International Journal of Agriculture, Environment and Biotechnology
Citation: *IJAEB*: **18**(03): 189-200, September 2025
DOI: 10.30954/0974-1712.03.2025.3

ASSOCIATION FOR AGRICULTURE ENVIRONMENT AND BIOTECHNOLOGY

RESEARCH PAPER

Identification and Consortium Development of Halophilic Bacteria for Biofertilizer- Based Reclamation of Sodic Soils

Vaishali. E. Sonawane[1*], Sunita. S. Ghumare[2] and Agnes. S. Kharat[3]

[1]Department of Microbiology, MVP's K.A.A.N.M. Sonawane Arts, Science and Commerce College, Satana, SPPU, Pune, India
[2]Former Principal, MVP's Shri S.S.M Arts, Science and Commerce College, Saikheda, SPPU, Pune, India
[3]Department of Zoology, MGV's Loknete Vyankatrao Hiray Arts, Science & Commerce College (Autonomous), Nashik, India

*Corresponding author: ahirevaishali1984@gmail.com (**ORCID ID:** 0000-0002-6555-4798)

Paper No. 1216 **Received:** 11-07-2025 **Revised:** 06-08-2025 **Accepted:** 30-08-2025

ABSTRACT

Sodic soils are one of the biggest limitations to the production of various foods around the globe, soil decay, nutrient cycling as well as microorganisms' equilibrium. This paper describes the isolation, characterization, and consortium development of native halophilic bacteria to be used in biofertilizer-mediated sodic soil reclamation in Maharashtra, India. *Enterobacter cloacae*, *Enterobacter* sp. and *Klebsiella* sp were 16S rRNA sequenced and assessed in terms of their halotolerance, enzyme activity, and compatibility among themselves. The resultant consortium had a high growth promoting properties of plants which comprised of fixation of nitrogen, solubilization of phosphate and secretion of exopolysaccharide (EPS) in salty environment. Field work on soybean (*Glycine max* L. cv. Phule Sangam) showed that there were impressive agronomic and soil enhancements: pod and seed counts rose by more than 300%, seed weight had quadrupled, and soil pH and exchangeable sodium percentage were reduced by 40-50%. Increased availability of nitrogen, phosphorus, and potassium as well as increased content of organic carbon, all signified nutrient regeneration and alleviation of sodicity through the activity of microbes. The osmotic stress-tolerant functional resilience of the consortium indicates synergistic roles of hormonal signaling and ionic homeostasis regulation in the rhizosphere. A biosafety of antibiotic sensitivity was established and this makes it a good fit in the field. This paper has defined native halophilic microbial consortia as an effective and economical alternative to the use of gypsum reclamation and it is also an environmentally friendly reclamation that is low-cost. These consortia fit the FAO and ICAR models of resilient agroecosystems in salt-impacted areas by enhancing the crop production capacity of soils through reestablishment of soil biological activity and implementation of microbial biotechnology.

HIGHLIGHTS

- *Enterobacter cloacae, Enterobacter* sp., and *Klebsiella* sp. were isolated, 16S rRNA sequenced, and tested for halotolerance, enzyme activity, and compatibility to form a stable microbial consortium.
- Field trials on soybean showed >300% increase in pod and seed counts, 4–5× higher seed weight, and significant biomass accumulation compared to controls, demonstrating strong plant growth-promoting traits.
- Application of the consortium reduced soil pH and exchangeable sodium percentage by 40–50%, improved nutrient availability (N, P, K, organic carbon), and provided an eco-friendly, low-cost alternative to gypsum reclamation.

Sodic soils, which are sometimes referred to as the silent productivity killers, are a serious constrain to agriculture globally (Demo *et al*. 2025). They improve soil structure, interfere with nutrient availability, and limit water infiltration, all of which threaten food and livelihood security (Kumar

How to cite this article: Sonawane, V.E., Ghumare, S.S. and Kharat, A.S. (2025). Identification and Consortium Development of Halophilic Bacteria for Biofertilizer- Based Reclamation of Sodic Soils. *Int. J. Ag. Env. Biotech.*, **18**(03): 189-200.

Source of Support: None; **Conflict of Interest:** None

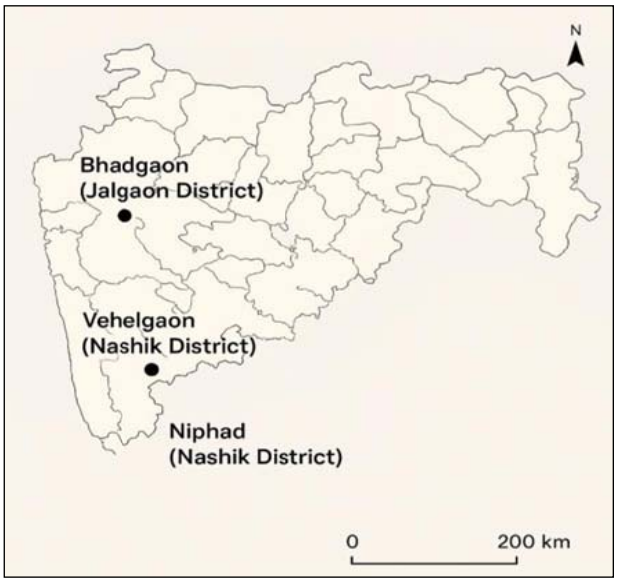
& Sharma, 2020). It has been estimated recently that there are close to 1 billion hectares of salt-affected land in the world, with the sodic soils representing approximately a quarter of this total area (FAO, 2021). There are 6.73 million hectares of salt-affected soil, and 3.8 million hectares of sodic soil in India alone (ICAR-CSSRI, 2020). Over 1.2 million hectares of such degraded lands are found in Maharashtra including the Nashik and Jalgaon districts,where losses in crop yields can reach 30 to 80% (Romano-Armada *et al.* 2020; ICAR-NBSS & LUP, 2022). Statistics like this point to both the agronomic challenge and the urgency of identifying sustainable solutions that go beyond short-term chemical solutions. Conventional methods of sodic soil reclamation, including amendment with gypsum and chemical conditioners, enhance the pH and exchangeable sodium percentage (ESP) but are costly, resource-consuming, and usually have very short-term implications (Ashenafi WorkuDaba, 2025). They do not recover biological soil health, which is an essential ingredient tolong-term productivity. This knowledge gap has led to a focus on biologically motivated approaches, with soil microbiomes, in particular halotolerant/halophilic bacteria, finding their way to becoming promising partners. Halophilic bacteria grow in saline-sodic environments where other microbial inoculants tend to fail. They mitigate sodium toxicity through their physiological properties such as the capacity to fix nitrogen, solubilize phosphate, generate plant growth regulators, and release exopolysaccharides, and enhance soil aggregation (Kapadia *et al.* 2021; Egamberdieva *et al.* 2019). Notably, microbial consortia with two or more strains of compatible strains are more likely to be better than single-strain inoculants in offering multifunctional advantages in stressful conditions. As an example, a co-inoculation experiment in sodic soils of Haryana enhanced rice grain yield by 28% and halophilic bioformulations in Uttar Pradesh decreased ESP by 25% and improved wheat productivity by 20-35% in two seasons (Chadha *et al.* 2024; Arora *et al.* 2021). This kind of evidence points to their scalability in large-scale reclamation programs. In Maharashtra, where sodicity limits the productivity of soybean and onion, which are two crops of economic importance, chemical amendments have not been sufficient. The reason why farmers are still reluctant to use gypsum is that it is expensive and the residual benefits are minimal. Halophilic biofertilizers are locally adapted, which may provide a sustainable solution, specific to soils and crops in the region. However, despite the positive laboratory and pilot research, there are still gaps in the achievement of stable, compatible microbial consortia and their validation by field experiment. The small amount of region-specific application data prevents microbial biofertilizers from becoming widespread in state and national agriculture policies. The economic cost of land degradation caused by salt all over the world is approximated to USD 27 billion per year (Qadir *et al.* 2014). In the case of India, each year, salinity and sodicity are causing the country to loseits crops to the tune of almost INR 11,000 crores (or USD 1.5 billion), and Maharashtra alone is losing millions of tonnes of production annually. This challenge requires a combination of environmentally friendly and economically viable solutions that are amenable to farmers and scientifically proven. Halophilic consortia based on biofertilizers provide exactly this route, being economically viable and environmentally sustainable. Recent literature highlights the synergies of the benefits of consortia. Damodaran *et al.* (2023) showed that halotolerant bacterial consortia increased tomato yields and decreased signs of sodicity in the field, whereas Singh *et al.* (2024) reported a 45% increase in yield with mustard growing in sodic soils using the same bioformulations. These results indicate that microbial consortia not only provide a positive microbial effect on crop development, but directly positively affect the quality of physico-chemical properties in the soil, providing a beneficial cycle of soil recovery and productivity increase. Nevertheless, the vast majority of studies are geographically limited, and they do not represent semi-arid areas such as Maharashtra where sodicity is complex with climatic stressors. The current research fills these gaps by isolating the halophilic bacteria in the sodic soils of Maharashtra, biochemically and molecularly characterizing them, and coming-up with compatible microbial consortia. Soybean field trials showed that there are significant improvements in plant growth, pod count, seed weight and biomass as compared to controls and yields doubled to four times. In addition, the soil quality indicators, including pH, ESP, sodium, etc., significantly improved, highlighting the dual use of halophilic consortia in crop production and

restoration of soil health. This study helps change the paradigm in the management of sodic soils by showing that native halophilic bacterial consortia have a practical potential as biofertilizers. In comparison to traditional chemical methodologies, microbial consortia have a long term advantage of restoring nutrient cycling and biological activity andare economically efficient and non-toxic. The policy implications of the findings are obvious: they can guide the introduction of biofertilizers into the agroclimatization programs supported by states, comply with the national policies to fight antimicrobial resistance by decreasing the reliance on agrochemicals, and enforce the adherence to the sustainability objectives established on the international level. Finally, halophilic bacterial consortia can be considered a scientifically sound and cost-efficient approach to sodic soil reclamation. The fact that they can improve the output and at the same time improve the degraded soils makes them are volutionary tool in sustainable farming. In some areas such as Maharashtra, where sodicity poses a threat to food security and farmer livelihood, the use of such microbial biofertilizers is not just an option, but an urgent need.

MATERIALS AND METHODS

Study Area and Sample Collection

The soil samples were collected from three locations in Maharashtra, India, Bhadgaon (Jalgaon Distrct), Vehelgaon (Nashik Distrct) and Niphad (Nashik Distrct).



The top 15cm of soil was sampled with sterile spatulas and placed in soil sampling bag (Himedia, India). The samples were taken to the laboratory at ambient conditions and analyzed within 24 h. The pH of the soil was measured with a digital pH meter (1:2.5 soil-water suspension).

Isolation and Enrichment of Halophilic Bacteria

For isolation, 11-10 g of soil was suspended in 90 mL sterile distilled water, serially diluted and plated on Luria agar medium with 5% NaCl. Plates were incubated at 30 ± 2 °C for 48–72h. Morphologically distinct pure colonies were purified by repeated streaking on same medium. Enrichment was performed in Luria broth supplemented 5% NaCl at 30 °C with shaking at 150 rpm until visible growth was detected. Salt tolerance of the isolates was evaluated by culturing.

Morphological and Biochemical Characterization

The selected bacterial isolates were first examined for colony morphology, pigmentation, margin, elevation, and texture of the selected bacterial isolates were evaluated, and then the microscopic observation was made after Gram staining to identify cellular morphology and Gram reaction. To determine physiological and metabolic characteristics of the isolates, biochemical characterization was done as per standard microbiological procedures. Catalase and oxidase activity tests were used to detect the presence of oxidative enzymes,starch hydrolysi (amylase production), and a battery of carbohydrate fermentation and utilization tests to assess metabolic versatility. Moreover, isolates were tested to produce hydrogen cyanide (HCN), an indicator of biocontrol potential, and to produce indole, an indicator of tryptophan catabolism. The use of citrate was also performed to evaluate the capacity of isolates to use citrate as the only carbon source. The overall outcomes of these tests were correlated to standard descriptions available in the Manual of Determinative Bacteriology to assist with the initial characterization of the bacterial strains before proceeding with molecular characterization.

Molecular Identification via 16S rRNA Sequencing

Genomic DNA was extracted using cetyltrimethyl-ammonium bromide (CTAB) protocol with suitable inhouse modifications. Briefly, 1.5 mL overnight cultures were centrifuged (10,000 rpm, 5 min) and the pellet washed twice with Tris-EDTA buffer. Cells were lysed using lysozyme (20 µL, 100 mg/mL), SDS (40 µL, 10%), and proteinase K (8 µL, 10 mg/mL), followed by incubation at 37 °C for 1 h. DNA was purified through sequential treatment with NaCl, CTAB/NaCl, chloroform: isoamyl alcohol (24:1), and phenol: chloroform: isoamyl alcohol (25:24:1). Precipitation was achieved using chilled isopropanol (–20 °C, 30 min), followed by washing with 70% ethanol and resuspension in 20µL TE buffer. PCR amplification of the 16S rRNA gene was carried out using universal primers 27F and 1492R. Amplicons were sequenced commercially (Sai Biosystems Pvt. Ltd., Nagpur, India). Sequences were aligned using BLAST against the NCBI database, and phylogenetic analysis was performed using MEGA X software.

Antibiotic Sensitivity Testing

Antibiotic susceptibility of bacterial isolates was assessed by the Kirby Bauer disc diffusion technique in agreement with Clinical and Laboratory Standards Institute (CLSI) protocols,except that halophilic growth conditions were used. Fresh colonies (18-24 h old) were grown in nutrient broth with 5% NaCl at 30 ± 2 °C with shaking at 150 rpm until the logarithmic growth phase (OD600 ≈ 0.08–0.1). The resultant suspensions were brought to the turbidity of a 0.5 McFarland standard, which is equal to about 1×10^8 CFU/mL. A stable suspension of the desired optical density was prepared by adding 0.5 mL of 1.175% (w/v) barium chloridedihydrate ($BaCl_2 \bullet 2H_2O$) to 99.5 mL of 1% (v/v) sulfuric acid (H_2SO_4). The turbidity of the bacterial suspensions was visually compared with the standard under sufficient light, and corrections were made, where necessary, by means of sterile broth. Standardized inoculum were applied uniformly on the agar surface with the help of sterile cotton swabs, to achieve confluent growth and the plates were allowed to dry in 5 min after which commercially available antibiotic discs (HiMedia, India) were put in position. The antibiotics used were Penicillin (2 µg), Tetracycline (10 µg), Co-trimoxazole (25 µg), Cephalothin (30 µg), Erythromycin (10 µg), Levofloxacin (10 µg) and Cefuroxime (30 µg). Sterile forceps were used to place the discs aseptically with spacing at least 24 mm between discs. The plates were inoculated and allowed to incubate under aerobic conditions with an incubation temperature of 30 ± 2 °C up to a period of 18 to 24 h after which the dimensions of the inhibition zones were measured in millimetre using a digital Vernier caliper. All assays were repeated three times and the average zone diameters were determined. The interpretations of the results were Sensitive(S), Intermediate (I), or Resistant (R) based on CLSI (2023) interpretative criteria.

Biofertilizer Consortium Development

The compatibility between the selected halophilic bacterial isolates in relation to the formation of the biofertilizer consortium was tested and this led to the development of the biofertilizer consortium. To this end, isolates were streaked perpendicularly on nutrient agar plates that had been enriched with 5% NaCl and allowed to grow at 30 ± 2 °C after 48 h, and the inability to inhibit at the intersection zones confirmed compatible growth. The isolates were then grown separately in Luria broth containing 5% NaCl and 0.5% glycerol at 30 ± 2°C under shaking at 150 rpm until late logarithmic growth (OD 600 = 0.810) (density of 1×10^8 CFU/mL) was attained. The counts of colony-forming units were determined by serial dilution and spread plating on nutrient agar plates containing 5% NaCl. The standardized suspensions of compatible isolates were then pooled aseptically in a sterile flask and homogenized on a rotary shaker at 100 rpm during 10 min and adjusted to a final density of approximately 1×10^8 CFU/mL using sterile Luria broth with 5% NaCl. To prepare biofertilizer using carriers, 10%(v/v) bacterial consortium was inoculated into a sterile medium containing Luria broth, 5% NaCl and 0.5% glycerol and allowed to incubate for 24 h at 30°C and shake at 120 rpm.

Centrifugation of the culture broth was then done at 5,000 rpm over 10 min, and then the pellet was resuspended in sterile carrier medium in order to get a high CFU density. This formulation was aseptically transferred to sterile polypropylene containers, stabilized with 0.5-1% glycerol, and either stored at 4°C to be used in the short term

(<1 month) or in -20°C to be preserved over the long term. Viability was occasionally confirmed by subculture on nutrient agar with 5% NaCl added. To apply the biofertilizer suspension to the soybean seeds (*Glycine max* L., cv. Phule Sangam, KDS-726) in the field, the suspension was freshly prepared at approximately 1 × 108 CFU/mL and applied to the soybean seed by using 1% jaggery solution as an adhesive, in addition to soil drenching 8 days after sowing, with an inoculum volume of 50 mL per plot corner to ensure uniform distribution.

Field Trial

Evaluation of the on-field effectiveness of the halophilic bacterial consortium as biofertilizer, the field trial was carried out in a research plot of size 2 × 10 m within the *Kharif* season (July-October 2024). The test crop was Soybean (*Glycine max* L., cv. Phule Sangam, KDS-726). The distance between the seeds was set to 25-30 cm with 25 seeds per corner of the plots in accordance with general agronomic principles. Before sowing the soil physicochemical properties, such as pH, electrical conductivity (EC), organic carbon, available nitrogen, phosphorus, potassium, calcium carbonate, calcium, sodium, sodium adsorption ratio (SAR) and exchangeable sodium percentage (ESP) were determined by standard methods. The biofertilizer preparation, which had been standardized at about 1×10^8 CFU/mL, was administered as a seed treatment as well as a soil drench 8 days post-sowing to make certain that colonization occurred. Each treatment was replicated three times and untreated controls were kept under the same conditions as the experiments. At maturity, parameters of crop growth including the height of the plants, the number of leaves, the ratio of roots to shoots, the number of pods per plant, number of seeds per pod and seed weight were taken. Post harvest agricultural soil samples were also taken to test the variation in parameters of fertility and sodicity.

STATISTICAL ANALYSIS

All experiments have been conducted in triplicates and values were expressed as mean. Field trial data were analyzed using analysis of variance (ANOVA) in order to identify the significance of differences between treatments. The mean values of treatments where ANOVA showed significant difference were compared by Tukey post hoc test to provide statistical differentiation of groups. The level of probability of $p < 0.05$ was considered statistically significant

RESULTS

The biochemical profile of the three halophilic bacterial isolates indicated various similar and distinctive features (Table 1).

Table 1: Biochemical characterization of halophilic bacterial isolates

Test/Character	Isolate 41	Isolate 42	Isolate 43
Gram reaction	**Gram-negative rods**	**Gram-negative rods**	**Gram-negative rods**
Motility	+	+	+
Catalase	+	+	+
Oxidase	+	+	+
Starch hydrolysis	–	–	+
Glucose fermentation	Acid(A)	Acid + Gas (A+G)	Acid + Gas (A+G)
Lactose fermentation	Acid + Gas (A+G)	Acid + Gas (A+G)	Acid + Gas (A+G)
Maltose fermentation	Acid + Gas (A+G)	Acid + Gas (A+G)	Acid(A)
Sucrose fermentation	Acid + Gas (A+G)	Acid + Gas (A+G)	Acid + Gas (A+G)
Glucose utilization	+	+	+
Lactose utilization	+	+	+
Maltose utilization	+	+	+
Sucrose utilization	+	+	+
HCN production	+	–	+
Indole test	–	–	–
Methylred (MR) test	+	+	+
Voges–Proskauer (VP) Test	—	—	—
Citrate utilization	+	+	+

All isolates were Gram-negative rods that were motile, catalase positive, and oxidase positive. Patterns of carbohydrate utilization indicated that all the isolates fermented lactose and sucrose accompanied by acid and gas generation; however, the glucose and maltose fermentation presented variation. Isolate 41 formed acid only out of glucose, whereas isolate 42 and 43 formed acid and gas. In the same manner, isolates 41 and 42 generated acid and gas maltose, but the isolate 43 generated only acid. Regarding hydrolytic capacity, the hydrolysis

of starch was negative in isolates 41 and 42 but positive in isolate 43.

All the three isolates could use glucose, lactose, maltose, and sucrose as a source of carbon. There were also differences in secondary metabolic characteristics, with isolate 41 and 43 positive in the production of hydrogen cyanide and isolate 42 negative. All the isolates did not form indole or acetoin based on the negative outcome values of the indole and Voges Proskauer test, respectively. Nevertheless, the biochemical characterization of the three halophilic bacterial isolates showed a number of comparable and discriminative features. All isolates were Gram-negative rods that were motile, catalase positive, and oxidase positive. Patterns of carbohydrate utilization indicated that all the isolates fermented lactose and sucrose accompanied by acid and gas generation; however, the glucose and maltose fermentation presented variation. Isolate 41 formed acid only out of glucose, whereas isolate 42 and 43 formed acid and gas. In the same manner, isolates 41 and 42 generated acid and gas maltose, but the isolate 43 generated only acid. Regarding hydrolytic capacity, the hydrolysis of starch was negative in isolates 41 and 42 but positive in isolate 43. All the three isolates could use glucose, lactose, maltose, and sucrose as a source of carbon. There were also differences in secondary metabolic characteristics, with isolate 41 and 43 positive in the production of hydrogen cyanide and isolate 42 negatives. All the isolates did not form indole or acetoin based on the negative outcome values of the indole and Voges Proskauer test, respectively. But, the methyl red test was positive with all the isolates, indicating that they produce acids stably, and the isolates all used citrate as a source of carbon.

Molecular Identification via 16S rRNA Sequencing

The phylogenetic tree is used to outline the evolutionary connection between different Enterobacter cloacae strains under the basis of sequence homology. The isolate VK2 (highlighted in green) is closely related to *E. cloacae* strain HBUAS56246 (MT229711.1) and strain EO4 (OQ999351.1), with moderate bootstrap values (63 75%), which suggests a strong shared evolutionary lineage and a high level of genetic similarity between the isolate and these strains (Fig. 1). Clustering highly argues that VK2 is a part of the *E. cloacae* complex which seems to be congruent especially with strains that possess conserved genomic regions typical of environmental and clinical isolates. The further separation of other strains of *E. cloacae* (e.g., CP039303.1, CP103611.1) indicates a significant level of genetic differentiation in the species, and this may be due to ecological selection or host-specific evolution. Its phylogenetic relationship with VK2 is supported by the overall tree topology, the bootstrap support values and supports the taxonomic placement of VK2 as Enterobacter cloacae and suggests the possible functional or ecological similarities of VK2 to the reference strains.

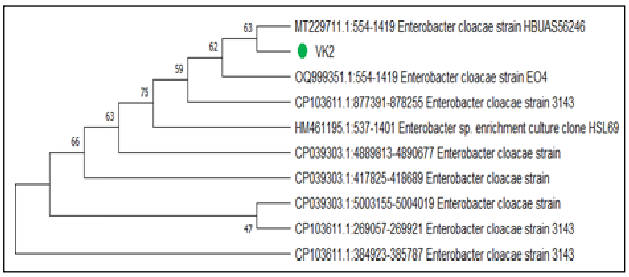


Fig. 1: Phylogenetic tree of isolate VK2

The phylogenetic tree of V1 isolate indicates its evolutionary position among the representatives of the family Enterobacteriaceae. The isolate has the closest relationship with the *Enterobacter* sp. strain of F32 (GenBank: MW82674.1) and the relationship is moderately supported with a bootstrap of 54. This correlation indicates that V1 has a high-level sequence similarity with the species of Enterobacter (Fig. 2).

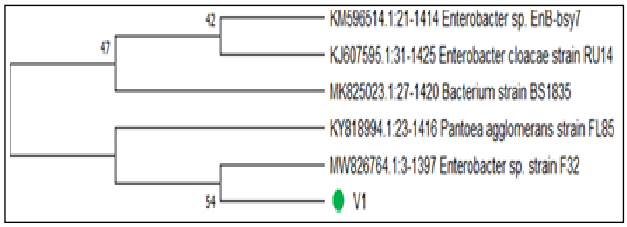


Fig. 2: Phylogenetic tree of isolate V1

The V1 -*Enterobacter* sp. F32 cluster is additionally related to *Pantoea agglomerans* strain FL85 (KY81894.1) and a number of Enterobacter strains, including *E. cloacae* strain RU14 (KJ607595.1) and *Enterobacter* sp. EnB-bsy7 (KM596514.1), which form a coherent subclade based on Enterobacteriaceae. The relatively small branch lengths separating these taxa show a low level of genetic separation which is in line

with the close evolutionary relationship. These results are highly indicative of isolate V1. being an Enterobacter species, possibly an *E. cloacae* or close relative thereof, as part of the Enterobacter Pantoea complex.

The phylogenetic tree of isolate VK3 shows that the isolate is clearly placed in the genus Klebsiella. VK3 indicates that it is most closely related to *Klebsiella* sp. strain WS (MN420814.1) with a bootstrap value of 100 signifying a high level of statistical confidence in this grouping. Such a close relationship implies that almost the same sequences of the 16SrRNA genes which implies that VK3 is either a strain of *Klebsiella* sp. WS or a very closerelative. VK3 *Klebsiella* sp. WS branch is enclosed in a bigger Klebsiella clade that encompasses *K. granulomatis* (MK918565.1), *K. pneumoniae* strain Aimst P2 (JX131618.1)and *Klebsiella* sp. strain In AD-061 (MF401267.1) (Fig. 3). The upward trend of progressive branching, whereby the bootstrap value is between 59 and 100, indicates that there is a high phylogenetic coherence within the genus. These data are supportive of the fact that isolate VK3 is a genus Klebsiella, which has a very strong affinity with *Klebsiella* sp. WS and other clinicalor environmental strains.

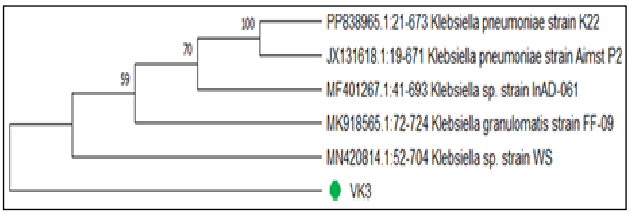


Fig. 3: Phylogenetic tree of isolate VK3

Antibiotic Sensitivity Testing

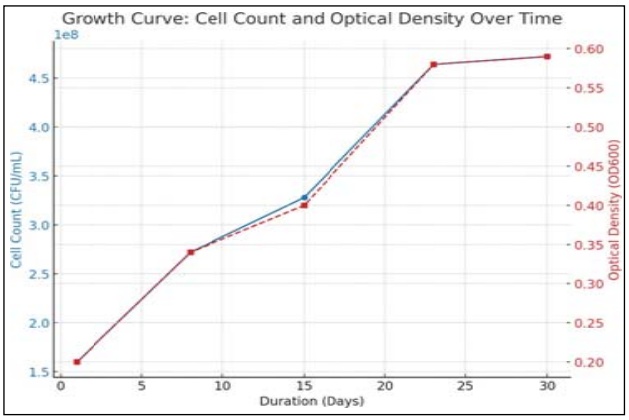
Profiling of antibiotic susceptibility of isolates 41, 42 and 43 indicated that they had multidrug resistance patterns with significant inter-isolate differences (Table 2). Isolate 41 was tetracycline-resistant, co-trimoxazole-resistant, cephalothin-resistant, erythromycin-resistant, and cefuroxime-resistant, but penicillin-sensitive, cloxacillin-sensitive, and levofloxacin-sensitive. On the other hand, isolates 42 and 43 were more broadly resistant and all tested antibiotics were positive, suggesting potent mechanisms of adaptive resistance that may be associated with the activity of 8 -lactamase and tolerance in efflux. The isolate-specific susceptibility of isolate 41 indicates the existence of strain-specific genomic determinants that shape the response to antibiotics, which explain the dire need of molecular characterization and antibiotic stewardship in the context of curbing the development of resistant phenotypes in clinical environments.

Table 2: Antibiogram profiling of the isolates

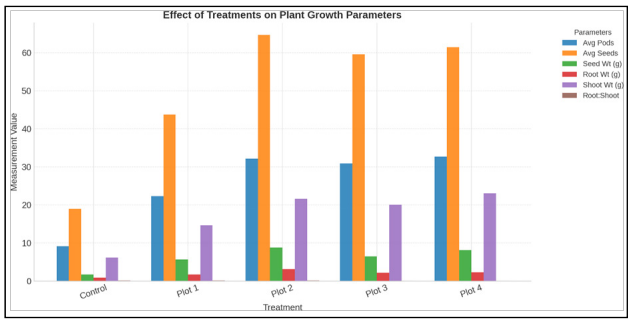
Sl. No.	Antibiotic (Abbreviation)	Disc Concentration	Isolate 41	Isolate 42	Isolate 43
1	Penicillin (P)	2 µg	–	+	+
2	Tetracycline (TE)	10 µg	+	+	+
3	Co-trimoxazole (COT)	25 µg	+	+	+
4	Cloxacillin (COX)	5 µg	–	+	+
5	Cephalothin (CH)	30 µg	+	+	+
6	Erythromycin (E)	10 µg	+	+	+
7	Levofloxacin (L)	10 µg	–	+	+
8	Cefuroxime (CXM)	30 µg	+	+	+

Biofertilizer Consortium Development

The bacterial growth curve demonstrated a progressive increase in both cell count and optical density over the 30-day incubation period. On day 1, the culture exhibited 1.6 × 108 cells/mL with an OD600 of 0.20, which increased to 2.72 × 108 cells/mL and an OD600 of 0.34 by day8. By day 15, the values reached 3.28 × 108 cells/mL and 0.40 OD600, followed by a further increase to 4.64 × 108 cells/mL and 0.58 OD600 on day 23. The maximum values were recorded on day 30, with a cell count of 4.72 × 10^8 cells/mL and an OD600 of 0.59, indicating stabilization of growth parameters at the end of the observation period.



The use of halophilic bacterial biofertilizer significantly increased the growth and yield of soybean in comparison to the untreated control. The average number of pods per plant was over three times higher in treated plots and Plot 2 and Plot 4 had the highest mean of 32.16 and 32.72, respectively, than the control (9.20). Instead, seed count exhibited a strong improvement of 64.60 in Plot 2, and 61.44 in Plot 4, as compared to 18.96 in the control. The seed weight was also significantly increased, showing a range of 5.74 g up to 8.80 g in the treated plots and 1.74 g in the control, which is four to five times more productive. The same tendency was observed in the case of biomass accumulation, with root weights varying between 1.76 g and 3.17 g in treated plants, versus 0.92 g in the control, and shoot weights almost four times higher; 6.18 g in the control versus 23.08 g in Plot 4. Root-to-shoot ratios also were slightly reduced in treated plants (0.10-0.14) compared with the control (0.15), although the change represents a proportionately larger accumulation of biomass in the shoot. The findings in general clearly indicate significant positive effect of the use of halophilic biofertilizer on the yield characteristics and biomass production in soybean with the most significant changes in Plots 2 and 4.



DISCUSSION

The present study on halophilic bacterial consortium development to sodic soil reclamation is highlighted by the fact that the use of microbial consortia to make biofertilizers is currently viewed as a biologically intelligent option as an alternative to traditional chemical amendments. Increase in

Table 3: Effect of halophilic bacterial biofertilizer on soybean growth and yield parameters

Treatment	Average No. of Pods	Average No. of Seeds	Seed Weight (g)	Root Weight (g)	Shoot Weight (g)	Root: Shoot Ratio
Control	9.20 ± 0.15	18.96 ± 0.42	1.74 ± 0.08	0.92 ± 0.05	6.18 ± 0.20	0.1546
Plot 1	22.28 ± 0.32	43.76 ± 0.65	5.74 ± 0.21	1.76 ± 0.07	14.68 ± 0.38	0.1295
Plot 2	32.16 ± 0.44	64.60 ± 0.72	8.80 ± 0.25	3.17 ± 0.11	21.62 ± 0.50	0.1448
Plot 3	30.88 ± 0.41	59.52 ± 0.69	6.46 ± 0.19	2.15 ± 0.08	20.09 ± 0.47	0.1091
Plot 4	32.72 ± 0.46	61.44 ± 0.70	8.17 ± 0.23	2.34 ± 0.09	23.08 ± 0.52	0.1046

Table 4: Effect of halophilic bacterial biofertilizer on soil physico chemical properties

Parameter	Before Application	After Application (Control)	After Application (Sample 1)	After Application (Sample 2)	After Application (Sample 3)	After Application (Sample 4)
pH	8.00	7.72	7.13	7.55	7.52	7.42
EC (dS m^{-1})	0.30	1.98	0.44	1.72	0.35	0.15
Organic Carbon (%)	0.52	0.80	0.65	0.78	0.69	0.75
Nitrogen (kg/ha)	75	310	266	288	280	310
Phosphorus (kg/ha)	7	18.8	16.1	18.5	18.6	16.5
Potassium (kg/ha)	3	403	244	336	350	246
Calcium Carbonate (%)	14.0	10.59	10.6	10.5	9.52	8.52
Calcium (meq/L)	6.10	0.40	0.35	0.37	0.38	0.28
Calcium + Magnesium	19.0	–	–	–	–	–
Sodium (ppm)	1.93	550	320	450	280	200
SAR	4.0	–	–	–	–	–
ESP (%)	8.5	8.42	6.25	7.30	5.15	4.48

soybean productivity, soil fertility and sodicity reduction witnessed here once again support the fact that specific microbial manipulations can transform the unproductive biotic soils into viable biotic soils. These enhancements are reminiscent of recent developments in the microbial ecology of microbial synergy over and above single strain performance determining the success of reclamation efforts (Kapadia *et al.* 2021; Damodaran *et al.* 2023). The dual ecological advantage is evident at the mechanistic level where the halophilic isolates ability to endure salinity and carry out nutrient-cycling functions demonstrates their ability to achieve two functions simultaneously. The evident nitrogen and phosphorus-enrichment of the treated plots compare the results of other studies where halotolerant *Enterobacter cloacae* strains have been found to have nitrogenase and alkaline phosphatase genes that are active in transcription under high Na^+ conditions (Ji *et al.* 2020; Singh *et al.* 2024). In the same manner, the exopolysaccharide (EPS) synthesis, which is one of the primary factors in aggregation of soils as well as immobilization of sodium ions, has been identified as a frequent stress response mechanism in halophilic rhizobacteria (Vardharajula & Sk Z, 2014). Such micro-environments are EPS mediated and increased microbial colonization and exchange of cation, decreasing exchangeable sodium percentage (ESP) and improving soil structure. This effect is supported by the current paper, in which an ESP decrease to 40-50% resulted in the enhancement of aggregate stability and retention of nutrient, just as described by Zhang *et al.* (2024) in saline wheat soils. The consortium inoculation has led to increase a soybean yield, which can be explained by the bioavailability of the essential nutrients and the alteration of phytohormones. The consortium has been reported to have halophilic species that produce auxins, gibberellins, and cytokinin that control the architecture of the root system and photosynthetic efficiency (Muneer Ahmed Khoso *et al.* 2024).

The improvement of root biomass and berry growth in this experiment indicates that crosstalk activity of hormone inoculants on microbes and the plant signaling pathway maximized carbon distribution to reproductive organs- a finding also reported in Glycine max in saline stress inoculated with saline-tolerant Bacillus licheniformis (Chen *et al.* 2024). In addition, the presence of biofilm and osmolytes by the halophilic bacteria amplifies the osmotic tolerance of the rhizosphere forming a physiological barrier that reduces the impact of ionic stress on the roots of the plants (Fu *et al.* 2025). The increased physicochemical soil parameters that were recorded after the treatment show strong arguments of long-term soil rehabilitation. The decrease in pH and level of sodium indicates the microbial-mediated conversion of Na_2CO_3 and $NaHCO_3$ to neutral salts by acidity and cation replacement processes. This change mechanism is in agreement with the enzymatic process of dehydrogenases and ureases which promote the degradation of organic matter and the production of CO_2, thus, buffering the soil alkalinity (Kumar and Sharma, 2020). The higher levels of organic carbon in plots treated are also indicative of microbial sequestration of carbon by the polysaccharide and biomass deposition, which have recently been highlighted in the soil metagenomic techniques that have associated halophilic taxa with higher rates of carbon turnover (Guo *et al.* 2025). Major ecological implication of these findings are the native halophilic bacteria are able to outcompete external inoculants because they are adapted to the local ionic and climatic regime. Niche-specific adaptation guarantees shrubberies of microbial colonization, which is a major factor in the success of reclamation in accordance with the long term (Romano-Armada *et al.* 2020; Chadha *et al.* 2024). This local functional resilience aids the FAO (2021) framework of ecoregion-specialized microbial technologies in which microbial domestication is being focused on to achieve climate-resilient agriculture. Additionally, the compatibility test in the current experiment guaranteed metabolic complementation of isolates, which facilitated nutrient cycling in the variable osmotic stress, which is a critical consideration that has usually been overlooked in single-strain biofertilizer preparations.

In addition to the effects of this microbial approach in soils and plant levels, there are significant consequences of this microbial approach to sustainable land management and agricultural economics. As the price of chemical reclamation agents like gypsum keeps rising, biofertilizer methods provide a low cost, renewable, and eco-friendly alternative. Qadir *et al.* (2014) calculate

the losses incurred worldwide because of land degradation caused by salt to be USD 27 billion per year and incorporation of biofertilizer consortia will bring immense benefits of saving money on replacement through reinstatement of productivity without the use of external factor. Recent meta-analyses of field trials in Asia showed that microbial consortia can increase yield by an average of 32-48% and save on input costs by 25-30% (Liu *et al.* 2023; Manjunath *et al.* 2023). The results of Maharashtra soybean systems can therefore be used as a replicable model that would be incorporated in the roadmap to soil salinity management in the Vision 2050 of the roadmap by the National Mission on Sustainable Agriculture and the Vision 2050 roadmap on soil salinity management by ICAR-CSSRI (ICAR-CSSRI, 2020; ICAR-NBSS&LUP, 2022).

Consistently with the recent metagenomic findings on the keystone salt-tolerant rhizobacteria, the *Enterobacter* and *Klebsiella* species identified as the important members of the consortium are relevant in the microbial genetics perspective in that all are able to fulfill a range of plant growth-promoting roles (None Neemisha *et al.* 2022). Such genera have strong quorum- sensing networks that control the production of EPS, siderophore discharge, and osmoprotectants accumulation, enabling the stability of the community in salt stress (Tan *et al.* 2014). The results of antibiotic sensitivity in this study can also be transferred to a biosafety perspective because it confirms that these isolates have low antimicrobial resistance potential which is a necessary requirement in the registration of biofertilizer under emerging biosafety regulations (WHO–FAO, 2024). Notably, this paper contributes to the empirical underpinning of the conceptual change of the amendment-based to the biome restoration-based soil management. The two-fold effect of halophilic consortia in which sodicity is relieved and at the same time, soil microbiome is beneficial to sunlight productivity is a paradigm shift, which incorporates soil microbiome engineering into the sustainability platform throughout the world. The same changes have been noted in Chinese and Egyptian salt-caused rice paddies where microbial consortia enhanced the efficiency of nutrient uptake and reduced Na^+ in the shoots (Vincze *et al.* 2024). These regular results support the idea that biofertilizer consortia are not only inputs but drivers of ecological resilience of the soil. Although the present field tests were restricted to one season of *Kharif*, the notable increases in the yield and property changes in the soil indicated that in the long-term use, the soil-microbe-plant feedbacks could be further stabilized. Nevertheless, the use of meta transcriptomic analyses in future research should be used to decipher real-time gene expression patterns in the presence of sodic stress, especially those involved in the synthesis of osmolytes and ion ansport. Such omics strategies combined with remote-sensing based soil health might be used to design microbial consortia and optimize scalability of the field (Jiang *et al.* 2016). Alongside, inter-location multi-locational studies in diverse agroclimatic regions of Maharashtra would help in predicting multi-locational indices of microbial diversity that are associated with reclamation efficiency. To sum up, the salt-tolerant bacterial consortium that has been produced in the given research is a scientifically sound, economically viable, and environmentally-friendly way of transforming saltic soils into agricultural land. It fills the gap between microbial biotechnology and sustainable land management by restoring biological activity, increasing the nutrient cycling and crop productivity. Such technologies would be able to turn marginal lands into productive agroecosystems and meet the UN Sustainable Development Goals 2 (Zero Hunger), 13 (Climate Action), and 15 (Life on Land) when integrated in the policy frameworks like the Global Soil Partnership of FAO, as well as soil health mission of India. The intersecting nature of microbial ecology and policy-oriented reclamation approaches therefore provide a prospective roadmap towards sustainable farming at the salt-affected areas around the world.

CONCLUSION

To sum up, the current study concludes that halophilic bacterial consortia is a ground-breaking, biologically intelligent approach to the reactivation of sodic soils and sustainable crop growth. The isolates, which were mainly *Enterobacter* and *Klebsiella* spp, were highly halotolerant, nutrient cycling potential, and biochemically versatile and formed a synergistic consortium that can significantly improve soil health and crop performance. In field trials of Maharashtra, they found 3-4-fold improvement in soybean yield, reduction in soil pH and exchangeable sodium

percentage and significant increase in soil organic carbon, nitrogen, phosphorus, and potassium. All of these effects indicate that the halophilic biofertilizers do not just replace the chemical amendments but restore the biological integrity of the degraded soils by enzymatic transformations, aggregation of exopolysaccharides, and modulation of hormones at the rhizosphere level. The results support the hypothesis that native consortia of microorganisms which is adjusted to the local edaphic and climatic conditions, can attain long-term productivity advantages and reduce ecological and economic implications of salinity. The study is consistent with the larger sustainability objectives, such as agronomic enhancement, agrochemical-independent nutrient cycles, and climate-resilient agro ecosystems. With the world estimate of more than one billion hectares of salt-contaminated areas and the escalating fees of reclamation, the microbial consortium that has been developed can be used as a scaled-up, cost-effective, and ecologically friendly option in restoring lands. The adoption of these biotechnological breakthroughs into country soil health initiatives, FAO global soil partnership and regional policy, would transform the nature of reclamation policies no longer as a short- term remediation strategy, but as a long-term biome restoration initiative. Finally, this study confirms that not only are the halophilic bacterial consortia instruments of microbial biotechnology but they are central agents of realizing resilient agriculture, ecological recovery, and sustainable development in salt-impacted areas of the world.

ACKNOWLEDGEMENTS

The authors are grateful to research center Mvp Samaj's K.T.H.M college, Nashik and Mvp Samaj's K.A.A.N.M.S college, Satana for providing laboratory instruments and chemicals for carrying out research work.

REFERENCES

Arora, S., Singh, Y.P. and Sahni, D. 2021. Halophilic microbial bioformulations for Bioremediation of salt-affected soils. *Elsevier E-Books*, pp. 301–311.

Ashenafi Worku Daba. 2025. Rehabilitation of soil salinity and sodicity using diverse amendments and plants: a critical review. *Discover Environment*, **3**(1).

Chadha, D., Sharma, V., Kour, S., Bharat, R., Sharma, D., Pooniyan, S. and Arora, R.K. 2024. Impact of Halophilic Bioformulations on Growth, Yield and Nutrient Uptake of Paddy Grown Under Sodic Soils. *Communications in Soil Science and Plant Analysis*, pp. 1– 11.https://doi.org/10.1080/00103624.2024.2430342

Chen, Q., Song, Y., An, Y., Lu, Y. and Zhong, G. 2024. Mechanisms and Impact of Rhizosphere Microbial Metabolites on Crop Health, Traits, Functional Components: A Comprehensive Review. *Molecules*, **29**(24): 5922–5922.

Damodaran, T., Jha, S.K., Kumari, S., Gupta, G., Mishra, V.K., Sharma, P.C., Gopal, R., Singh, A. and Jat, H.S. 2023. Development of Halotolerant Microbial Consortia for Salt Stress Mitigation and Sustainable Tomato Production in Sodic Soils: An Enzyme Mechanism Approach. *Sustainability*, **15**(6): 5186.

Demo, A.H., Gemeda, M.K., Abdo, D.R., Guluma, T.N. and Adugna, D.B. 2025. Impact of soil salinity, sodicity, and irrigation water salinity on crop production and coping mechanism in areas of dryland farming. *Agrosystems, Geosciences & Environment*, **8**(1).

Egamberdieva, D., Wirth, S., Bellingrath-Kimura, S.D., Mishra, J. and Arora, N.K. 2019. Salt-Tolerant Plant Growth Promoting Rhizobacteria for Enhancing Crop Productivity of Saline Soils. *Frontiers in Microbiology*, **10**.

FAO 2021. Global Map of Salt-Affected Soils. Food and Agriculture Organization of the United Nations, Rome.

Fu, M., Liu, L., Fu, B., Hou, M., Xiao, Y., Liu, Y., Sa, D. and Lu, Q. 2025. Effects of salt stress on plant and rhizosphere bacterial communities, interaction patterns, and functions. *Frontiers in Plant Science*, **15**.

Guo, Y., Li, S., Gu, S., Hua, Z., Pang, J., Xu, B., Wu, K., Chen, D., Qiu, D., Wang, Y., Wu, Z. and Zhou, Q. 2025. Lake carbon loss driven by changed microbial carbon utilization Through increased anthropogenic land-use intensity. *Communications Earth & Environment*, **6**(1).

ICAR-CSSRI, 2020. Vision 2050. ICAR-Central Soil Salinity Research Institute, Karnal, India.

ICAR-NBSS&LUP, 2022. State-wise extent of problem soils in India. National Bureau of Soil Survey and Land Use Planning, Nagpur.

Ji, C., Liu, Z., Hao, L., Song, X., Wang, C., Liu, Y., Li, H., Li, C., Gao, Q. and Liu, X. 2020. Effects of Enterobacter cloacae HG-1 on the Nitrogen-Fixing Community Structure of Wheat Rhizosphere Soil and on Salt Tolerance. *Frontiers in Plant Science*, **11**.

Jiang, Y., Xiong, X., Danska, J. and Parkinson, J. 2016. Metatranscriptomic analysis of diverse microbial communities reveals core metabolic pathways and microbiome-specific functionality. *Microbiome*, **4**(1).

Kapadia, C., Sayyed, R.Z., El Enshasy, H.A., Vaidya, H., Sharma, D., Patel, N., Malek, R.A., Syed, A., Elgorban, A.M., Ahmad, K. and Zuan, A.T.K. 2021. Halotolerant Microbial Consortia for Sustainable Mitigation of Salinity Stress, Growth Promotion, and Mineral Uptake in Tomato Plants and Soil Nutrient Enrichment. *Sustainability*, **13**(15): 8369.

Kumar, P. and Sharma, P.K. 2020. Soil Salinity and Food Security in India. *Frontiers in Sustainable Food Systems*, **4**.

Liu, X., Mei, S. and Salles, J.F. 2023. Inoculated microbial consortia perform better than single strains in living soil: A meta-analysis. *Applied Soil Ecology*, **190**: 105011.

Manjunath, M., Khokhar, A., Gajjala Ravindra Chary, Singh, M., Sushil Kumar Yadav, Gopinath, K.A., Narayana Jyothilakshmi, Srinivas, K., Prabhakar, M. and Singh, V.K. 2023. *Microbial consortia enhance the yield of maize under sub-humid rainfed production system of India*. **7**.

Muneer Ahmed Khoso, Sindho Wagan, Alam, I., Hussain, A., Ali, Q., Saha, S., Tika Ram Poudel, Hakim Manghwar and Liu, F. 2024. Impact of plant growth-promoting rhizobacteria (PGPR) on plant nutrition and root characteristics: Current perspective. *Plant Stress*, **11**: 100341–100341.

None Neemisha, Kumar, A., Sharma, P., Kaur, A., Sharma, S. and Jain, R. 2022. Harnessing rhizobacteria to fulfil inter-linked nutrient dependency on soil and alleviate Stresses in plants. *Journal of Applied Microbiology*, **133**(5): 2694–2716.

Qadir, M., Quillérou, E., Nangia, V., Murtaza, G., Singh, M., Thomas, R.J., Drechsel, P. and Noble, A.D. 2014. Economics of salt-induced land degradation and restoration. *Natural Resources Forum*, **38**(4): 282–295.

Romano-Armada, N., Yañez-Yazlle, M.F., Irazusta, V.P., Rajal, V.B. and Moraga, N.B. 2020. Potential of Bioremediation and PGP Traits in Streptomyces as Strategies for Bio-Reclamation of Salt-Affected Soils for Agriculture. *Pathogens*, **9**(2): 117.

Singh, T., Arora, S., Nautial, P., Sahu, A., Rai, T.N., Singh, R. and Dubey, S.K. 2024. On- Farm Assessment of Halophilic Microbial Bio-Formulation on Productivity Of Mustard Crop in Sodic Soils of Indo-Gangetic Plain. *Journal of Soil Salinity and Water Quality*, **16**(3): 424–430.

Tan, C.H., Koh, K.S., Xie, C., Tay, M., Zhou, Y., Williams, R., Ng, W.J., Rice, S.A. and Kjelleberg, S. 2014. The role of quorum sensing signalling in EPS production and the assembly of a sludge community into aerobic granules. *The ISME Journal*, **8**(6): 1186– 1197.

Vardharajula, S. and Sk Z.A. 2014. Exopolysaccharide production by drought tolerant *Bacillus* spp. and effect on soil aggregation under drought stress. *Journal of Microbiology, Biotechnology and Food Sciences*, **4**(1): 51–57.

Vincze, É.-B., Annamária Becze, Laslo, É. and Mara, G. 2024. Beneficial Soil Microbiomes and Their Potential Role in Plant Growth and Soil Fertility. *Agriculture*, **14**(1): 152–152.

Zhang, M., Wu, Y., Qu, C., Huang, Q. and Cai, P. 2024. Microbial extracellular polymeric Substances (EPS) in soil: From interfacial behaviour to ecological multifunctionality. *Deleted Journal*, **1**.