MINISTRY OF EDUCATION AND TRAINING

VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY

GRADUATE UNIVERSITY OF SCIENCE AND TECHNOLOGY

Le Thi Hue

STUDY ON THE MOISTURE-RETAINING AND SALINITY-REDUCING POTENTIAL OF CORAL SAND USING SALT-TOLERANT, EXOPOLYSACCHARIDE-PRODUCING BACTERIA ISOLATED FROM THE TRUONG SA (SPRATLY) ARCHIPELAGO

SUMMARY OF DISSERTATION ON APPLIED BIOLOGY

Major: Biotechnology

Code: 9 42 02 01

Ha Noi - 2025

This dissertation was completed at: Graduate University of Science and Technology, Vietnam Academy of Science and Technology.

Scientific Supervisor:
1. Supervisor 1: Prof. Dr. Le Mai Huong
2. Supervisor 2: Dr. Vu Duy Nhan

Referee 1: Assoc. Prof. Dr. Truong Quoc Phong
Referee 2: Assoc. Prof. Dr. Pham Bich Ngoc

The dissertation is examined by Examination Board of Graduate University of Science and Technology, Vietnam Academy of Science and Technology at 14:00 h (22/12/2025)

The dissertation can be found at:
1. Graduate University of Science and Technology Library
2. National Library of Vietnam

INTRODUCTION

1. Rationale of the study

The Truong Sa (Spratly) archipelago holds strategic importance but is characterized by an arid climate, freshwater scarcity, and nutrient-poor, alkaline, and saline coral sand soils, which severely limit agricultural development. Under the growing impacts of climate change and salinization, rehabilitating coral sand into productive soil has become an urgent priority. Environmentally friendly biological solutions, especially the use of exopolysaccharide (EPS)-producing bacteria, have recently attracted considerable interest due to their capacity for water retention, soil aggregation, and salt adsorption. However, studies on indigenous EPS-producing bacteria for coral sand improvement in Vietnam remain scarce.

Therefore, this dissertation entitled **"Study on the moisture-retaining and salinity-reducing potential of coral sand using salt-tolerant, exopolysaccharide-producing bacteria isolated from the Truong Sa (Spratly) archipelago"** was conducted to isolate and characterize EPS-producing salt-tolerant strains and evaluate their potential in enhancing the moisture retention and salinity reduction of coral sand, contributing to sustainable agriculture on Vietnam's offshore islands.

2. Research objectives

- To isolate, select, and analyze the genomic characteristics of salt-tolerant, exopolysaccharide (EPS)-producing bacterial strains obtained from the Truong Sa (Spratly) Archipelago.
- To evaluate the moisture-retaining and salinity-reducing capacities of EPS extracted from the selected bacterial strain.
- To preliminarily assess the application potential of the selected bacterial strain for the improvement of coral sand at pot-scale experiments.

3. Research contents

1. Isolation and selection of indigenous bacterial strains from the Truong Sa (Spratly) Archipelago with salt tolerance and exopolysaccharide production ability.

2. Whole-genome sequencing and prediction of biosynthetic pathways for EPS and genes related to salt tolerance in the selected bacterial strain.

3. Investigation of culture media and cultivation conditions for EPS production from the selected bacterial strain.

4. Purification and structural characterization of EPS produced by the selected bacterial strain.

5. Evaluation of the moisture-retaining and salinity-reducing capacities of EPS from the selected bacterial strain.

6. Evaluation of the coral sand improvement efficiency of the salt-tolerant, exopolysaccharide-producing bacterial strain under pot-scale experimental conditions.

CHAPTER 1. OVERVIEW OF RESEARCH

1.1. Overview of arid and saline soils

Global climate change has been accelerating the expansion of arid and saline lands, posing a serious threat to global food security. These areas, which account for approximately 40% of the Earth's surface, are characterized by low rainfall, high evaporation rates, poor nutrient content, and the accumulation of soluble ions such as Na^+ and Cl^-. The causes originate from both natural factors (weathering, seawater intrusion) and anthropogenic activities (improper irrigation and the use of saline water). The combination of these factors increases soil osmotic pressure, reduces the uptake of water and nutrients by plants, and leads to ion toxicity, thereby negatively affecting crop productivity and soil ecosystem functions.

1.2. Status of arid and saline soils in Vietnam

In Vietnam, arid and saline soils are mainly distributed in the Mekong River Delta, the central coastal provinces, and offshore islands such as the Truong Sa (Spratly) Archipelago. Notably, coral sand in Truong Sa represents a unique soil type of marine biogenic origin, containing up to 95% $CaCO_3$, with a high alkaline pH (8–9), coarse texture, and large porosity but

extremely low water- and nutrient-holding capacities (CEC < 5 meq/100 g, moisture < 5%). Strong evaporation, irregular rainfall, saline groundwater, and a poor native microbial community further exacerbate the conditions, rendering coral sand on the floating islands of Truong Sa unsuitable for agricultural production without appropriate soil improvement measures.

1.3. Overview of methods for the amelioration of saline and arid soils

Various methods have been developed for the amelioration of saline and arid soils, including: (i) Mechanical measures, such as salt leaching and soil mulching; (ii) Biological and biotechnological approaches, such as the addition of organic matter, biochar, beneficial microorganisms, and EPS-producing bacteria to enhance soil nutrient content and moisture retention; (iii) Physicochemical treatments, such as the use of gypsum and bentonite in combination with mulching to replace Na^+ ions and improve water-holding capacity; and (iv) Integrated approaches, which combine biological, chemical, and physical measures to optimize soil improvement efficiency and ensure long-term sustainability.

In Vietnam, problems of soil degradation, salinization, and aridity have become increasingly severe due to climate change, seawater intrusion, and unsustainable land use practices. In recent years, several soil amelioration strategies have been tested and applied in the field, including physicochemical treatments, the addition of organic materials, and the integration of biological inoculants–particularly EPS–producing microorganisms–which have yielded promising experimental results.

1.4. Overview of salt-tolerant, exopolysaccharide-producing bacteria and their applications in the amelioration of arid, saline soils

Salt-tolerant bacteria are bacteria capable of growing under both non-saline and high-salinity conditions, commonly isolated from saline soils, coral islands, and salt lakes. These bacteria not only survive in environments with elevated NaCl concentrations but also produce bioactive compounds, such as EPS, which enhance their tolerance to osmotic and salt stress.

Numerous studies have demonstrated that EPS produced by halotolerant bacteria can mitigate Na^+ toxicity, improve soil physical properties, and help plants withstand salinity stress.

Salt tolerance in bacteria is generally attributed to four well-established mechanisms: (1) Regulation of osmotic pressure through Na^+ efflux and intracellular accumulation of K^+/Cl^-; (2) Stabilization of the cell envelope via acidic surface structures; (3) Accumulation of compatible solutes; and (4) EPS production forming a biofilm layer that shields cells against environmental stress.

EPS-producing bacteria are widespread among both Gram-positive and Gram-negative groups. Among them, *Bacillus velezensis* is recognized as a promising species due to its capacity to synthesize diverse EPS types–including levan, glucomannan, and acidic EPS–at concentrations ranging from 0.504 to 75.5 g/L. The EPS of *B. velezensis* exhibits structural diversity, often composed of 2–4 monosaccharide units. This species has been reported to enhance soil water retention, adsorb salts and heavy metals, form gel-like matrices, and promote plant growth under saline stress, highlighting its strong potential for application in the improvement of saline, arid soils. Other EPS-producing microorganisms, such as *Bacillus safensis*, *Paenibacillus polymyxa*, and *Rhizobium meliloti*, have also been studied and applied in soil amelioration programs worldwide. In Vietnam, research has mainly focused on EPS-producing yeasts such as *Lipomyces* for soil improvement.

EPS are extracellular polysaccharides secreted during microbial growth that protect cells from environmental stress while playing key roles in biofilm formation and microbe–microbe or microbe–soil interactions. EPS may exist as homopolysaccharides (HoPS) or heteropolysaccharides (HePS) and are synthesized through four major biosynthetic pathways: Wzx/Wzy-dependent, ABC transporter–dependent, synthase–dependent, and extracellular sucrase–dependent pathways. EPS biosynthesis is closely linked to sugar uptake and metabolism, typically initiated through central

metabolic routes such as glycolysis or the pentose phosphate pathway. The composition, structure, and bioactivity of EPS are influenced by environmental conditions–particularly the carbon source–as well as the genetic characteristics of the producing bacteria.

1.5. Whole-genome sequencing

Whole-genome sequencing (WGS) is a high-throughput sequencing technique that determines the complete genetic information of an organism. It serves as a key tool in genomics, genetics, personalized medicine, and applied microbiology.

In recent years, WGS has become an indispensable approach in studying salt-tolerant microorganisms, particularly for exploring their genetic potential in soil improvement and bio-product development. Both in Vietnam and worldwide, WGS has been applied to analyze salt-tolerant bacterial strains to elucidate their mechanisms of salt tolerance, adaptive capacity, and plant growth-promoting traits. Several EPS-producing bacterial species, such as *Bacillus velezensis*, *B. subtilis*, *Pseudomonas* spp., and *Rhizobium* spp., have been fully sequenced to identify gene clusters responsible for EPS biosynthesis, assess safety and metabolic potential, and examine their adaptation to adverse environments as well as their applications in agriculture, industry, and biomedicine.

The overview highlights that coral sand in the Truong Sa (Spratly) Archipelago is saline, arid, and nutrient-poor, posing major challenges for soil rehabilitation. Biological approaches using indigenous EPS-producing, salt-tolerant bacteria offer great potential to improve soil aggregation, enhance moisture retention, and naturally reduce salinity. However, related studies in Vietnam remain limited, particularly at the genome level. This dissertation therefore isolates, sequences, and characterizes indigenous EPS-producing bacteria from Truong Sa coral sand to elucidate their genetic basis and evaluate their potential for sustainable soil improvement in coastal and island ecosystems.

CHAPTER 2. MATERIALS AND METHODS

2.1. Research Materials

Research subjects: Nine soil samples were collected from three islands: Truong Sa Dong (coded TSD), Da Tay A (coded DTA), and Truong Sa Lon (coded TSL). Coral sand used for experimental trials was collected from Truong Sa Lon Island on April 29, 2024, by V. D. N.

2.2. Research methods

2.2.1. Soil sampling method

Soil samples were collected in accordance with TCVN 7538-2:2005 (ISO 10381-2:2002) - Soil quality - Sampling - Part 2: Guidance on sampling techniques.

2.2.2. Microbiological methods

2.2.2.1. Isolation and screening of salt-tolerant, exopolysaccharide-producing bacteria

Field surveys showed that soils on the floating islands of the Truong Sa Archipelago contained 0.45–1.27% NaCl, and plants were often irrigated with brackish water due to freshwater scarcity. To isolate salt-tolerant, EPS-producing bacteria for coral sand improvement, soil samples were cultured on LB medium supplemented with 3% NaCl, equivalent to average seawater salinity.

Ten grams of soil were serially diluted (10^{-1}–10^{-5}) in sterile physiological saline, and 100 µL aliquots were spread on LB agar with 3% NaCl, then incubated at 30°C for 45–48 hours. Microbial density was determined following TCVN 11039-1:2015. Colonies exhibiting distinct morphological characteristics were recorded, and those with shiny, wet, mucoid appearances capable of forming viscous filaments longer than 5 mm were selected as potential EPS-producing strains.

2.2.2.2. Screening of salt-tolerant bacterial strains with high exopolysaccharide production

The screening was conducted following the methods of Deka *et al.*

(2019) and Cheng *et al.* (2020). The bacterial isolates were cultured in LB medium containing 3% NaCl, after which EPS was extracted and quantified. Strains producing the highest EPS yield were selected for further analysis

2.2.2.3. Assessment of carbon source utilization by the selected strain

The ability of the selected *Bacillus* strain to utilize various carbon sources was examined using the API 50CHB test kit (BioMérieux, France), which evaluates the fermentation profile of 49 different carbohydrates.

2.2.2.4. Evaluation of salt and heavy metal tolerance of the selected strain

The selected bacterial strain was cultivated in LB medium supplemented with different concentrations of NaCl or $MgSO_4$ (0–17.5%, w/v) (A), with heavy metals Cd, Hg, Cr, Co, and As at concentrations ranging from 1 to 128 µg/mL (B), and with Pb, Zn, and Fe from 150 to 1050 µg/mL. Growth was assessed based on turbidity of the culture or the formation of an opaque white biofilm layer on the surface of the wells.

2.2.2.5. Investigation of the effects of individual media and culture conditions on exopolysaccharide production

The selected bacterial strain was cultured in six different media–LB, NB, YPM, ISP2, YM, and TB–to determine the medium that yielded the highest EPS production. Subsequently, the effects of various factors, including carbon and nitrogen sources, mineral salts, temperature, pH, shaking speed, and inoculum rate, were examined to identify the conditions that maximize EPS yield.

2.2.2.6. Optimization of environmental factors influencing EPS biosynthesis

Three key environmental factors with the most significant impact on EPS production were selected for optimization using the response surface methodology (RSM).

2.2.3. Bacterial classification methods

The bacterial identification was based on morphological, physiological, and biochemical characteristics, as well as on 16S rRNA gene sequence analysis compared with sequences available in GenBank.

2.2.4. Whole-genome sequencing

The selected bacterial strain was cultured, and genomic DNA was extracted and sent to Novogen AIT (Singapore) for WGS using the Illumina NovaSeq 6000 platform with paired-end reads of 150 bp in length.

2.2.5. Bioinformatics analysis methods

Bioinformatics analyses included the processing of raw sequencing reads and de novo genome assembly, phylogenetic tree construction, functional annotation, and comparative analysis of gene clusters.

2.2.6. Biochemical methods

2.2.6.1. Extraction of exopolysaccharides

The EPS extraction procedure was carried out according to the method described by Vu et al. (2021).

2.2.6.2. Purification of exopolysaccharides

EPS purification was performed following the protocol of Zhao et al. (2019). Proteins were removed using trichloroacetic acid, EPS was precipitated with cold acetone, and the precipitate was dialyzed to remove monosaccharides and salts. Further purification was achieved using a Sephadex G-75 column, followed by freeze-drying to obtain purified EPS.

2.2.6.3. Determination of carbohydrate content

The carbohydrate content was determined according to the method of Dubois (1956), using a standard calibration curve based on D-glucose.

2.2.6.4. Determination of total protein content

Total protein concentration was quantified following the Bradford method, with bovine serum albumin (BSA) used as the standard.

2.2.6.5. Determination of the molecular weight and structural characteristics of exopolysaccharides

- Surface morphology of EPS was examined using SEM.
- Elemental composition was analyzed by EDS.
- Molecular weight of EPS was estimated from its intrinsic viscosity.

- Monosaccharide composition was determined using HPLC.
- Fourier Transform Infrared (FT-IR) spectroscopy was performed at the Military Technical Academy to identify functional groups.

2.2.6.6. Evaluation of the effect of carbon sources on the monosaccharide composition of exopolysaccharides

EPS yield and composition were determined in media similar to the optimal culture condition but supplemented with glucose or sucrose at concentrations of 0%, 1%, and 5%.

2.2.6.7. Measurement of Zeta potential

The zeta potential of EPS samples was measured using a Zetasizer Nano ZS90 (Malvern Instruments, UK) at the Institute of Materials Science, VAST, based on the dynamic light scattering (DLS) method.

2.2.6.8. Determination of exopolysaccharides characteristics under stress conditions

EPS yield and composition were evaluated in cultures grown under salt (NaCl) and/or heavy metal stress conditions.

2.2.7. Experimental methods

2.2.7.1. Evaluation of the salinity-reducing capacity of free water in soil

Five experiments (E1–E5) were designed using coral sand amended with 3% bentonite and 10% organic substrate to form a composite soil: E1 (negative control): composite soil + water; E2: composite soil + optimized medium; E3: composite soil + 70 mg EPS + NaCl; E4: composite soil + culture broth of the EPS-producing bacterial strain grown under optimal conditions. After 24 h of incubation, 250 mL of water was added to each sample for extraction, and the salinity of the resulting soil filtrate was measured.

2.2.7.2. Determination of the water-holding capacity of exopolysaccharides

Fifty milligrams of crude EPS (extracted from the culture supernatant of the bacterial strain grown under optimal conditions) were soaked in

distilled water, and water absorption was evaluated after 1, 2, and 4 hours to determine hydration capacity.

2.2.7.3. Determination of the water-retention capacity of soil treated with exopolysaccharides and exopolysaccharide-producing bacterial culture

The experiment was designed according to the method of Vardharajula et al.:

- E1: 50 g coral sand + 10 mL distilled water.
- E2: 50 g composite soil + 10 mL distilled water.
- E3: 50 g composite soil + 10 mL culture of the selected bacterial strain (10^9 CFU/mL).
- E4: 50 g composite soil + 10 mL distilled water + 70 mg crude EPS.
- E5: 50 g composite soil + 10 mL distilled water + 140 mg crude EPS.

The corresponding soil samples from the five experiments above were analyzed for water-holding capacity, drainage rate, and aggregation ability, following the "drop-counting method" described by Brischke et al. (2019).

2.2.7.4. Evaluation of the moisture-retaining and salinity-reducing efficiency of salt-tolerant, exopolysaccharide-producing bacteria on Basella alba under pot-scale conditions

The experiment was conducted in a net house at the Faculty of Agronomy, Vietnam National University of Agriculture, following Cheng et al. (2020) and Kasotia et al. (2016), with seven treatments: T1 – coral sand; T2 – coral sand + organic substrate; T3 – T2 + EPS-producing bacteria; T4 – T2 + bentonite; T5 – T2 + bentonite + EPS-producing bacteria; T6 – T2 + bentonite + NaCl irrigation; T7 – T2 + bentonite + EPS-producing bacteria + NaCl irrigation. Each treatment had three replicates with one *Basella alba* plant per pot. Pots were irrigated every three days with 50 mL of water or 1% NaCl solution (T6, T7). After 30 days, plant and soil samples were collected to assess plant growth, SPAD value, water-retention capacity, soil aggregation, carbohydrate content, and soil salinity.

2.2.8. Statistical analysis

All experiments were conducted in triplicate. Statistical differences among treatments were determined using one-way ANOVA followed by Tukey's pairwise comparison test, performed with Minitab Statistical Software. Differences were considered statistically significant at $p < 0.05$.

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Isolation and selection of indigenous salt-tolerant, exopolysaccharide-producing bacteria from the Truong Sa archipelago

3.1.1. Isolation and screening of salt-tolerant, exopolysaccharide-producing bacterial strains

From nine soil samples collected across three islands of the Truong Sa Archipelago, ten bacterial strains capable of growing in 3% NaCl and producing EPS were successfully isolated and selected. Among them, four strains originated from Truong Sa Dong Island, four from Truong Sa Lon Island, and two from Da Tay A Island.

3.1.2. Selection of salt-tolerant strains with high exopolysaccharide production

Four strains–DTA1, TSL6, TSD5, and TSL5–were selected for their EPS production capacity, which was significantly higher than that of the other bacterial strains ($p < 0.05$). Among them, strain DTA1 exhibited the highest EPS yield (1.31 g/L).

3.1.3. Identification of high exopolysaccharide-producing, salt-tolerant strains

The 16S rRNA gene sequences of the isolates TSD5, DTA1, TSL5, and TSL6 showed the highest similarity to *B. velezensis* NR116240 (99.86%), *B. velezensis* NR075005 (99.79%), *Priestia megaterium* MG491526 (100%), and *B. velezensis* NR075005 (99.82%), respectively. Among these, strain *B. velezensis* DTA1 demonstrated the highest EPS yield and robust growth and was therefore selected for further detailed studies.

3.1.4. Biological characteristics of strain DTA1

3.1.4.1. Utilization of carbon sources

Strain DTA1 was able to utilize 28 out of 49 tested carbon sources. It exhibited strong metabolic activity toward several sugars, particularly D-glucose, D-fructose, D-mannose, D-cellobiose, D-sucrose, and esculin.

3.1.4.2. Salt and heavy metal tolerance

Strain DTA1 tolerated up to 12.5% NaCl and 10% $MgSO_4$. It also showed high tolerance to heavy metals, withstanding concentrations of up to 900 μg/mL for Pb and Zn, 600 μg/mL for Fe, and up to 64 μg/mL for Cr and As. The tolerance levels for Cd, Hg, and Co were 32 μg/mL, 2 μg/mL, and 4 μg/mL, respectively, indicating variable resistance among tested metals.

3.2. Whole-genome sequencing and prediction of exopolysaccharide biosynthesis pathways and salt-tolerance-related genes in strain DTA1

3.2.1. Genome DNA quality assessment

The genomic DNA of strain DTA1 met all quality criteria for concentration, purity, quantity, and integrity, ensuring suitability for WGS.

3.2.2. General genome features

WGS analysis revealed that the assembled genome of *Bacillus velezensis* DTA1 was 3,901,259 bp in size, with a GC content of 46.5%, comparable to the reference genome of *B. velezensis* FZB42.

3.2.3. Phylogenetic analysis and taxonomic position of strain DTA1

Combined analyses based on 16S rRNA gene sequencing and core genome comparison confirmed that strain DTA1 belongs to the species *B. velezensis*.

3.2.4. Sugar Transport Systems in Bacillus velezensis DTA1

A total of 22 phosphotransferase system (PTS) transport proteins responsible for various sugars were identified in the genome of strain DTA1, classified into 11 distinct PTS systems. Regarding specific sugar permease transport systems, proteins specific for arabinose, maltose, and multiple-

sugar permeases were detected. In addition, ABC transporter subunits facilitating the uptake of maltose and maltodextrin were also identified. These findings suggest that *B. velezensis* DTA1 is capable of utilizing cellobiose, glucoside, glucose, mannose/mannitol, fructose, sucrose, arabinose, and N-acetylglucosamine, with cellobiose, sucrose, and arabinose being the most likely preferred substrates.

3.2.5. Nucleotide sugar biosynthesis pathways in Bacillus velezensis DTA1

The DTA1 genome contains 29 genes encoding key enzymes involved in converting diverse carbon sources into nucleotide sugars. The strain is predicted to synthesize 8 types of nucleotide sugars, including UDP-N-acetylglucosamine, UDP-N-acetylgalactosamine, UDP-N-acetylmannosamine, dTDP-glucose, dTDP-rhamnose, UDP-glucose, UDP-glucuronate, and UDP-galactose. Based on WGS analysis and API® 50CHB biochemical testing, *B. velezensis* DTA1 is predicted to utilize nine carbohydrates–cellobiose, glucoside, glucose, mannose, mannitol, fructose, sucrose, arabinose, and N-acetylglucosamine–as carbon sources for metabolic and EPS biosynthetic processes.

3.2.6. Exopolysaccharide biosynthesis pathways in B. velezensis DTA1

Two gene clusters associated with EPS biosynthetic pathways were identified in the DTA1 genome: the Wzx/Wzy-dependent pathway and the extracellular synthesis pathway mediated by the enzyme levansucrase.

3.2.7. Stress-resistance-related genes in Bacillus velezensis DTA1

The DTA1 strain harbors a wide range of genes involved in various stress responses, including osmotic stress (*degU*, *degS*, *sodA*, *dnaK*, etc.), heavy metal stress (*czcD*, *cadA*, *chrA*, *chrB*, *ydpP*, *mneP*, *mntP*, *mgtE*, *corA*, and *asrB*), and oxidative stress (*nsrR*, *tpx*, *bcp*, and *hmpA*). These genes collectively contribute to the strain's ability to tolerate saline and heavy metal stress conditions.

3.3. Study on culture media and conditions for exopolysaccharide production by *B. velezensis* DTA1

3.3.1. Effects of environmental and culture conditions on exopolysaccharide production

Single-factor experiments showed that TB medium containing 5% sucrose and 3% NaCl at pH 8, shaken at 150 rpm and incubated at 30 °C for 72 h, produced the highest EPS yield (≈ 27.30 g/L). The strain tolerated wide ranges of pH (4–10), temperature (5–50 °C), and salinity (0–12.5%), with optimal production under alkaline–saline conditions typical of coral sand soils. Therefore, pH, NaCl, and sucrose concentrations were selected as key factors for further optimization.

3.3.2. Optimization of exopolysaccharide production using the response surface methodology

A second-order polynomial model was established using RSM to evaluate the effects and interactions of NaCl concentration (X_1), pH (X_2), and sucrose concentration (X_3) on EPS yield. The model predicted an optimal yield of 32.78 g/L at 3.27% NaCl, pH 8.46, and 7.26% sucrose. Experimental validation under these conditions produced 32.80 g/L, confirming the accuracy and reliability of the model.

3.3.3. Effect of carbon sources on the monosaccharide composition of exopolysaccharides produced by B. velezensis DTA1

Results presented in Table 3.1 indicate that the type of carbon source significantly affected both the yield and the monosaccharide composition of EPS synthesized by *B. velezensis* DTA1. The EPSDTA1 consisted of 3 to 5 types of monosaccharides, which is notably higher than previous reports for *B. velezensis* EPSs (2–4). When glucose was used as the carbon source, the EPS contained three monosaccharides, predominantly mannose. In contrast, supplementation with sucrose–particularly at a concentration of 5%–not only

increased EPS yield but also introduced two additional monosaccharides, fructose and N-acetylglucosamine.

These findings demonstrate that EPS biosynthesis in *B. velezensis* DTA1 is strongly dependent on the type of carbon source provided and suggest that sucrose supplementation activates EPS synthesis through two distinct pathways (Wzx/Wzy-dependent and levansucrase-dependent).

Table 3.1. EPS characteristics from *B. velezensis* strains

***B. velezensis* strain**	**Source of added sugar (%)**	**EPS yield (g/L)**	**Monosaccharide component ratio in EPS**	**References**
DTA1 (TB medium, pH 8.46, with NaCl 3.27%)	Glucose 1	21.42±0.32	Glucose : rhamnose : mannose (1.00 : 9.56 : 59.94)	This study
	Glucose 5	23.29±0.34	Glucose : rhamnose : mannose (1.00 : 3.70 : 745.16)	
	Sucrose 1	23.93±0.30	Glucose : rhamnose : fructose : mannose : N-acetylglucosamine (1.00 : 17.81 : 2.23 : 1.86 : 24.45)	
	Sucrose 5	30.39±0.42	Glucose : rhamnose : fructose : mannose : N-acetylglucosamine (2.61 : 12.86 : 7.09 : 1.00 : 12.30)	
	No added sugar	16.35±0.30	Glucose : rhamnose : mannose (1.00 : 5.49 : 3.92)	
TSD5	No added sugar	1.02	Glucose : rhamnose : mannose (1.00 : 1.83 : 14.20)	Le et al. (2025)
VTX20	Sucrose 20	75.5±4.8	Fructose, glucose	Vu et al. (2021)
AG6	Sucrose 10	5.79	Xylose : galactose : galacturonic acid (2.0 : 0.5 : 2.0)	Alharbi et al. (2023)

HY23	No added sugar	2.8	Mannose : glucose (82 : 18)	Zou et al. (2024)
KY471306	Molasses 12	7.88	Glucose, mannose and galactose	Moghannem et al. (2018)
MHM3	Sucrose 5	5.8	Glucuronic acid, glucose, fructose and rhamnose (4.00 : 2.00 : 1.00 : 0.13)	Mahgoub et al. (2018)
OM03	Glucose 5	0.594	Mannose (63.52%) and glucose	Chirakkara, Abraham (2023)

3.4. Purification and structural characterization of exopolysaccharide from *Bacillus velezensis* DTA1

3.4.1. Purification of EPSDTA1

EPSDTA1 cultured under optimal conditions was purified by Sephadex chromatography, yielding 51.25% with 97.07% purity.

3.4.2. SEM analysis of exopolysaccharide surface morphology

SEM images of EPSDTA1 (×500, ×1000) showed a dense three-dimensional network of long, intertwined fibrils, forming a compact matrix that contributes to its high viscosity and strong water-holding capacity.

3.4.3. Estimation of molecular weight of EPSDTA1

The molecular weight of the EPS was estimated based on its intrinsic viscosity (0.9556 dL/g) and was calculated to be approximately 1.9×10^5 Da.

3.4.4. Monosaccharide composition of EPSDTA1

HPLC analysis of purified EPSDTA1 revealed five monosaccharides: glucose, rhamnose, fructose, mannose, and N-acetylglucosamine (in a ratio of 2.59 : 12.75 : 8.49 : 1.00 : 12.18). These sugars contain hydrophilic functional groups such as hydroxyl, carbonyl, and amide, which contribute to water affinity and metal-binding capacity.

3.4.5. FT-IR analysis of functional groups

FT-IR spectra revealed that EPSDTA1 contains abundant hydroxyl, carbonyl, and carboxyl groups, which contribute to its strong hydrophilicity and ability to bind salts and metal ions such as Na^+, Pb^{2+}, and Cd^{2+}. These findings, consistent with HPLC and SEM results, indicate that EPSDTA1 has high potential for water retention, salinity reduction, and heavy metal adsorption.

3.5. Evaluation of the moisture-retaining and salinity-reducing capacity of EPSDTA1

3.5.1. Assessment of salt and metal adsorption capacity of EPSDTA1

3.5.1.1. Zeta potential of EPSDTA1

EPSDTA1 exhibited a high negative zeta potential (–30 mV), indicating a strong ability to adsorb cations.

3.5.1.2. Characteristics of exopolysaccharides under stress conditions

Table 3.2. Effects of salt and/or heavy metal stress conditions on the growth and EPS production of bacterial strain

TB medium, pH 8.46, with sucrose 72.6 g/L	**OD600**	**EPS yield (g/L)**	**Total sugar (% of EPS)**	**Total protein (% of EPS)**
without NaCl	22.93±0.35	19.27 ± 0.26	19.34 ± 0.46	12.06±0.33
3% NaCl	20.61±0.26	30.78 ± 0.38	40.81±0.56	25.90±0.88
1% NaCl, 1 µg/mL $Co(NO_3)_2$, 1 µg/mL $Cd(NO_3)_2$	18.27±0.34	29.33± 0.39	37.83±0.52	26.58±0.54
1% NaCl and $FeSO_4$, $CuSO_4$, Ag_2SO_4, $ZnCl_2$, $MgCl_2$, $MnSO_4$ salts all at 1 µg/mL	16.89±0.39	28.73±0.41	41.29±0.52	28.26±0.55

Values are presented as mean ± SD; n = 3.

Although bacterial growth decreased under stress, EPS yield and its carbohydrate and protein contents increased markedly compared with the

control (Table 3.2). Under non-stress conditions, EPS formed loose particles (1–5 μm in diameter), whereas at 3% NaCl, it exhibited a denser structure with adhesive colloidal layers filling the interparticle spaces. EDS spectroscopy showed that sodium and chlorine contents in salt-induced EPS increased from 0.73% to 9.03% and from 0.52% to 2.04%, respectively, indicating strong adsorption of Na^+ and Cl^- ions–particularly Na^+–through negatively charged functional groups (hydroxyl, carboxyl, carbonyl, amino, and phosphonate) on the polymer. Under combined salt and heavy-metal stress, EDS also detected Na^+, K^+, Mg^{2+}, Ca^{2+}, and trace metals such as Co^{2+}, Cd^{2+}, Mn^{2+}, Fe^{2+}/Fe^{3+}, Cu^{2+}, Zn^{2+}, and Ag^+, confirming the broad cation-binding capacity of EPSDTA1.

3.5.1.3. Ability of EPSDTA1 to reduce soil solution salinity

As predicted, the addition of EPS or the culture supernatant of *B. velezensis* DTA1 markedly reduced the concentration of soluble salts in soil pore water. Notably, in treatment T3, 1 mg of EPS adsorbed approximately 0.25 mg of salt. This result corroborates structural analyses showing that EPSDTA1 possesses numerous polar and negatively charged functional groups, along with a highly negative zeta potential (–30 mV), which facilitates cation binding. EDS evidence further confirmed the adsorption of Na^+ and Cl^- ions, explaining the observed salinity reduction in the treated soil solution.

3.5.2. Study on the moisture-retaining ability of EPSDTA1 and Bacillus velezensis DTA1 on Truong Sa coral sand

3.5.2.1. Moisture-retaining capacity of EPSDTA1

The WHC of EPSDTA1 increased rapidly within the first two hours, from 1001.8% (1 h) to 1762.2% (2 h), corresponding to 1 g of EPS absorbing 17.62 g of water, and subsequently reached saturation ($p > 0.05$). This value is considerably higher than several previously reported ranges (496–882%), highlighting the contribution of its three-dimensional network structure, high

molecular weight, and abundance of hydrophilic groups, which enhance water absorption and retention efficiency.

3.5.2.2. Moisture-retaining capacity of coral sand treated with EPSDTA1 and the culture broth of B. velezensis DTA1

Both EPSDTA1 and the culture broth of *B. velezensis* DTA1 significantly enhanced the WHC of coral sand. EPS addition reduced water loss by 4.95–7.83 times and increased WHC 1.6–2.0 times, reaching 66.31% compared to 33.17% in the control (coral sand). One gram of EPS retained up to 158.7 g of water, exceeding previously reported values. This improvement is attributed to the polymer's large molecular weight and hydrophilic, network-like structure that binds soil particles, enhances aggregation, and maintains long-term moisture. The combined use of organic substrate, bentonite, and EPS further doubled the WHC of coral sand.

3.6. Evaluation of the effectiveness of *B. velezensis* DTA1 in improving coral sand into cultivable soil under pot-scale experiment conditions

To validate the application potential of *B. velezensis* DTA1 in the amelioration of coral sand, a pot-scale experiment using *Basella alba* was conducted under net house conditions at the Vietnam National University of Agriculture.

3.6.1. Determination of exopolysaccharide production and soil aggregation

3.6.1.1. Results of soil carbohydrate content determination

The carbohydrate content, serving as an indicator of EPS, differed markedly among treatments. Control coral sand (T1) contained 212.77 µg/g, while organic amendments (T2, T4) increased it to 633.75–642.24 µg/g. Inoculation with *B. velezensis* DTA1 (T3, T5) further raised the content to 960.79–1045.34 µg/g, nearly fivefold higher than the control. Under salt stress (T7), EPS reached 1120.8 µg/g–5.27 times higher than T1 and 1.54

times higher than T6–indicating that strain DTA1 enhanced EPS synthesis as a salt-adaptive response.

3.6.1.2. Soil aggregation ability

Microscopic observations of the seven soil treatments revealed that in T1, T2, T4, and T6, soil particles were relatively loose and mostly smaller than 2000 μm. In contrast, samples TN3, TN5, and TN7 (supplemented with the EPS-producing strain DTA1) exhibited pronounced aggregation, forming clusters >2000 μm; particularly in TN5 and TN7, aggregates reached approximately 8000 μm. This aggregation is attributed to the EPS produced by strain DTA1, which acts as a binding matrix and contributes to enhanced water retention within these soil aggregates.

3.6.2. Evaluation of soil moisture retention at the time of vegetable harvest

The control coral sand (T1) had the lowest moisture content, while treatments containing organic substrate, bentonite, and *B. velezensis* DTA1 (T3, T5, T7) retained significantly more water ($p < 0.05$). Treatment T7 (substrate + bentonite + EPS + NaCl) maintained the highest moisture level (38.33–31.0%), about 2–2.5 times that of the control T1. These findings confirm the synergistic effect of EPS, bentonite, and NaCl in improving water retention and highlight their strong potential for rehabilitating saline, arid coral sand soils in coastal and island regions.

3.6.3. Evaluation of soil salinity reduction after harvest

Measurements of soil salinity after harvest revealed that in treatment T7 (saline irrigation + EPS-producing *B. velezensis* DTA1), the salt concentration was lower than in the control T6 (saline irrigation, without *B. velezensis* DTA1), although the difference was not statistically significant ($p > 0.05$). This trend is consistent with previous findings, demonstrating that EPS from strain DTA1 possesses salt-adsorbing properties, thereby reducing the concentration of free salts in the soil.

3.6.4. Evaluation of growth parameters of Basella alba

Table 3.3. Growth parameters of *Basella alba* under seven treatments evaluating the water-retaining and salinity-reducing effects of EPS-producing bacteria in pot-scale coral sand experiments

Treatment	Root length (cm)	Root width (cm)	Root-ball weight (g)	Shoot height (cm)	Shoot weight (g)	Number of leaves
T1	13.6±1.93^{b}	7.83±1.04$^{a.b.c}$	61.6±22.5^{c}	14.67±2.25$^{a.b}$	10.67±0.87^{d}	8±1$^{a.b}$
T2	15±1.5$^{a.b}$	6.83±1.61$^{b.c}$	64.9±20.1$^{b.c}$	16.33±2.75$^{a.b}$	14.73±1.21^{c}	7±1.73$^{b.c}$
T3	23.43±5.69^{a}	10.67±0.58$^{a.b}$	184.7±52.8$^{a.b}$	20.33±4.54$^{a.b}$	22.83±0.93$^{a.b}$	10±1^{a}
T4	15.93±2.7$^{a.b}$	9.67±1.63$^{a.b.c}$	173.4±46.6$^{b.c}$	18.83±2.84$^{a.b}$	20.67±0.9^{b}	10.33±0.58^{a}
T5	16.5±4.27$^{a.b}$	12.83±3.62^{a}	302.1±85.3^{a}	26.43±10.97^{a}	25.23±1.01^{a}	10.33±0.58^{a}
T6	8.63±4.05^{b}	5.33±1.26^{c}	60.17±8.98^{c}	12.23±0.93^{b}	10.2±0.85^{d}	5.33±0.58^{c}
T7	10.33±2.52^{b}	4.67±1.26^{c}	96.03±6.56$^{b.c}$	14.77±1.75$^{a.b}$	11.63±1.03^{d}	6.33±0.58$^{b.c}$

As shown on Table 3.3, the addition of organic substrate, bentonite, and *Bacillus velezensis* DTA1 markedly enhanced plant growth, with the best performance observed in the combined treatment (T5). Plants in T5 developed stronger roots, taller stems, broader canopies, and the highest biomass. Treatments with EPS or bentonite plus substrate (T3, T4) also improved growth compared to the substrate alone (T2) and the untreated control (T1). Under saline irrigation, the addition of strain DTA1 (T7) produced healthier, greener plants than without DTA1 (T6), indicating that the EPS-producing strain *B. velezensis* DTA1 not only improved soil structure and moisture retention but also enhanced plant salt tolerance on coral sand.

3.6.5. Evaluation of SPAD index

The SPAD index, an indicator of chlorophyll content and nitrogen status, was used to assess leaf health and soil amendment efficiency. Across seven treatments, SPAD values generally increased over time (15–30 days), except under saline stress (T6, T7). The addition of organic substrate (T2)

and its combinations with *B. velezensis* DTA1 (T3) or bentonite (T4) significantly improved SPAD compared with the control. The combined treatment (T5) achieved the highest SPAD value (45.67), 1.26–1.60 times higher than the control T1. Under saline conditions, SPAD in T6 declined sharply, whereas in T7 containing the EPS-producing strain, it remained stable and higher than T6. These results demonstrate the synergistic effect of organic substrate, bentonite, and *B. velezensis* DTA1 in enhancing chlorophyll content and mitigating salt stress.

A field trial at Dam Bay Station (Hon Tre Island) further verified that coral sand amended with EPS-producing, phosphate-solubilizing, and nitrogen-fixing bacteria plus organic substrate and bentonite improved soil fertility and increased vegetable yield by 4–5 times over untreated sand. Overall, the EPS and *B. velezensis* DTA1 plays a key role in improving soil moisture retention, reducing salinity, enhancing plant tolerance, and boosting crop productivity in coral sand environments.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

1. A bacterial strain, *Bacillus velezensis* DTA1, exhibiting tolerance to salinity and heavy-metal stress, was isolated and selected.

2. Sequencing the whole genome of *Bacillus velezensis* DTA1 and predicting metabolic pathways involved in EPS biosynthesis and tolerance to salt and metal stress.

3. Some physiological and biochemical characteristics, along with the EPS biosynthetic capability of *Bacillus velezensis* DTA1, were determined; the EPS produced by this strain was isolated and characterized.

4. The potential application of *Bacillus velezensis* DTA1 for coral sand improvement was preliminarily evaluated.

Recommendations

1. Further research should be conducted on the biological characteristics of *Bacillus velezensis* DTA1, along with the development of an EPS-producing microbial preparation for soil remediation.

2. Evaluating the effectiveness of coral sand improvement for the cultivation of various green plants and vegetables.

3. Implement pilot-scale field trials on coral islands in the Truong Sa (Spratly) Archipelago and in other arid and saline-affected areas.

NOVEL CONTRIBUTIONS OF THE DISSERTATION

1. This dissertation represents the first study in Vietnam to investigate drought and salt tolerance of the native bacterial strain *Bacillus velezensis* DTA1, from the genetic level to biological characteristics.

2. The dissertation demonstrated the potential application of *Bacillus velezensis* DTA1 for coral sand improvement.

LIST OF PUBLICATIONS RELATED TO THE DISSERTATION

1. **Le Thi Hue***, Vu Duy Nhan, Le Mai Huong, Do Thi Tuyen (2024), Selection of salt-tolerant bacterial strains capable of producing exopolysaccharide from coral islands in Khanh Hoa, *TNU Journal of Science and Technology*, 229(13), pp. 386-393. doi.org/10.34238/tnu-jst.10566. (ACI)
2. **Thi Hue Le***, Duy Nhan Vu, Thi Hoai Phuong Nguyen, Mai Huong Le, Cong Tinh Nguyen, Thi Tuyen Do, Van Thang Le, Mai Phuong Pham, Dinh Duy Vu* (2025), Amending coral soil using exopolysaccharide from salt-tolerant *Bacillus velezensis* TSD5 bacteria from an atoll in Vietnam, *Journal of Applied Biology & Biotechnology* 13(5), pp. 151-162. doi: 10.7324/JABB.2025.229116. (Scopus, Q3)
3. **Thi Hue Le***, Hoang Tuan Dinh, Duy Nhan Vu, Mai Huong Le, Cong Tinh Nguyen, Quang Cuong Hoang (2025), Characterization and exopolysaccharide production of *Bacillus velezensis* DTA1 from Vietnamese atoll soil, *Journal of degraded and mining lands management*, 12(4), pp. 8305 - 8314, doi:10.15243/jdmlm.2025.124.8305. (Scopus, Q3)
4. **Le H.T.***, Tran T.T.T.*, Nguyen S.T., La D.D., Vu N.D., Le M.H., Nguyen H.T. (2025), Genomic insights into salt-tolerant, exopolysaccharide-producing *Bacillus velezensis* DTA1 isolated from coral island soil in Vietnam: Implications for soil remediation, *One Ecosystem*, 10, pp. e158806. doi: 10.3897/oneeco.10.e158806. (ESCI, Scopus, Q1)