

Isolation of Fluorescent *Pseudomonas* spp. from Degraded Agricultural Soils in the Daloa Region: Salt Stress Resistance Capacity

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Abstract Salinisation due to intensive agriculture and geological alteration reduces soil microbial activity, creating unfavorable conditions for plant growth. This study aimed at screening degraded banana plantation soils from Digbapia and Zokoguhé (Daloa, Côte d'Ivoire) for fluorescent *Pseudomonas* spp. able to tolerate saline stress. Isolates were recovered, assayed for extracellular enzymatic activities, and tested for growth across a NaCl and KNO₃ gradient up to 10%. Soil samples were collected from six degraded banana plantation sites. Fluorescent *Pseudomonas* spp. were isolated using culture techniques on media King's A and B. Isolates were subsequently assayed for amylase, cellulase, protease and lipase activities and evaluated by monitoring growth in media supplemented with NaCl and KNO₃ at concentrations ranging from 0 to 10%. Eight *Pseudomonas* species coded C226, C211, C127, C213, C212, C112R, C2211 and C129 were isolated from these soils. Among these isolates, C112R and C226 showed amylolytic activity, while C212, C213 and C127 showed cellulolytic activity. For high concentrations (8 to 10%), in KNO₃ between 791.27 and 989 mM then in NaCl between 1368.9 and 1711.1 mM, the isolates able to grow were C129, C213, C127, C2211 and C112R. These species are the best candidates for resistance to salt stress. They could serve as plant biostimulants under saline conditions, contributing to soil restoration.

Keywords: salinity, *Pseudomonas*, enzymes, degraded soil, agriculture

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1. Introduction

Sustainable agriculture is increasingly recognized as an essential approach for addressing global challenges such as food security, environmental degradation, and climate change [1]. This focuses on multiple interlinked factors, with dynamic soil microbial activity playing a pivotal role in enhancing soil fertility and health, crop productivity and reinforcing ecosystem resilience [2,3].

In many global south countries particularly Côte d'Ivoire, food security remains a critical challenge owing to a rapidly growing population with 2.5% rate [4]. According to projections by the National Statistics Agency (Anstat) and the World Health Organization (WHO), Côte d'Ivoire's population is expected to rise from 31.7 million in 2025 [4] to nearly 56 million by 2050 [5], so almost doubling in a quarter-century. Regarding the

significant growth rate, there is an imperative to boost food production both quantitatively and qualitatively. However, meeting this demand is often hampered by constraints. Indeed, over the last five years, yields of key staple crops particularly cassava and plantain have declined across major production zones in Côte d'Ivoire, driving up prices of their derivative products. These crops are furthermore subject to numerous biotic and abiotic threats in the areas where they are produced [6]. This decline in agricultural productivity can generally be attributed to various factors, including soil degradation, climate change and pest pressure. With regard to the factors limiting agricultural productivity, the issue of soil quality deserves special attention because of the many ecosystem services such as supply, regulation, it provided [7].

Soil degradation is a multifaceted process driven by interacting biophysical and socio-economic forces. It appears as declines in soil quality and in associated water, fauna, and flora resources, and it impairs essential

ecosystem services such as carbon storage and water purification. This deterioration lowers soils' productive capacity and the overall utility of natural resources [7]. Since the twentieth century, pressures to achieve food self-sufficiency have prioritized ever-greater, faster production, often at the environment's expense. Practices associated with this push namely agricultural intensification and uncontrolled domestic and industrial waste disposal have accelerated soil decline and promoted processes such as salinisation [8].

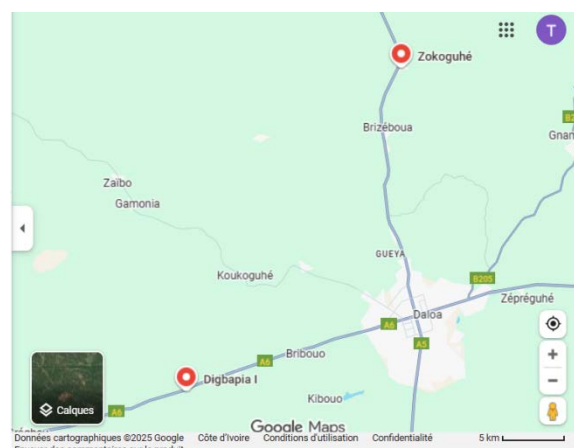
Salinisation process, whether arising from natural sources like geological material, groundwater, and surface water, or from human activities such as irrigation and excessive fertilization, raises soil salt concentrations and undermines soil function. High salt content reduces microbial activity and creates conditions hostile to plant growth, with clear negative impacts on agricultural productivity and ecosystem health [8,9]. Soil is a living system rich in microbial diversity that play an essential role in all biogeochemical processes, particularly those affecting the carbon and nitrogen cycles. Bacteria members of the genus *Pseudomonas* function as plant growth promoting rhizobacteria by mobilizing nutrients (nitrogen, phosphorus, and minerals), modulating plant hormones, inducing plant defense responses, and producing phytoprotective siderophores [10]. Exploiting the biological and chemical properties of these strains under saline conditions can increase crop resilience and productivity with low ecological impact, aligning with organic farming in a sustainable manner. This study, forming part of efforts to improve crop production in degraded and saline soils aims to identify biostimulant microorganisms, particularly fluorescent *Pseudomonas* spp. that are able to tolerate and grow under extreme abiotic stresses. Specifically, the objectives were isolating fluorescent *Pseudomonas* spp. in degraded agricultural soils, researching the enzymatic activities (cellulolytic, amylolytic, proteolytic, lipolytic) of the isolates, evaluating the growth of the isolates obtained under various concentrations of NaCl and KNO₃.

2. Materials and Methods

2.1. Study Site

The study was conducted in the villages of Digbapia (6°50'N, 6°38'W) and Zokoguhé (7°47'N, 6°27'W), located in the department of Daloa in central west of Côte d'Ivoire (Figure 1). These villages are among the main agricultural areas supplying Daloa with foodstuffs. Daloa, capital of the Hautassandra region, covers 15,205 km² and has a population of 421,879. The climate in Daloa and its surrounding villages Digbapia and Zokoguhé is humid tropical, with annual rainfall of 1,200 to 1,600 mm and two rainy seasons (April to mid-July and mid-September to November) separated by two dry periods (December to March and mid-July–mid-September) [11,12]. Vegetation consists of formerly dense forest now degraded by the expansion of cash crops such as cocoa, coffee, oil palm and rubber, and by uncontrolled exploitation of forest species [13]. Soils are ferralitic with a thin humus horizon,

high organic matter content, and are generally suitable for a wide range of crops [14].



Caption :  Sampling sites

Figure 1. Study site at village of Digbapia located at 6°50'N, 6°38'W and Zokoguhé at 7°47'N, 6°27'W ((A) Map of Côte d'Ivoire and (B) Map of the department of Daloa) [15]

2.2. Materials

The study material comprised degraded soil samples collected from banana plantations in the villages of Digbapia and Zokoguhé. The soil had a compacted appearance, and plant growth was slow.

2.3. Methods

2.3.1. Soil Samples Collection

Sampling was carried out in February 2024 on two banana plantations: site S1 (Digbapia) and site S2 (Zokoguhé). Each plantation covered areas ranging from 2 to 5 hectares. At S1, three soil samples (S1.1, S1.2, S1.3) were collected at points P1.1 (N 6°49'40.467", W 6°38'11.0886"), P1.2 (N 6°49'39.90792", W 6°38'16.6632") and P1.3 (N 6°49'39.06588", W 6°38'11.2668"), with sampling points spaced at least 100 m apart. At S2, three soil samples (S2.1, S2.2, S2.3) were collected at points P2.1 (N 7°43.99216", W 6°27'55.78524"), P2.2 (N 7°2'59.99216", W 6°27'55.78524") and P2.3 (N 7°2'58.25112", W 6°28'1.92792") using the same procedure as at S1. At each point the soil surface was cleared of plant and animal debris and a sample of approximately 500 g was taken at a depth of 15–20 cm using a hoe. Samples were placed in sterile plastic bags, labelled, and transported to the laboratory for analysis.

2.3.2. Preparation of Culture Media

Culture media used for analysis, namely buffered peptone water, King A, King B, plate count agar (PCA), Kligler-Hajna, milk agar and carboxymethyl cellulose (CMC) agar were prepared according to the manufacturers' instructions

2.3.3. Microbial Isolation

Preparation of stock solutions, inoculation of agar plates, and cultivation and quantification of microorganisms were

carried out according to ISO 6887-2: 2017 and to Yusran [16] method with some modifications. Practically, 10 g of the soil samples were suspended in 90 ml of sterile buffered peptone water (Difco, Becton Dickinson, Sparks, MD, USA), stirred for 30 minutes, then allowed to stand for 10 minutes. Tenfold serial dilutions of soil suspension, ranging from 10¹ to 10⁵, were prepared, spread on King A and King B and then all incubated at room temperature for 48 hours for isolation and determination of microbial counts. After isolation from each soil sample, presumptive colonies of *Pseudomonas* sp. bacteria with fluorescent appearances and exhibiting distinct macroscopic characteristics were taken with an ose needle and purified by placing them in 5 mL of sterile water and making a suspension and then by streaking onto King A and King B agar plates. Petri dishes were incubated for 48 hours at room temperature and colonies that formed were identified. Confirmation and identification of *Pseudomonas* species was carried out through a series of morphological observations and conventional biochemical reactions. Several characteristics such as morphology, coloration, catalase and oxidase reactions, respiratory type, and ability to ferment glucose on Kligler-Hajna media were examined on purified isolates.

2.3.4. Enzymatic Activity Screening in *Pseudomonas* Isolates

Certain enzymes such as cellulase, amylase, protease, and lipase, important for soil organic matter degradation were screened in bacterial isolates identified as *Pseudomonas*. Detection media were agar supplemented with carboxymethylcellulose (CMC) for cellulase, Gause agar with starch for amylase, skimmed milk agar for protease, and Sierra agar supplemented with Tween 20 for lipase. Prepared plates were surface inoculated using spots from 24 hour liquid cultures of each isolate and incubated at 30 °C for 24 hours. After incubation, enzymatic activities were revealed as follows:

Cellulase and amylase: Plates were flooded with Lugol's iodine for 30 seconds; a clear halo around colonies indicated substrate hydrolysis. For amylase, an intact starch layer produced a blue black colour with iodine, whereas starch hydrolysis produced a clear zone.

Protease and lipase: Activity was indicated directly by clear halos around colonies on skimmed milk agar and Sierra agar with Tween 20, respectively.

The diameter of lysis zones was measured and used to compare and select the most active isolates.

2.3.5. Evaluation of the Growth of Fluorescent *Pseudomonas* spp. in Saline Conditions

The effect of salt on the growth of fluorescent *Pseudomonas* isolates was evaluated on King B agar supplemented with NaCl or KNO₃ at 0%, 2%, 4%, 6%, 8% and 10%. King B plates containing each salt concentration were prepared and streaked with 24-hour bacterial cultures. Plates were incubated at 37°C for 24 hours. After incubation, growth at each salt concentration was compared to the control and quantified according to the method observed by Ouina et al. [17] with some modifications (Table 1). The percentages 0%, 2%, 4%, 6%, 8% and 10% correspond respectively for NaCl to the molarities 0 mM, 342.2 mM, 684.4 mM, 1026.7 mM,

1368.9 mM and 1711.1 mM, and for KNO₃ to 0 mM, 197.82 mM, 395.63 mM, 593.45 mM, 791.27 mM and 989 mM.

Table 1. Growth quantification grid for *Pseudomonas*

Growth intensity of isolates compared to that of the control	Number assigned
No growth	0
Growth of isolates less than 50%	1
Growth estimated at 50% of the control	2
Growth greater than 50%	3

2.3.6. Statistical Analysis

The microbial count data obtained in the laboratory were analysed using Statistica 7.1 (StatSoft, Tulsa, USA). A one-way analysis of variance (ANOVA) was performed to compare mean microbial loads between soil samples and between sampling sites. Significant differences were evaluated at the 5% level ($P < 0.05$) using Fisher's least significant difference (LSD) test.

3. Results

3.1. Bacterial Load in Soil Samples

The abundance of *Pseudomonas* and related genera shown in Figure 2 was evaluated in soil samples collected from sites S1 and S2. Bacterial loads differed between soil samples while remaining within homogeneous groups ($P < 0.05$). Soil sample S1.2 contained the highest load at 9.8×10^6 CFU/g, whereas the lowest load was observed in sample S2.3 at < 1 CFU/g. Furthermore, soils from sites S1 and S2 contained on average 6.1×10^6 CFU/g and 3.2×10^6 CFU/g of presumptive *Pseudomonas*, respectively.

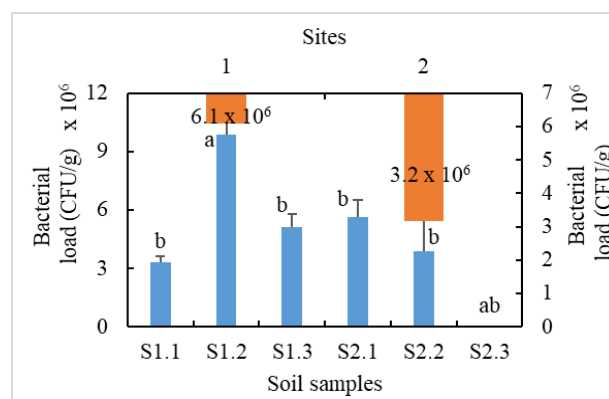


Figure 2. Abundance of *Pseudomonas* and related genera in soil samples from sites S1 and S2. Values are expressed as CFU/g; bars with different letters indicate significant differences ($P < 0.05$). Error bars represent standard deviation

3.2. Diversity of *Pseudomonas* spp. in Soil Samples from Sites S1 and S2

After isolation on King's A and King's B media from soil samples, 15 presumptive *Pseudomonas* colonies were selected based on distinct macroscopic characteristics. Isolates were described by colony appearance, gram reaction, fluorescence, oxidase and catalase reactions, pigment production, motility, and other biochemical tests

used for preliminary identification. From these 15 presumptive *Pseudomonas* bacteria, 8 isolates showed the morphological, cultural and biochemical characteristics summarized in Table 2. These isolates, designated C226, C211, C127, C213, C212, C112R, C2211, and C129, are listed in Table 3.

3.3. Enzymatic Activities of Isolated *Pseudomonas* spp.

Amylolytic, cellulolytic, proteolytic, and lipolytic activities were assessed for each of the eight *Pseudomonas* isolates. Only strains C112R and C226 showed amylolytic activity, with enzyme indices of 0.9 and 1.1 respectively, while cellulase activity was detected in C212, C213, and C127, with enzyme indices of 1.3, 1.3, and 1.25 respectively (Figure 3; Table 4).

Table 2. Characteristics of presumptive *Pseudomonas* spp. isolates

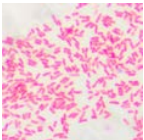
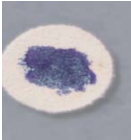
				
Gram-negative bacillus	Catalase positive	Oxydase positive	Strict aerobe	Glucose not degraded

Table 3. *Pseudomonas* spp. isolates from the investigated sites

Sites	Isolates
Site S1	<i>Pseudomonas</i> C127, <i>Pseudomonas</i> C112R, <i>Pseudomonas</i> C129
Site S2	<i>Pseudomonas</i> C226, <i>Pseudomonas</i> C211, <i>Pseudomonas</i> C213, <i>Pseudomonas</i> C2211, <i>Pseudomonas</i> C212

Table 4. Enzymatic activities screened in the different strains

Isolates of <i>Pseudomonas</i>	Activities					
	Amylolytic		Cellulolytic		Proteolytic	Lipolytic
	EA	EI	EA	EI	EA	EA
C112R	+	0.9	-		-	-
C2211	-		-		-	-
C212	-		+	1.3	-	-
C129	-		-		-	-
C213	-		+	1.3	-	-
C127	-		+	1.25	-	-
C211	-		-		-	-
C226	+	1.3	-		-	-

EA: enzymatic activity; EI: enzymatic indice; + and -: presence and absence of EA

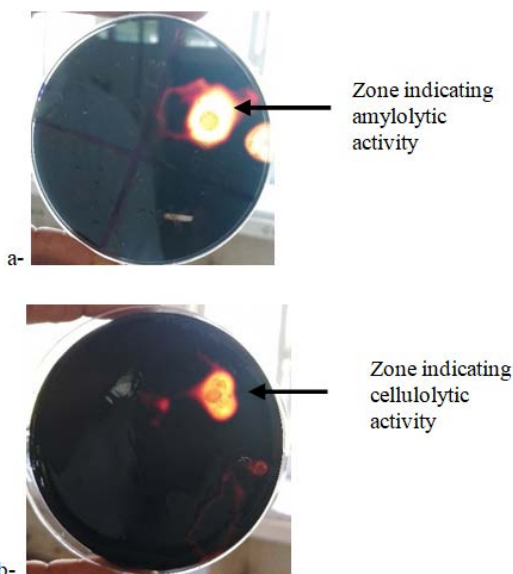


Figure 3. Enzymatic activities detected in the various isolated *Pseudomonas* species (a- Amylolytic activity, b- Cellulolytic activity)

3.4. Tolerance of *Pseudomonas* spp. Isolates to NaCl and KNO₃

Isolates C226, C211, C127, C213, C212, C112R, C2211 and C129 were cultivated with varying concentrations of NaCl (0 mM, 342.2 mM, 684.4 mM, 1026.7 mM, 1368.9 mM and 1711.1 mM) and KNO₃ (0 mM, 197.82 mM, 395.63 mM, 593.45 mM, 791.27 mM and 989 mM) and showed different tolerance levels. Growth quantification is provided in Figure 4, and the effects of NaCl and KNO₃ on the growth of *Pseudomonas* spp. from sites S1 and S2 are presented in Figures 5 and 6 respectively.

In presence of NaCl, Evaluation of survival of the eight *Pseudomonas* spp. isolates grown with NaCl showed decreased survival beginning at 684.4 mM for C2211, C211, C226, C213 and C217, and at 1026.7 mM for the other isolates. Complete growth inhibition occurred for C226 at 1368.9 mM and for C129, C213 and C127 at 1711.1 mM. C2211 and C112R exhibited the lowest inhibition (33.33%) at the highest NaCl concentrations tested (1368.9 mM and 1711.1 mM). None of the NaCl concentrations completely eliminated isolates C212, C211, C2211 or C112R.

In presence of KNO₃, all isolates except C129 grew at tested salt concentrations, but growth levels varied. C2211, C112R and C212 were inhibited at KNO₃ concentrations of 593.45 mM and above, while C226, C211, C127, C213 and C129 were sensitive at the lower concentration of 197.82 mM, with initial inhibition of 33% relative to the control. Only C129 experienced complete growth inhibition at 593.45 mM; none of the other isolates were completely eliminated by the KNO₃ concentrations tested. At the highest KNO₃ concentrations (791.27 mM and 989 mM), C2211 and C112R exhibited the lowest inhibition rates, at 33.33%.

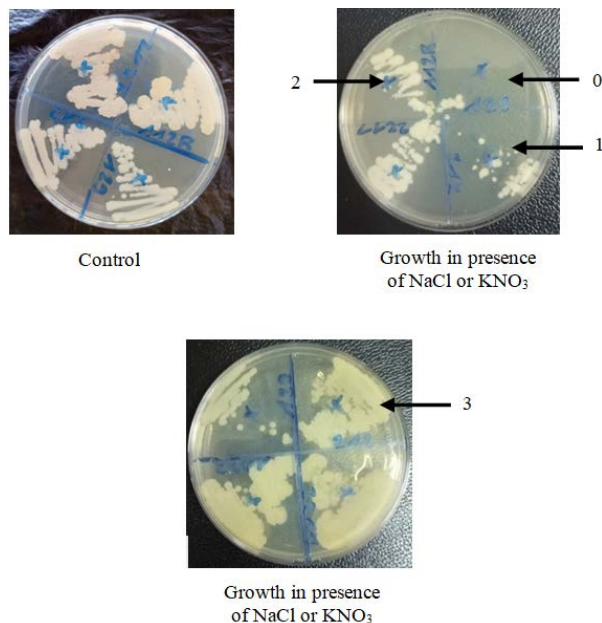


Figure 4. Quantification of the growth level of *Pseudomonas* spp. isolates relative to the control (growth on King's B with 0% NaCl or KNO₃)

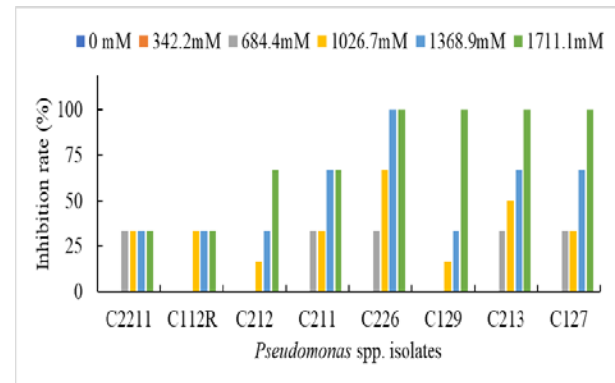


Figure 5. Effect of different NaCl concentrations on *Pseudomonas* growth

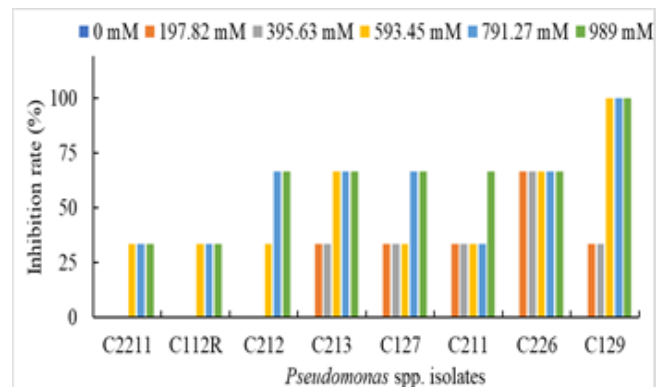


Figure 6. Effect of different KNO₃ concentrations on *Pseudomonas* growth

4. Discussion

Soil salinization is a process characterised by the progressive accumulation of soluble salts in the soil profile, resulting from a combination of anthropogenic and natural factors. Various contributors include intensive agricultural practices such as irrigation with saline groundwater or surface water and excessive fertilization alongside uncontrolled domestic and industrial effluent discharge and the degradation of geological substrates. This phenomenon, whether of natural origin or induced by human activity, leads to a significant decline in soil microbial activity, thereby creating suboptimal conditions for plant growth and productivity [8-9]. In the context of climate change and the increasing need for sustainable agricultural systems, the application of biostimulant micro-organisms has emerged as a promising strategy. Among these, *Pseudomonas* spp. capable of exhibiting notable adaptability to saline stress through physiological and metabolic modulation, making them valuable allies in promoting plant resilience and maintaining soil health under salinized conditions.

The isolation of *Pseudomonas* spp. and the assessment of their abundance in soil samples S1.1, S1.2, S1.3, S2.1, S2.2 and S2.3 revealed significant differences ($P < 0.05$) in bacterial abundances that can be attributed to variability in management between sites. Agricultural practices such as intensive cultivation and crop succession that can

differently affect the soil microbial community. Such practices disrupt soil microbial balance by reducing microbial biodiversity and increasing soil vulnerability [18,19]. The presence of bacteria *Pseudomonas* spp. in the soils of sites S1 and S2 is related to their saprophytic nature of agricultural soils [20]. The mean load of *Pseudomonas* spp. in the site S1 (6.1×10^6 UFC/g) and site S2 (3.2×10^6 UFC/g) are close to that (4.1×10^6 UFC/g) revealed by Aagot et al. [21] in a polycyclic aromatic hydrocarbon (PAH)-contaminated soil. These bacterial isolates are involved in bioremediation processes, contributing to the degradation of environmental pollutants and the restoration of soil health [22].

Using morphological traits, respiratory enzyme activity, and glucose assimilation profiles, eight *Pseudomonas* isolates coded C226, C211, C127, C213, C212, C112R, C2211, and C129 were identified from bacterial colonies grown on King's A and B media, derived from soil samples collected at sites S1 and S2. *Pseudomonas* spp. are widely recognized as native inhabitants of agricultural soils, particularly within the rhizosphere, where they represent one of the most prominent bacterial groups. Their presence in the rhizosphere of various crops, including banana, soybean, and maize, has been well documented [23,24,25]. Among them, fluorescent *Pseudomonas* strains are known for their mutualistic interactions with plants, contributing to growth, nutrition, and disease suppression through diverse phyto-beneficial mechanisms [10]. The detection of these strains in soils from S1 and S2 underscores their agronomic relevance and potential role in supporting crop productivity in these environments.

The eight *Pseudomonas* strains isolated from soil samples were evaluated for their cellulolytic, amylolytic, proteolytic, and lipolytic activities. The enzymes responsible for these functions are of agronomic importance, as they contribute to the mineralization of organic matter and enhance nutrient availability for plants within agroecosystems. None of the isolates exhibited proteolytic or lipolytic activity, indicating an absence of protease and lipase production. This finding contrasts with existing literature that highlights the enzymatic potential of *Pseudomonas* species to produce proteases and lipases [24,26,27]. This may be due to an inherent inability of the strains to produce these enzymes, potentially resulting from genetic variability. Regarding cellulase and amylase activity, cellulolytic activity was observed exclusively in strains C212, C213, and C127, while strains C112R and C226 demonstrated amylolytic activity. The latter suggests their ability to hydrolyze starch, the primary storage polysaccharide in plants. According to Voisin et al. [28], amylase activity plays a critical role in energy production required for nitrogen fixation in root nodules. These results are consistent with findings by Klinfoong et al. [29], who reported amylase production in *Pseudomonas* strains isolated from mangrove soils in Thailand.

The cellulolytic activity of *Pseudomonas* strains C212, C213, and C127 was confirmed through the degradation of carboxymethyl cellulose (CMC). The enzyme responsible for this reaction, cellulase, facilitates the breakdown of organic matter, leading to humus formation and inhibition of spore germination [30]. Cellulose is

quantitatively the most abundant polysaccharide among carbon sources. Its decomposition yields simple sugars that are readily utilized by most soil microorganisms, playing a key role in soil fertilization processes [31]. The presence of these cellulolytic and agronomically valuable isolates C212, C213, and C127 represents a beneficial asset for the soils at sites S1 and S2. Similar findings were reported by Goel et al. [32], who identified cellulase activity in *Pseudomonas* strains isolated from landfill soils in India.

According to Adjanohoun et al. [33], microorganisms act as the "terrestrial engine" driving all biogeochemical processes. In the rhizosphere, they perform a wide array of essential functions, including the decomposition of plant, animal, microbial residues, and organic waste through the breakdown of carbon sources (e.g., lignolysis, cellulolysis), the synthesis of humus (stable and bound organic matter), the mineralization and immobilization of nitrogen, sulfur, and phosphorus, and the enhancement of soil structure through aggregate stabilization. In the present study, 5 out of the 8 *Pseudomonas* isolates recovered from soil samples namely C212, C213, C127, C112R, and C226 exhibited enzymatic activities of agronomic interest. Harnessing the functional potential of these strains under saline conditions may offer promising solutions for improving crop productivity and soil health within the framework of sustainable agriculture.

The tolerance assessment of the eight *Pseudomonas* isolates cultured in the presence of NaCl and KNO₃, as shown in Figures 6 and 7, revealed a relative variability in salt tolerance among the strains. At the highest concentrations of KNO₃ (791.27 mM [8%] to 989 mM [10%]) and NaCl (1368.9 mM [8%] to 1711.1 mM [10%]), the isolates capable of growth included C129, C213, C127, C2211, and C112R. These isolates exhibit higher tolerance to NaCl than *Pseudomonas* strains isolated from the onion rhizosphere, which grow at NaCl concentrations between 0 and 0.51 M [34]. According to [35], *Pseudomonas* spp. respond to saline stress through diverse mechanisms, notably by producing osmoprotectants such as glycine betaine, proline, etc, by contributing to indirect antioxidant defense by limiting the production of reactive species associated with salt stress. Notably, three of these strains also showed enzymatic activities of agronomic interest. Considering the role of soil salinity as a major constraint on agricultural productivity [36], *Pseudomonas* strains C129, C213, C127, C2211, and C112R exhibited traits consistent with resistance to saline stress under degraded soil conditions.

5. Conclusion

To address the challenges of sustainable agriculture in extreme environments, this study aimed to identify biostimulant microorganisms, particularly *Pseudomonas* spp., capable of thriving under saline conditions. In this context, bacterial load assessments in degraded soils from the study sites in Daloa revealed 6.1×10^6 CFU/g at site S1 (Digbabia) and 3.2×10^6 CFU/g at site S2 (Zokoguhé). Eight *Pseudomonas* spp. isolates coded C226, C211, C127, C213, C212, C112R, C2211, and C129 were recovered from these soils. Among them, strains C112R and C226

exhibited amylolytic activity, while C212, C213, and C127 demonstrated cellulolytic activity. Under high salt concentrations ranging from 8% to 10%, corresponding to 791.27 nM-989 mM KNO₃ and 1368.9mM–1711.1 mM NaCl, the isolates capable of growth included C129, C213, C127, C2211, and C112R. These strains are considered promising candidates for resilience to salt stress in degraded soils, with potential applications (biostimulation, biorestauration, etc), in sustainable crop production systems.

Declaration of Conflict of Interest

The authors have not declared any conflict of interests.

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