 (Galactose)		

Scientific production: Publications

PUBLICATIONS

- *Articles in press*

Waste valorization as low-cost media engineering for auxin production from the newly isolated *Streptomyces rubrogriseus* AW22: Model development (2023).

Wiem Alloun, Mohammed Berkani, Akila Benaissa, Amin Shavandi, Maroua Gares, Camellia Danesh, Delloula Lakhdari, Ayman A. Ghfar and Noreddine Kacem Chaouche. *Chemosphere, Science Direct, Elsevier*. 326: 138394. *https://doi.org/10.1016/j.chemosphere.2023.138394*

Box-Behnken design optimization of xylanase and cellulase production by *Aspergillus fumigatus* on *Stipa tenacissima* biomass (2023). Maroua Gares, Akila Benaissa, Serge Hiligsmann, Radia Cherfia, Sigrid Flahaut, Wiem Alloun, Hadjer Djelid, Samah Chaoua and Noreddine Kacem Chaouche. *Mycologia.* 115:1-19. *https://doi.org/10.1080/00275514.2023.2205331*

Phenolic profile and bioactivity of the aerial part and roots of *Mentha rotundifolia* L. grown in two different localities in northeastern Algeria: A comparative study (2022). Hadjer KCIES, Abdelouahab Yahia, Mohamed Bagues, Lynda Gali, Fatiha Mekircha, Wiem Alloun and Kameleddine Nagaz. *Biocatalysis and Agricultural Biotechnology*. *Elsevier*. 47:10258. *https://doi.org/10.1016/j.bcab.2022.102581*

The study of the industrial aptitude of *Aspergillus fumigatus* strain for xylanase production (2023). Maroua GARES, HILIGSMANN Serge, Mahmoud KASSEM AL SAYED, Wiem ALLOUN and Noreddine KACEM CHAOUCHE. *Egyptian Journal of Chemistry*. *https://doi.org/10.21608/EJCHEM.2023.174799.7195*

- *Online review papers and book chapters*

Chapters 4, 21 and 25 in the Book: Arbuscular Mycorrhizal Fungi: For Nutrient, Abiotic and Biotic Stresses Management in Rice" CRC Press, Taylor & Francis Group. ISBN 978103240641, 232 Pages 17 B/W Illustrations (July 25, 2023): Arbuscular Mycorrhizal Fungi: For Nutrient, Abiotic and Biotic Stress (routledge.com) https://doi.org/10.1201/9781003354086

Chapter 4. Arbuscular Mycorrhizal Fungi: A Sustainable Approach for Enhancing Phosphorous and Nitrogen Use Efficiency in Rice Cultivation.

Authors: Wiem Alloun and Debasis Mitra

Chapter 21. AM Fungi Production Upscaling, Government Regulations, Marketing and Commercialization.

Authors: Wiem Alloun, Somya Sinha and Debasis Mitra

Chapter 25. Rice-Mycorrhizal Interaction: Enhances the Biocontrol Efficiency Through Integrated Approaches

Authors: Wiem Alloun, Izdihar Ferhat, Hadjer Kecies, Aya Rehouma and Abdelkader Mahrouk.

Chapter 8. Nanoparticles for the Improved Horticultural Crop Production. (2023). In book: Engineered Nanoparticles in Agriculture.

Authors: Debasis Mitra, Wiem Alloun, Shraddha Bhaskar Sawant, Edappayil Janeeshma, Pradeep Kumar Das Mohapatra, Periyasamy Panneerselvam. De Gruyter academic publishing. Engineered Nanoparticles in Agriculture (degruyter.com). https://doi.org/10.1515/9781501523229-009

CONFERENCES AND SEMINARS

Wiem ALLOUN, David CANNELLA, Amin SHAVANDI and N. KACEM CHAOUCHE. Biocontrol and phytostimulation potential of Actinobacteria strains for further applications on wheat crops. FEMS Online Conference on Microbiology 2020. on 28th – 31st October 2020. https://fems-microbiology.org/opportunities/fems-conference-on-microbiology-2020/

Wiem ALLOUN and N. KACEM CHAOUCHE. *Streptomyces* isolates from Algerian ecosystems shown antifungal activity against wheat phytopathogens. International seminar on "Genome and wheat sequencing", 28 -29 January 2019, UMC 1 University, Algeria.

https://ensbiotech.edu.dz/servicepratique/evenement/1262-international-seminar-genome-and-wheat-sequencing

Wiem ALLOUN and N. KACEM CHAOUCHE. Biological control of wheat root rot caused by *Fusarium* species by Streptomyces strains isolated from different Algerian niches. International seminar on "Genome and wheat sequencing", 28 -29 January 2019, UMC 1 University, Algeria. https://ensbiotech.edu.dz/servicepratique/evenement/1262-international-seminar-genome-and-wheat-sequencing

SYMPOSIA

Wiem ALLOUN and N. KACEM CHAOUCHE. "Next-Generation Microbial Inoculants for Sustainable Agriculture: Status, Challenges and Needs. " 26th Annual Symposium of the Belgian Society for Microbiology, "Microbiology at the 2020 horizon", held online on October 16, 2020.

https://belsocmicrobio.be/events/

Wiem ALLOUN, Amin SHAVANDI, David CANNELLA and N. KACEM CHAOUCHE. "Selection of Actinobacteria strains for Biocontrol and plant growth promoting activities: Applications on wheat crops. "25th Annual Symposium of the Belgian Society for Microbiology, "Microbes without Frontiers", organized on October 18th, 2019 jointly with the National Committee for Microbiology held at the Royal Academies for Science and the Arts of Belgium (RASAB), in Brussels, Belgium.

https://belsocmicrobio.be/wp-content/uploads/2021/12/2019 abstract_book.pdf

Wiem ALLOUN, KARA ALI Mounira and N. KACEM CHAOUCHE. Exploration of Algerian ecosystems for the selection of antagonistic bacteria against wheat pathogens in the region of Constantine, Algeria. 2nd Meeting of: Réseau Filière Blé dur (*Triticum durum*) "Stratégies et perspectives" (19 March, 2018). Brothers Mentouri of Constantine 1 University. Algeria.

Wiem ALLOUN, Maroua Gares, Hadjer KECIES and Noredine KACEM CHAOUCHE. Assessment of rhizospheric actinobacteria strains as potential biopesticides and fertilizers for further applications on wheat crops.1st National Seminar on : Applications Thérapeutiques et Agroalimentaires des Substances Naturelles, October 18th, 2022. Centre Universitaire BOUSSOUF Abdelhafid, Mila. 1er Séminaire National sur - المركز الجامعي عبد الحفيظ بوالصوف (centre-univ-mila.dz)

Author : Wiem ALLOUN	**Viva voce on 20th July, 2023**

Thesis/Dissertation submitted for obtaining the degree of Doctor of Science (D. Sc.) 3rd Cycle LMD

Entitled: Exploration of Algerian ecosystems for the selection of actinobacteria belonging to the genus *Streptomyces* developing potentialities of PGPR and antagonists of wheat phytopathogens: Modeling of bioactive metabolites

Abstract

This work aims to isolate Actinobacteria strains with a growth promotion ability and the biocontrol potential of *Fusarium culmorum*, the wheat root rot-causing fungi. The exploration of terrestrial and aquatic Algerian ecosystems, i.e. the wheat rhizosphere in the Tiffeche region (Souk-Ahras) and the aquatic sediments of Lake Oubeira (El Taref), respectively, resulted in 102 native isolates. Therefore, 37 have morphological and cultural characteristics similar to the genus *Streptomyces*. These isolates were screened for their plant growth-promoting traits. These activities consist of the production of hydrogen cyanide (HCN), ammonia (NH_3), and indole-3-acetic acid (IAA), as well as *in vitro* antagonism against *F. culmorum*. Among the auxin family, IAA constitutes a crucial phytohormone regulating specific tropic responses of plants and functions as a chemical signal between host plants and their symbionts. IAA derived from Actinobacteria grown on agricultural waste represents a more economical alternative than its synthetic homologous. Rhizospheric isolate AW 22 was positive for HCN and NH_3 production, growth inhibition of *F. culmorum* with an index of 67.320±8.99% and high IAA content of 23.999±1.126 µg. mL^{-1} in standard growth conditions on yeast-tryptone broth (YTB) amended with 0.2% (w/v) L-Tryptophan. Thus, the AW22 isolate was selected for a polyphasic chemotaxonomic characterization and the optimization of the production process of this phytohormone. Molecular and phylogenetic analysis identified isolate AW22 as *Streptomyces rubrogriseus*, and its sequence was deposited in Genbank under accession ID OP176004. Analysis of the putative IAA produced by *S. rubrogriseus* AW22 on YTB using thin-layer chromatography (TLC) and (HPLC) revealed R*f* values equal to 0.69 and a retention time of 3.711 min, equivalent to the authentic IAA. Artificial intelligence-based approaches (i.e. Behnken design from response surface methodology (BBD-RSM) with artificial neural networks (ANNs) coupled with the genetic algorithm (GA)) were employed to bioengineer *in vitro* and *silico* a suitable medium for maximum IAA bioproduction. According to the Box Behnken Design matrix, data were based on empirical studies involving the inoculation of AW22 in various cultural conditions and low-cost feedstocks notably, the spent coffee grounds (SCGs). Four input variables comprising L-Trp (X1), incubation T° (X2), initial pH (X3) and SCG concentration (X4) were screened via Plackett-Burman design (PBD) and served as BBD and ANN-GA inputs. The IAA yield constituted the output variable (Y in µg. mL^{-1}). Upon training the model, the optimal conditions suggested by the ANN-GA model were X1= 0.6%, X2= 25.8°C, X3 = 9, X4=30%). An R^2 of 99.98%, adding to an MSE of $1.86x10^{-5}$ at 129 epochs, postulated higher reliability of the ANN-GA approach in predicting responses, compared with BBD-RSM modeling exhibiting an R^2 of 76,28%. Using the process parameters generated by ANN-GA AW 22 achieved a maximum IAA yield of 188.290±0.38 µg. mL^{-1}. This optimization resulted in a 4.55-fold and 4.46-fold increase in IAA secretion after eight days of incubation, corresponding to ANN-GA and BBD-RSM models, respectively. These results confirm the validity of both models in maximizing IAA yield from the multifunctional *S. rubrogriseus* AW22 isolated for the first time in Algeria.

Keywords. *Streptomyces*; Indole-3-acetic acid (IAA); Artificial intelligence (AI); Response surface methodology (RSM); Artificial neural networks (ANNs); Mathematical Modeling.

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