Assiut University Journal of Multidisciplinary Scientific Research (AUNJMSR)
Faculty of Science, Assiut University, Assiut, Egypt.
Printed ISSN 2812-5029
Online ISSN 2812-5037
Vol. 2 (1): 1- 19 (2025)
https://aunj.journals.ekb.eg

Production and Optimization of Bacterial Exopolysaccharides for Mitigation of NaCl-Salinity in *Vicia faba* seedling growth

Naeima M. H. Yousef[1], Eman E. Ali[1], Huwida A. A. Abdel-Kader[1], Eman S. E. Aldaby[1*]

[1]Department of Botany & Microbiology, Faculty of Science, Assiut University, 71516 Assiut, Egypt.
[*]Corresponding author email: emanaldaby@aun.edu.eg

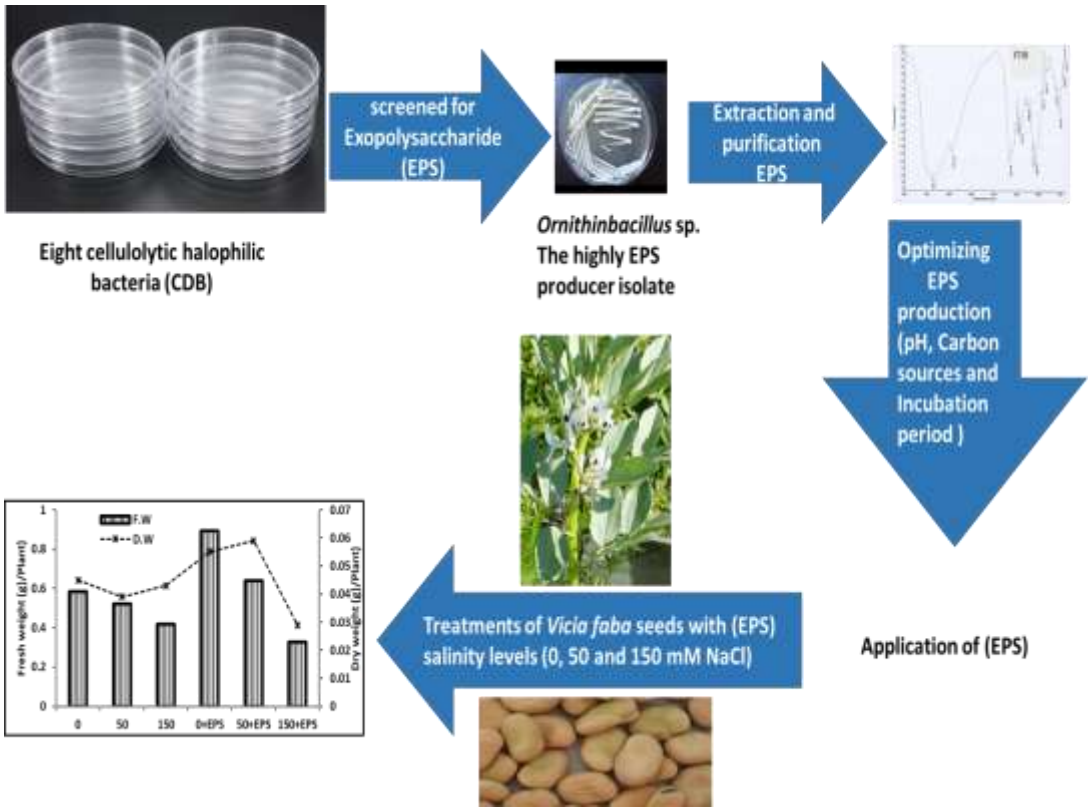
ARTICLE INFO
Article History:
Received:2024-11-10
Accepted:2024-12-09
Online: 2025-1-20

Keywords:
Exopolysaccharides, Halophilic bacteria, Salinity, *Vicia faba*

ABSTRACT

Exopolysaccharides (EPS) are high molecular weight polymers produced by several microorganisms, have unique properties to adapt to harsh environmental conditions, and play an important role in mitigation of abiotic stress. This work aimed to study the production of EPS by halophilic bacteria and their application on seed germination under salinity stress. Eight cellulolytic halophilic bacteria were screened for exopolysaccharides production. The highly EPS producer isolate was molecularly identified as *Ornithinbacillus* sp. strain BM10, accession number MZ648228. This bacterial strain produced about 7.32± 0.33 g L^{-1} of EPS at optimum growth conditions. The effect of bacterial EPS on seeds germination and early seedling growth of *Vicia faba* (Giza 843) at different salinity levels (0, 50 and 150 mM NaCl) has been studied. The morpho-physiological characteristics and the growth parameters of faba bean seedlings were negatively affected by NaCl stress. The EPS treatment (soaking for 6 hours) could alleviate salt stress on seeds germination (with a 20% increase in seed germination at 50 and 150 mM NaCl). As well as, water content, fresh and dry weight of seedling were enhanced. Also, photosynthetic pigment contents were increased by applying EPS. Chlorophylls (a + b) were improved by 165% in seedlings treated with bacterial EPS and grown in 150 mM NaCl. The Bacterial EPS can improve seed germination in stress environments and increase yield due to the role of EPS in salt stress reduction. Nevertheless, more research authentication would be needed before large scale/field application.

GRAPHICAL ABSTRACT



INTRODUCTION

Exopolysaccharides (EPS) are organic polymers exuded into the surrounding environment by microorganisms. These macromolecules can be detected in capsular material or as a scattered slime that has no specific cell connection [1]. In the marine environment, bacterial exopolysaccharide is required for formation of aggregates, adhesion to or colonized on surfaces, biofilm formation, and nutrient sequestration in the marine environment. Microbial polysaccharides assist the cell in colonizing hydrophobic surfaces and building biofilms, as well as coping with extreme circumstances such as salinity and temperature. Thus, EPS provides protection for bacterial and ecosystem stability [2- 4]. The attention to EPS has increased significantly, as they are used for many commercial applications in different industrial fields [5].

The majority of the studies have focused on discovering extremophiles that produce extracellular polysaccharides (EPS), assuming that these microbes feed on environmental extremes such as salt, acidity, temperature, pressure, and dryness. In order to adapt to such harsh circumstances, EPS is anticipated to possess certain special qualities as well

[6, 7]. There have been multiple reports of moderately halophilic bacterial species producing EPS. The polymers that these bacteria produce have potential uses as metal-binding, emulsifying, and jellying agents [8]. In the pharmaceutical business, sulfated EPS also offers intriguing uses due to its anticancer [9], antiviral [10, 11], and anticoagulant [12, 13] qualities.

Plant growth and biomass are affected by salt stress, due to lack of nutrients and water, as well as the expenditure of energy to fight the cytotoxic effects of NaCl [14]. Microorganism colonization potential is also hampered [15, 16]. Some plant growth-promoting rhizobacteria PGPR can secrete (EPS) to keep themselves and their plant hosts from environmental variations and other abiotic stresses like drought, heavy metal pollution, or salinity [17]. Plants can increase salt tolerance from EPS-producing microbes by binding to sodium ions in the soil and preventing these ions from reaching the stem, reducing Na ions absorption from soil and increasing nutrient uptake by the roots [18]. The EPS could be binding sodium ions from saline medium, thus relieving salt stress for geminating seeds [19]. More significantly, the capacity of marine microorganisms to produce polysaccharides at higher sugar concentrations is crucial for the creation of an economically viable polysaccharide manufacturing process because of their notable osmotic tolerance [20].
Salinity is one of the major limiting factors for crop growth, and germination is the most critical and key stage for successful crop establishment [21], it would be essential to investigate whether EPS application could promote better seed germination in the presence of salts in the medium.
Therefore, the present study was undertaken to screen, identify EPS producing halophilic bacteria and study the effects of bacterial exopolysaccharides (EPS) on seeds germination and early seedling growth of *Vicia faba* (faba bean) at different salinity levels of *Vicia faba* plants at different salinity levels.

MATERIALS AND METHODS

1. Screening of cellulolytic halophilic bacteria (CDB) isolates for EPS production

Eight cellulolytic halophilic bacteria (CDB) isolated from Wadi El-Natrun, Egypt were used in the current study according to Yousef et al. [22]. The CDB isolates were screened for exopolysaccharide (EPS) production.
For determining the EPS producing ability of the bacterial isolates, the bacterial isolates were grown on nutrient broth medium containing 5% NaCl at 30°C for 2 days. The growing bacterial isolates were inoculated in EPS production broth medium containing sucrose 25 g, $(NH_4)_2NO_3$ 2.8 g, KH_2PO_4 0.325 g, K_2HPO_4 0.325 g, $MgSO_4$ 0.2 g, distilled water 1L, pH 7. The bacterial cultures were inoculated in 50 mL of the medium in conical flasks (250 mL) in three replicates. Then, the flasks were incubated at 30° C with shaking at 100 rpm for 24 hours, followed by incubation in static incubator for 2-3 days [23]. The EPS collected and dried in an oven at 60 ºC then the dry weight was determined.

2. Extraction and purification of EPS

For production of EPS, the highly producer bacterial isolate was inoculated in EPS production broth medium. After incubation, the white pellicle on the surface of the culture medium was collected by centrifugation at 4000 rpm for 15 minutes. The EPS were purified as described by Gomaa and Yousef 202 [8]. Double volume of cold ethanol 70% was added to each conical flask and kept at 4 °C overnight, then centrifuged at 10,000 rpm for 10 minutes, and the pellet was dried in an oven at 60 °C.

3. Optimizing EPS production

Factors affecting EPS production by highly producer isolate were investigated using one factor at a time method. The optimized parameters were various carbon sources (sucrose, lactose, glucose, mannitol, and fructose). Studies were also performed to evaluate the influence of different incubation periods (1, 2, 3, 4, 5, and 6 days) at pH 7 and 30 ± 2ºC. To maximize EPS production by selected bacterial isolate, the medium was enriched with the best concentration of the most suitable carbon source and incubated at the best incubation period. All experiments were performed at pH 7 and 30 ± 2ºC.

4. Molecular identification of the highly producer bacterial isolate

The bacterial isolate that showed the highest EPS production was molecular identified by partial sequencing of the 16s rRNA gene using universal primers 27F and 1492R. The sequencing was performed by Solgent Company (South Korea). The obtained 16S rRNA gene sequences were compared with recognized (16S rDNA) sequences in the GenBank database through the National Center for Biotechnology Information's basic local alignment search tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST). The strain's sequence has been deposited in the GenBank nucleotide sequence database [22].

5. Fourier transformed infrared (FTIR) spectroscopy analysis of the produced EPS

Functional groups of the purified EPS were determined using Fourier transformed infrared (FTIR) spectroscopy. According to Yousef 2014 [24], infrared spectra of the purified EPS were recorded in the 4000–400 cm^{-1} region using an FTIR system (Thermo Scientific Nicolet iS10).

6. Application of Exopolysaccharides (EPS):-

➢ Treatment of *Vicia faba* seeds with the produced Exopolysaccharides (EPS)

Vicia faba seeds (Giza 843) were surface sterilized with ethyl alcohol 95% for 5 minutes, then with sodium hypochlorite 2% for 5 minutes, and the sterilized seeds were washed several times with distilled water. The seeds were subdivided into two groups:

- **The first group:** (reference control) contains the seeds that have been soaked in water only.
- **The second group:** contains the seeds that have been soaked in bacterial EPS (0.4g/L) for 6 hours.

The seeds were distributed in sterilized petri dishes and treated with NaCl (0, 50 and 150 mM) for each group. Seeds without NaCl were soaked in water as a control. Three replicates for each were applied.
The petri dishes were incubated for 7 days, and response of the seeds to various salinity levels and bacterial EPS was assessed. Then the percentage of germination (daily), fresh and dry weight, shoot and root length, vigor index and photosynthetic pigments in 7 days old seedlings were also determined.

- **Calculation of germination percentage:**

The percentage of germination was calculated daily [25] using the following formula:

Germination (%) = No. of germinated seeds/ total No. of seeds × 100

- **Shoot and root lengths**

The lengths of shoot and root of the plant under different treatments were recorded in mm and the shoot/root ratio was calculated.

- **Vigor index**

The following equation was used to calculate vigor index of the seedlings [26]:

Vigor index = (shoot length + root length) × germination percentage/100

- **Fresh weight, dry weight and water content**

Fresh weight of seedlings was immediately determined, oven dried at 80° C for 2 days to assess dry weight of the seedlings. Water content was calculated.

- **Estimation of photosynthetic pigments**

After 7 days of germination, 0.05 g fresh leaves were homogenized in 5 ml ethanol for photosynthetic pigments extraction [27].

RESULTS

1- Production of exopolysaccharides (EPS) by halophilic bacteria

The ability of eight cellulolytic halophilic bacterial isolates (CDB) to produce EPS was investigated. The bacterial isolates were grown on EPS producing medium containing sucrose as carbon source at 35ºC and pH 7. The results in (Figure1) showed that, among the eight tested isolates, the bacterial isolate CDB6 showed maximum EPS production (5.56 mg/mL) followed by the isolates CDB3 and CDB1 (4.23 and 3.93 mg/mL, respectively), while minimum EPS production was released by the isolate CDB4, about 0.58 g/mL (Figure 1).

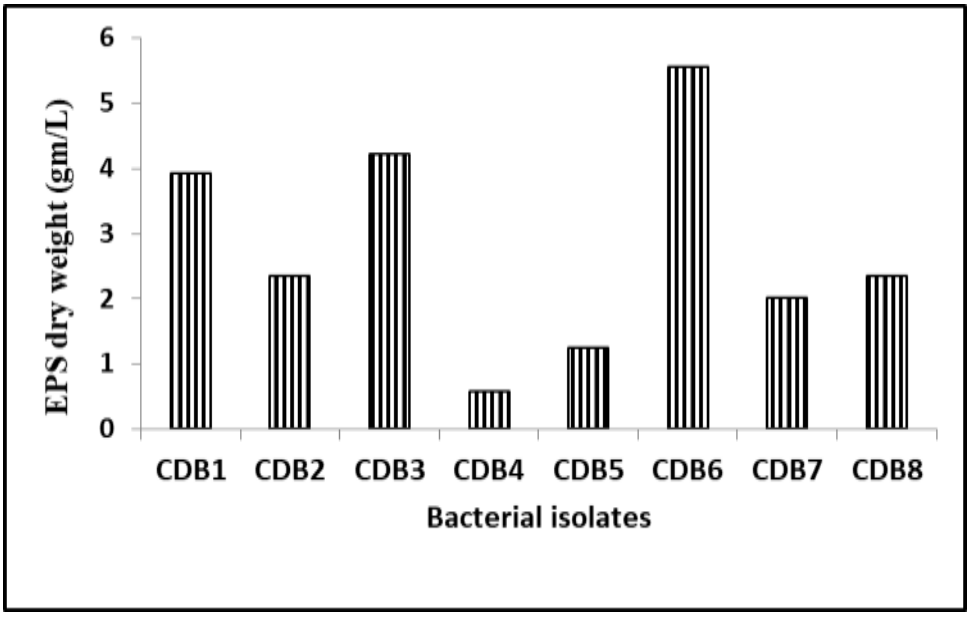


Figure 1: Production of EPS by CDB bacterial isolates on sucrose as a carbon source.

2- Production of EPS on different carbon sources

The highly EPS producer bacterial isolates (**CDB3 and CDB6)** were separately inoculated in different carbon sources (glucose, fructose, lactose, mannitol and sucrose) about 20 gm/L and incubated at 30 °C for 3 days. The results showed that the highly EPS production was observed on mannitol substrate followed by sucrose (Figure 2).

The purpose of the screening was to identify the essential carbon nutrients that affect EPS formation. Among the studied carbon sources, mannitol brought about the highest production of EPS by CDB3 isolate followed by sucrose for CDB6 (7.32 g L^{-1} and 5.94 g L^{-1} respectively), as shown in figure (2).

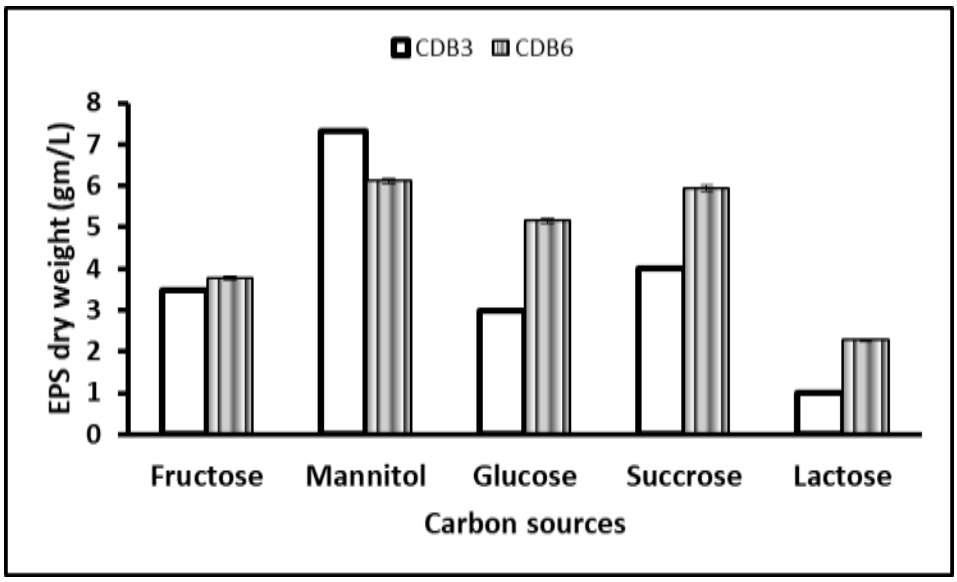


Figure 2: Effect of different carbon sources on the production of EPS by bacterial isolates CDB3 and CDB6.

3- Optimization of EPS production:

- **Effect of incubation periods on the EPS production by bacterial strain CDB3**

The suitable incubation period for EPS production by CDB3 was tested. The EPS production rate of the bacterial strain was measured as dry weight using 20 g/L mannitol as a sole carbon source at pH 7. The results represented in figure (3) revealed that, the highest EPS production was recorded at the fourth day. The total sugars of EPS were determined at the optimum production conditions (20 g/L mannitol, pH 7 and at 30 ºC).

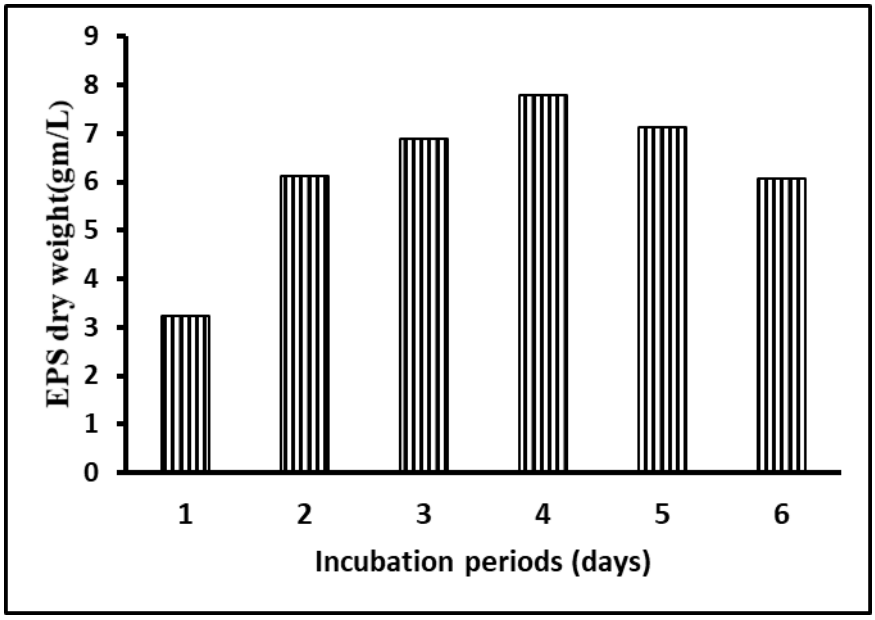


Figure 3: Production of EPS by CDB3 with using mannitol as carbon source for six days

4- Genotypic characterization of the EPS producer bacterial isolate

The highest producer bacterial isolate **CDB3**, for that molecular identification of bacterial isolate CDB3 was done based on the partial 16S rRNA gene sequences. The alignment results by using BLASTN search analysis showed that: the Sequencing of the 16S rRNA gene of the selected isolate CDB3 had the 16S rRNA gene with 97% matching to nucleotides of *Ornithinbacillus scapharcae* (MZ314100.1), *Ornithinbacillus californiensis* strain 9A (KJ575049.2) and 96% to *Ornithinbacillus bavariensis* strain WSBC (NR_044923.1). So, the halophilic isolate CDB3 was identified based on the sequence analysis of 16S rRNA genes as *Ornithinbacillus* sp. strain BM10 and deposited in GenBank under accession number MZ648228 [22].

5- Characterization of EPS by FTIR

The FTIR spectrum analysis of exopolysaccharides produced by CDB3 at the optimizing conditions was determined (Figure 4). The broad band at 3385.17 cm^{-1} and the signal at 2933.38 cm^{-1} represented the OH and CH stretching vibrations that confirmed the nature of polysaccharides. The peak observed at 1651.99 cm^{-1} may correspond to the ring stretching of mannose and galactose. While the band at 1454.76 cm^{-1} was related to the symmetric CH_2 bending vibration. The beak at 1255.29 cm^{-1} confirmed the presence of sulfated groups. The absorption beak at 1069.53 cm^{-1} was related to the glycosidic linkage (C\\O\\C) stretching vibration.

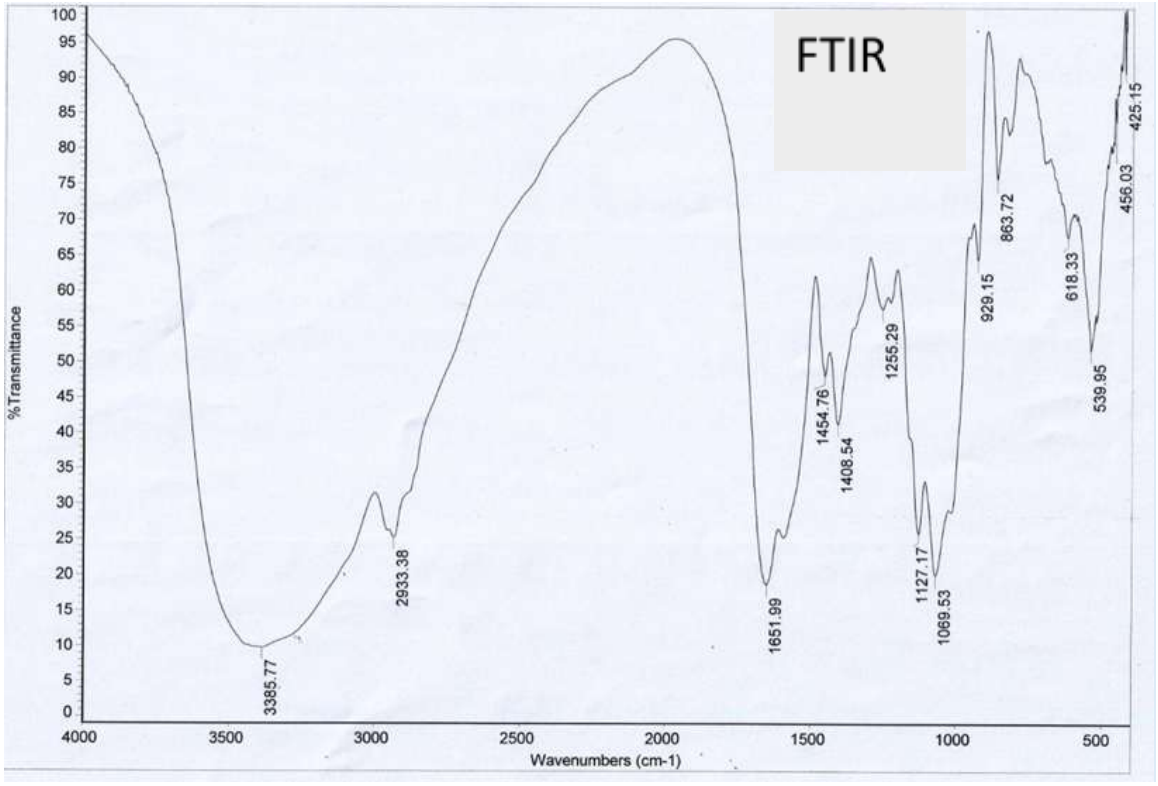


Figure 4: FTIR spectrum of EPS produced by CDB3.

6- Application of bacterial exopolysaccharide on *Vicia faba* seed germinated under salt stress

Table (1) shows the percentage of germination of the two tested groups of *Vicia faba* seeds: w the first group is the reference soaked in water, the second which soaked in bacterial EPS for 6 hours; and then seeds were germinated in 50 and 150 mM NaCl. Control was represented by seeds that soaked in water without NaCl and. The results indicated that, salinity has an obvious effect on seed germination, where germination percentage diminished with increasing salinity. The percentage of germination was similar in the first (soaking in H_2O) and second groups (soaking in EPS) where it recorded 80%, 60% and 0% at 0, 50 and 150 mM NaCl, respectively. In the second day, the germination percentage was relatively higher at seeds soaked in EPS than the reference group, reaching 100% at 50 mM NaCl, compared to 80% in reference group, while in 150 mM NaCl it reached 80% compared with 60% in reference group. It was noticed that, there is 20% improvement in seed germination with EPS application. On the third day, the rate of germination reached 100% in all treatments (Table 1).

Table 1: Percentage of germination (%) of *Vicia faba* seeds grown in (0, 50 and150 mM NaCl) for 7 days, after soaking in water or bacterial EPS.

Treatments		Seed Germination (%)		
	NaCl mM	**1 Day**	**2 Day**	**3 Day**
Seeds soaked in water	**0**	80	100	100
	50	60	80	100
	150	0	60	100
Seeds soaked in EPS	**0**	80	100	100
	50	60	**100**	100
	150	0	**80**	100

Figure (5) illustrate fresh and dry weight (g/plant) of variously treated *Vicia faba* seeds. Fresh and dry weight declined with salinity treatments. Soaking in EPS increased fresh and dry weight in seedling at 0 and 50 mM NaCl, compared with the reference group. It was recorded 22 and 37 % dry weight increase in 0 and 50 mM, respectively than in the reference group. High salinity condition (150 mM NaCl) decreased fresh and dry weight in the two groups.

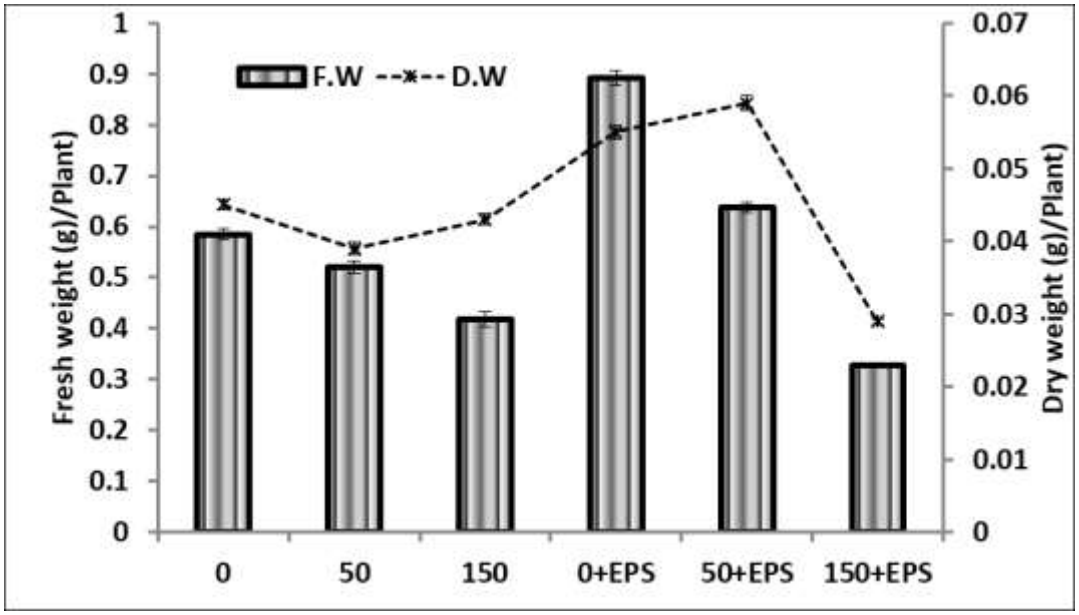


Figure 5: Fresh and dry weight (g/plant) of *Vicia faba* seedling grown in (0, 50 and 150 mM NaCl) for 7 days after soaking in water or bacterial EPS. Presented values are means of three replicates± SE.

Salinity (50 and 150 mM NaCl) has a noticeable effect on the shoot and root length of *Vicia faba* in the two groups. That, root length decreased to about half of the length which was documented at the corresponding control in the first group. Interestingly, the treatment of *Vicia faba* seeds with EPS enhanced shoot length at NaCl treatments by 68,

74.6 and 75 % respectively than that recorded in the first group. Soaking with EPS improved shoot/root ratio at all treatment at 150 mM NaCl (Figure 6).

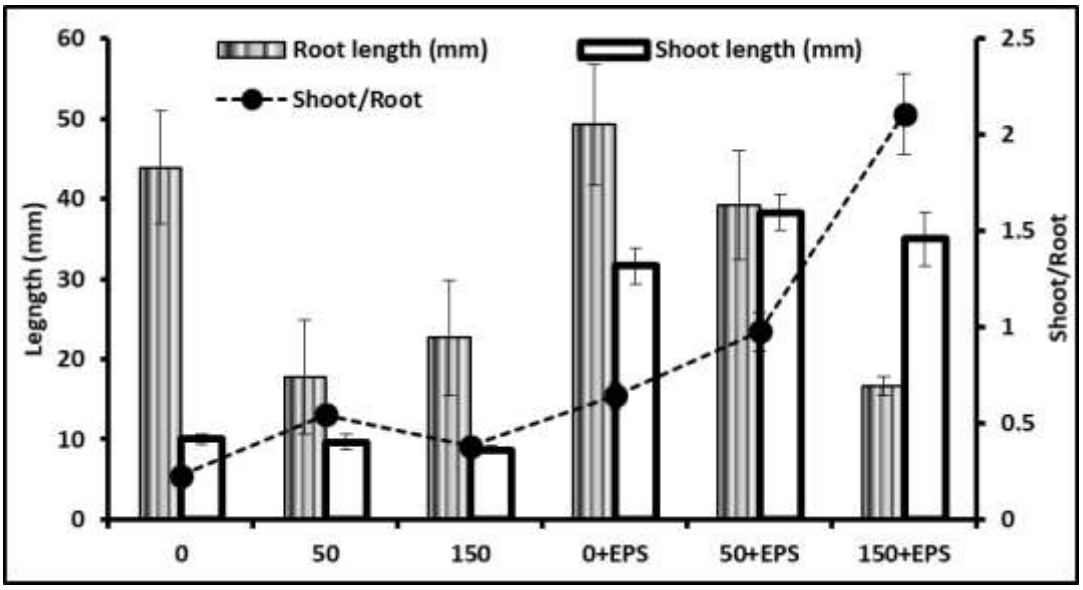


Figure 6: Shoot length, root length (mm) and shoot/ root ratio of *Vicia faba* seedling grown in (0, 50 and 150 mM NaCl) for 7 days after soaking in water or bacterial EPS. The presented values are means of three replicates ± SE.

Regarding to water content, salinity (50 and 150 mm NaCl) has adverse effect. On the other hand, water content was alleviated by soaking the seed in EPS at either 0 or 50 mm NaCl Figure (7). But the vigor index decreased to about half value at 50 and 150 mM NaCl, where they recorded 27 and 31%, respectively in comparison with control (54%). Soaking *Vicia faba* seeds with EPS has clearly improved vigor index at 0, 50 and 150 mM NaCl, up to 33, 65, 39%, respectively.

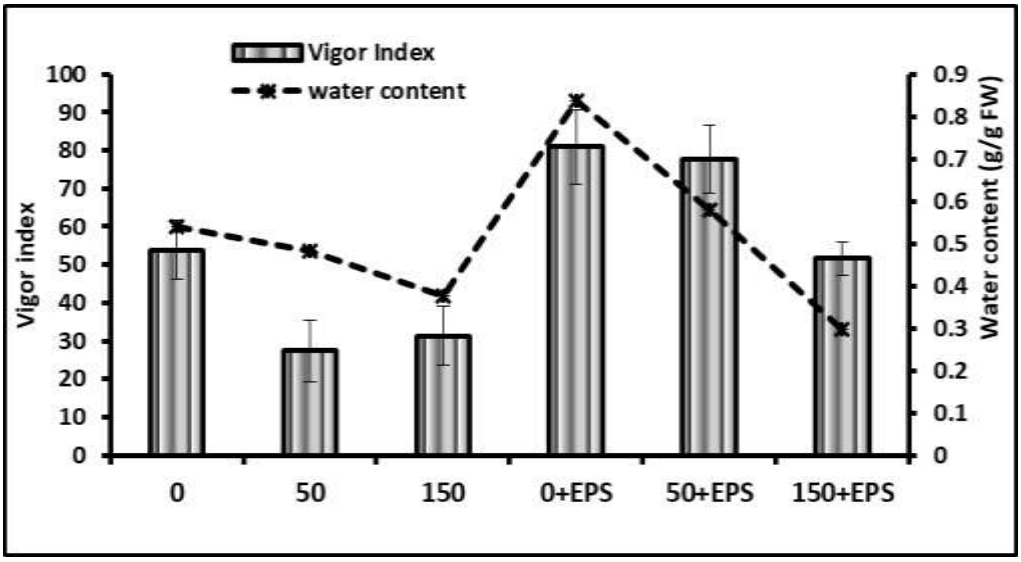


Figure 7: Water content (g/g fresh weight) and vigor index of *Vicia faba* seedling grown in (0, 50 and 150 mM NaCl) for 7 days after soaking in water or bacterial EPS. The presented values are means of three replicates ± SE.

Table (2) represents pigments content (Chlorophyll a, Chlorophyll b and Carotenoids) expressed as µg.gm^{-1} fresh weight of variously treated *Vicia faba* seeds. Pigments content severely diminished with salinity treatments in the two groups, but increased in seeds soaked in EPS under salinity treatments. Chlorophyll a+b values were higher at 50 and 150 mM NaCl than its counterpart in the reference group (37%, 165% increase, respectively). Also, carotenoids content increased by 12% and 78% at respectively 50 and 150 mM NaCl, using the EPS application.

Table 2 Chlorophyll (a+b) and carotenoids as µg.gm^{-1} leaves fresh weight of *Vicia faba* seeds germinated in (0, 50 and150 mM NaCl) after soaking in water or bacterial EPS.

Treatments		**Pigment contents**			
EPS	**NaCl mM**	**Chl a**	**Chl b**	**Chl.a+b**	**Carotenoids**
Seeds Soaked in water	**0**	4.598	1.235	5.833	4.266
	50	1.335	0.175	1.51	1.847
	150	0.89	0.12	1.01	1.227
Seeds Soaked in EPS	**0**	4.205	0.995	5.2	4.018
	50	1.705	0.365	2.07	2.067
	150	1.915	0.675	2.675	2.186

DISCUSSION

The EPS are extracellular metabolites, manufactured by some prokaryotic (bacteria, cyanobacteria, archae and actinomycetes) and some eukaryotic organisms (algae and fungi). Recently, there is a need for utilizing microorganisms to produce polysaccharides with several bioactivities [8]. Carbon source has a crucial role in the EPS production. This varies depending on the microbial strain; however, sucrose is usually used as a substrate to stimulate EPS production. In the studied carbon sources, mannitol brought the highest production of EPS by bacterial strain CDB3, followed by sucrose. However, mannitol does not appear to be a suitable carbon source for *Brevibacillus parabrevis* to produce EPS [28].

The current results revealed that most of the cellulolytic halophilic isolates produced high amounts of EPS from sucrose supplied in the medium. Similarly, sucrose induced

production of a high yield of EPS by some bacterial isolates [8,28,29]. Early trials by *Rhodothermus marinu*s showed that the usage of disaccharides as a carbon source mostly caused higher EPS production [30]. Lactose, galactose and glucose were not the best carbon source by *Stenotrophomonas maltophilia* for EPS production [28]. Janczarek and Skorupska [31] showed that using glucose as a sole carbon source had an inappropriate impact on EPS production by *Rhizobium leguminosarum*. While *Brevibacillus parabrevis* has high glucose assimilating ability for EPS production [32].

The incubation period of the microorganism for EPS production is a critical factor for optimizing the production. The results revealed that, the highest EPS production was observed in the fourth day of incubation. Also, Goma and Yousef [8] found that EPS production by moderately halophilic *Virgibacillus salarius* increased highly in the fifth day. The total sugars of EPS reached 81% at the optimum production conditions.

At the optimal conditions, the FTIR spectrum of EPS produced by CDB3 was determined. Broad band spectrum at 3385.77 cm^{-1} and the weak signal at 2933.38 revealed the OH and CH stretching vibrations, confirming the composition of a polysaccharide. The peak at 1651.99 cm^{-1}may correspond to galactose and mannose ring stretching [34, 35,8].

A symmetric CH_2 bending vibration was given to the band at 1454.76 cm^{-1} [36]. The presence of sulfated groups was confirmed by the tiny beak at 1255.29 cm^{-1} [37]. The glycosidic linkage (C\O\C) stretching vibration was also blamed for the high absorption at 1069 cm^{-1} [38].

Exopolysaccharide (EPS) is long chain biopolymer, contains repeating units of sugar moieties associated via glycosidic linkages [20]. The composition of polysaccharides can be used to distinguish between homo-polysaccharides, which include a single type of monosaccharide, and hetero-polysaccharides, which are made up of several residues and typically have a repeating unit and a regular backbone structure. These can contain up to 10 monomers and either organic or inorganic substituents, including phosphate, succinic, acetic, sulfate, lactic, and pyruvic acids, and can be linear or branched [39].

Many crops fail to germinate in saline circumstances, resulting in non-homogeneous germination and/or immature seedling development. High salt concentrations reduce the quantity of water accessible required for seed germination seedlings growth, respiration, photosynthesis, disrupt protein metabolism and harm the structure of several enzymes and macromolecules [40- 42]. Seed priming with EPS increased germination and get better physiological and biochemical properties. This improved growth and induced tolerance may also allow the seedlings to deal with extra environmental obstacles. The effect of exopolysaccharide (EPS) produced by cellulolytic-halophilic bacteria on germination of *Vicia faba* seeds was investigated under various salt concentrations. Our findings demonstrated that salinity has a negative impact on seed germination, which decreased as salinity increased. Osmotic imbalance and water scarcity are caused by increasing salt concentration [43, 44]. That, under saline conditions, there was an overabundance of reactive oxygen species (ROS) in the cytoplasm, resulting in ions toxicity in stressed plants [45]. When these poisonous ions can't be removed by plants,

they cause cellular structures to become distracted [45] that subsequently limit root and shoot growth. Furthermore, reserve cell division and expansion have been identified as a cause of reduced plant growth and vigor under salt stress.

A beneficial effect of bacterial EPS on seed germination was observed, with a 20% increase in seed germination at 50 and 150 mM NaCl. This acclaims that the seeds were able to withstand the elevated salt concentrations by using EPS produced by cellulolytic-halophilic bacterial isolates. In this regard, Arora et al 2010 [19] revealed that at 110 mM NaCl, the germination of wheat and maize was inhibited (54 and 32%, respectively), This implies that EPS plays a unique role in reducing the negative effects of salinity on seed germination.

Fresh and dry weight, as well as shoot and root length, of treated *Vicia faba* seeds all were reduced by salinity compared with control. These results are similar to that obtained by other researchers [46-48]. The reduction in growth and yield could be attributed to inhibit cell division, cell enlargement, and expansion [49]. NaCl affects the permeability of the plasma membrane and increases the influx of external ions (Na^+), the effluence of cytosolic solutes in plant cells and hardening of the cell wall [50, 51]. Interestingly, soaking of faba beans seeds in EPS increased dry weight and water content, as well as root length, at 0 and 50 mM NaCl, compared to the control group. In this regard, Ashraf et al 2004 [52] found that inoculating wheat seedlings with exopolysaccharides-producing bacterial strains significantly improved the dry weight of wheat seedlings cultivated in a moderately salty environment, with roots (149–522%) and shoots (85–281%). Bhagat et al 2021 [18] reported that bacterial exopolysaccharides ameliorate salinity stress by chelating free Na+ ion, making it available to plants, enhancing biofilm formation, and contributing to water retention.

According to the findings of Amna et al 2019 [53], EPS matrix produced by bacterial inoculation improved the root length of wheat seedlings under salt stress by lowering salt uptake through roots through trapping Na+ ions in it, this maintaining ionic balance of the plant [54, 55].

Salinity treatments severely repressed chlorophyll a +b in the reference set (٧٤% and 82% decrease in 50 and 150 mM, respectively), compared to the control (0 NaCl). but they improved in seeds soaked in 50 and 150 mM NaCl in the presence of EPS, compared to the reference set, with (37 and 165 %) increase in chlorophyll a+b respectively. Salinity reduced chlorophyll content in leaves at seven-day old seedlings by 15% in wheat, 76 percent in maize, and roughly 36% in rice, according to the study of Arora et al 2010 [19]. However, using EPS had a significant impact on salinity reduction, as evidenced by the recovery of photosynthetic pigment content. At 55 mM Na^+, there was 30-53 % increase in chlorophyll content in the leaves of the three crops, and at 110 mM Na^+, there was a 22-56 % increase. Salinity stress (NaCl) causes more quick pigment degradation or a reduction in chlorophyll production. However, plant growth promoting rhizobacteria (PGPR) enhanced the recognition of photosynthetic pigments, boosting the development of antioxidant enzymes that quench excessive ROS and maintain membrane integrity [56].

Salinity also influenced carotenoids content; *Vicia faba* plants subjected to higher levels of NaCl stress had lower carotenoids content in all treatments. When plants were grown under salt stress, their carotenoid concentration was lower than when they were grown without it. Similar findings on wheat plants were reported by [57]. EPS increased the

concentration of carotenoids, which function as antioxidants to stabilize and protect photochemical processes during photosynthesis in a stressful environment.
When comparing with a reference control, soaking *Vicia faba* seeds in EPS improved salinity resistance by increasing water content, fresh and dry weight, and photosynthetic pigments content.

CONCLUSION

The current research showed that halophilic bacteria are potent exopolysaccharides (EPS) producer strains. The strain *Ornithinbacillus* sp. may be considered highly favorable option as future cheap sources for the EPS production and offer important biotechnological potential. Additionally, it has been found that application of the EPS (soaking for 6h) on seeds grown under salt stress, could improve seeds germination, photosynthetic pigment contents and enhance the fresh and dry weight, shoot and root length and water content of seedling. Finally, the current study provide the prospect of evaluating the capability of EPS as a biofertilizer in field conditions to alleviate salt stress in germinating seeds and growing plants.

ABBRAVIATIONS

EPS: Exopolysaccharides; **PGPR**: plant growth-promoting rhizobacteria; **CDB**: cellulolytic decomposed bacteria; **FTIR**: Fourier transformed infrared.

REFERENCES

1) I. W., Sutherland. Biosynthesis of microbial exopolysaccharides, Adv Microbial Phys., (1982); 23:79-150.
2) Z .Chi, Y. Fang, Exopolysaccharides from Marine Bacteria, Journal of Ocean University of China (Oceanic and Coastal Sea Research), (2005); 4:67-74.
3) K. M. Lynch, A. Coffey, E. K., Arendt, Exopolysaccharide producing lactic acid bacteria: Their techno-functional role and potential application in gluten-free bread products, Food Research International, (2017), 110:52-61.
4) C. M. Nichols, J. Guezennec , J. P. Bowman, Bacterial exopolysaccharides from extreme marine environments with special consideration of the Southern Ocean, sea ice, and deep-sea hydrothermal vents: a review. Marine biotechnology, (2005), 7: 253-271.

5) E. Zannini, D. M. Waters, A. Coffey and E. K. Arendt, Production, properties and industrial food application of lactic acid bacteria-derived exopolysaccharides. Appl Microbiol Biotechnol. (2016), 100:1121–1135.
6) A. Poli, H. k. Kaza, B .Gürleyendag, G. Tommonar, G. Pieretti , E.T. Öner, B. Nicolaus, High level synthesis of levan by a novel *Halomonas* species growing on defined media Carbohydrate Polymers, (2009), 78: 651-657.

7) A. Poli , G. Anzelmo , B. Nicolaus, Bacterial exopolysaccharides from extreme marine habitats: production, characterization and biological activities. Marine Drugs, (2010), 8 :1779-1802.
8) M. Gomaa and N. Yousef. Optimization of production and intrinsic viscosity of an exopolysaccharide from a high yielding *Virgibacillus salarius* BM02: Study of its potential antioxidant, emulsifying properties and application in the mixotrophic cultivation of Spirulina platensi. International Journal of Biological Macromolecules, (2020), 149(1): 552-561.
9) K. Inoue, M. Saito, T. Kanai , T. Kawata , N. Shigematsu , T. Uno , Kouichi Isobe Cui-Hua Liu and Hisao Ito, Anti-Tumor Effects of Water-Soluble Propolis on a Mouse Sarcoma Cell Line *In Vivo* and *In Vitro*, The American Journal of Chinese Medicine, (1988), 36: 03.
10) A. Oren, Industrial and environmental applications of halophilic microorganisms. Environmental Technology, (2010), 31: (9) 8- 825-834.

11) W. Zheng, C. Chen, Q. Cheng, Y. Wang, C. Chu, Oral administration of exopolysaccharide from *Aphanothece halophytica* (Chroococcales) significantly inhibits influenza virus (H1N1)-induced pneumonia in mice. International Immunopharmacology, (2006), 6 1093-1099.
12) T. Nishino, G. Yokoyama, K. Dobashi, M. Fujihara, T. Nagumo, Isolation, purification, and characterization of fucose-containing sulfated polysaccharides from the brown seaweed *Ecklonia kurome* and their blood-anticoagulant activities. Carbohydrate Research, (1989), 186 :119-129.
13) Sutherland IW, Biotechnology of Microbial Exopolysaccharides, (1990), (Vol. 9). Cambridge University.
14) M. Yildiz, I. Poyraz., A. Çavdar, Y. Özgen, R. Beyaz, Plant Responses to Salt Stress. In (Ed.), Plant Breeding - Current and Future Views. IntechOpen, (2020).
15) F. Jahromi, R. Aroca, R. Porcel and J. M.Ruiz-Lozano, Influence of salinity on the *in vitro* development of *Glomus intraradices* and on the *in vivo* physiological and molecular responses of mycorrhizal lettuce plants, Microb. Ecol. (2008), 55: 45-53.

16) N. M. H. Yousef and N. A. Hussein, Impacts of inoculation with *rhizobium leguminosarum* and arbuscular mycorrhizae and phosphate on *vicia faba* l. grown in salt stressed soil. Bangladesh j. bot., (2017), 46(2): 599-605.
17) R. J. L. Morcillo, M. Manzanera, The Effects of Plant-Associated Bacterial Exopolysaccharides on Plant Abiotic Stress Tolerance. Metabolites, (2021), 11(6):337.

18) N. Bhagat, M. Raghav , S. Dubey , N. Bedi, Bacterial Exopolysaccharides: Insight into Their Role in Plant Abiotic Stress Tolerance. J Microbiol Biotechnol, (2021), 28;31(8):1045-1059.
19) M. Arora, A. Kaushik,N. Rani, C. P. Kaushik, Effect of cyanobacterial exopolysaccharides on salt stress alleviation and seed germination. J Environ Biol., (2010), 31: 701.
20) A. Mehta, C. Sidhu, A. K. Pinnaka, A. R. Choudhury, Extracellular polysaccharide production by a novel osmotolerant marine strain of *Alteromonas macleodii* and its application towards biomineralization of silver. Plos One, (2014), 9 (6): e98798.
21) A. Soltani, M. Gholipoor, E. Zeinali, Seed reserve utilization and seedling growth of wheat as affected by drought and salinity. Environ. Exp. Bot., (2006), 55:195-200
22) N. M. H. Yousef, E. S. E. Aldaby, E. E. Ali, Optimization of the cellulose enzyme production by *Brevibacterium halotolerans* isolated from Wadi-El Natrun, Egypt. Assiut Univ. J. of Botany and Microbiology, (2020), 49:14-33.
23) E. Prabhavati, K. V. Mallaiah, Extracellular polysaccharide production by *Rhizobium* sp. *nodulating* Macrotyloma uniflorum (Lam.) Verdc, Asian Journal of Bio Science, (2009), 4: 20-23.
24) N. M. H. Yousef, Characterization and antimicrobial activity of silver nanoparticles synthesized by rice straw- utilizing bacterium (*Lysinibacillus fusiformis*). International Journal of Development Research, (2014), 4: 1875-1879.
25) H. Manmathan, N. L. Lapitan, Measuring germination percentage in wheat. Bioprotocol, (2013), 3 (16): e866.
26) A. S. Dahindwal, B. P. S. Lather, J. Singh, Efficacy of seed treatment on germination, seedling emergence and vigor of cotton (Gossypium hirsutum) genotypes. Seed Res, (1991), 19:59-61.
27) L. Arvola, Spectrophotometric determination of chlorophyll-a and phaeopigments in ethanol extractions. Ann Bot Fennici, (1981), 18: 221-227.
28) M. H. Abd‐ Alla, S. R. Bashandy, N. A. Nafady, A. A. Hassan, Enhancement of exopolysaccharide production by *Stenotrophomonas maltophilia* and *Brevibacillus parabrevis* isolated from root nodules of *Cicer arietinum* L. and *Vigna unguiculata* L. (Walp.) plants. Rendiconti Lincei. Scienze Fisichee Naturali. (2018).
29) T. C. L. Castellane, M. V. F. Lemos, E. G. deMacedo Lemos, Evaluation of the biotechnological potential of *Rhizobium tropici* strains for exopolysaccharide production. Carbohydr Polym., (2014), 111:191–197.
30) R. R. Sardari, E. Kulcinskaja, E. Y. Ron, S. Björnsdóttir, H. Friðjónsson, G. O. Hreggviðsson, E. N. Karlsson, Evaluation of the production of exopolysaccharides by two strains of the thermophilic bacterium *Rhodothermus marinus*. Carbohydr Polym., (2017), 156:1–8.
31) M. Janczarek, A. Skorupska, *Rhizobium leguminosarum* bv. *Trifolii* rosR gene expression is regulated by catabolic repression. FEMS Microbiol Lett., (2009), 291:112–119.

32) K. Nouha, S. Yan, R. D. Tyagi, R. Y. Surampalli, EPS producing microorganisms from municipal wastewater activated sludge. Pet Environ Biotechnol. (2015), 7:1.
33) A. Malick, N. Khodaei, N. Benkerroum, S. karboune, Production of exopolysaccharides by selected *Bacillus* Strains: optimization of media composition to maximize the yield and structural characterization. Int.J.Bio.Macromol, (2017), 102: 539-549.
34) K. Kavita, A. Mishra, B. Jha, Isolation and physic-chemical characterization of extracellular polymeric substances produced by the marine bacterium Vibrio parahaemolyticus, Bio fouling, (2011), 27:309-317.
35) P. R. Rani, M. Anandharaj, P. Sabhapathy. D. A. Ravindran, Physicochemical and Biological characterization of novel exoplysaccharide produced by *Bacillus tequilensis* FR9 isolated from chicken. Int. J. Biol. Macromol, (2017), 96:1-10.
36) A. Spatareanu, M. Bercea, T. Budtova,V. Harabagiu, L. Sacaresc and S. Coseri. Synthesis, Characterization and Solution behavior of oxidized pullulan. Carohydr, Polym., (2014), 111: 63-71.
37) X. Wang, C. E. Sharp, G. M. Jones, S. E. Grasby, A. L. Brady and P. F. Dunfield, Stable-isotope probing identifies uncultured planctomycetes as primary degraders of a complex heteropolysaccharide in soil. Appl. Environ. Microbiol. (2015), 81: 4607–4615.
38) M. S. Asker, H. Osama El Sayed, G.M. Manal, M. Y. Shaymaa, S. M. Sahar, S. S Manal, S. Mohamed, El Awady, M. A. Salma, M. A. Mostafa, Production of exopolysaccharides from novel marine bacteria and anticancer activity against hepatocellular carcinoma cells (HepG2) Bulletin of the National Research Centre, (2018), 42:30.
39) C. Delbarre-Ladrat, S. Corinne, L. Lou, Z. Agata, C. Sylvia, Exopolysaccharides produced by marine bacteria and their applications as glycosaminoglycan-like molecule. Frontiers in Chemistry, (2014), 226464-2296.
40) A. S. Ali, A. A. Elozeiri. Metabolic Processes during Seed Germination. In (Ed.), Advances in Seed Biology, IntechOpen (2017).
41) M. A. Ahanger, N. S. Tomar, M. Tittal, S. Argal, R. M. Agarwal, Plant growth under water/salt stress: ROS production; antioxidants and significance of added potassium under such conditions, Physiology and molecular biology of plants: An international journal of functional plant biology, (2017), 23: 731–744.
42) Y. Ma, M. C. Dias, H. Freitas, Drought and Salinity Stress Responses and Microbe-Induced Tolerance in Plants Frontiers in Plant Science, (2020), 23: 731–744.
43) G. Ilangumaran, D. L. Smith, Plant Growth Promoting Rhizobacteria in Amelioration of Salinity Stress: A Systems Biology Perspective. Frontiers in Plant Science, (2017), 8 :1768 .
44) M. Hasanuzzaman, K. Nahar, M. Fujita, Plant response to salt stress and role of exogenous protectants to mitigate salt-induced damages. In *Ecophysiology and Responses of Plants under Salt Stress*; Ahmad, P, Springer: New York, NY, USA, (2013), 25–87.
45) R. Munns, M. Tester, Mechanisms of salinity tolerance. Annu. Rev. Plant Biol., (2008), 59:651–681.

46) M.A. Fathalla, Effectiveness of exopolysaccharides and biofilm forming plant growth promoting rhizobacteria on salinity tolerance of faba bean (*Vicia faba* L.). Afr. J. Microbiol. Res., **(**2018), 12:399–404

47) J. L Castanares, C.A. Bouzo, Effect of exogenous melatonin on seed germination and seedling growth in melon (*Cucumis melo* L.) under salt stress. *Hortic. Plant J.*, (2019), 5: 79–87.

48) M. F. Abd El-Ghany, M. Attia, Effect of Exopolysaccharide-Producing Bacteria and Melatonin on Faba Bean Production in Saline and Non-Saline Soil. *Agronomy*, (2020), 10: 316.

49) A. A. Radi, F. A. L. Farghaly, A. M.Hamada, Physiological and biochemical responses of salt-tolerant and salt-sensitive wheat and bean cultivars to salinity. *J. Biol. Earth Sci.*, (2013), *3*: 72–88.

50) G. J. Allen, R. G.Wyn Jones, R. Leigh, Sodium transport measured in plasma membrane vesicles isolated from wheat genotypes with differing K^+/Na^+ discrimination traits. Plant Cell Environ., (1995**),** 18: 105–115.

51) M. Nabil, A. Coudret, Effects of sodium chloride on growth, tissue elasticity and solute adjustment in two *Acacia nilotica* subspecies. *Physiol. Plant*, (1995**),** *93*:217–224.

52) M. Ashraf, S. Hasnain, O. Berge, T. Mahmood, Inoculating wheat seedlings with exopolysaccharide-producing bacteria restricts sodium uptake and stimulates plant growth under salt stress. Biol. Fertil. Soils, (2004), 40: 157–162.

53) B. Amna, Din, S. Sarfraz, Y. Xia, M. A. Kamran, M. T. Javed, T. Sultan, M. Farooq, H. Munis, H. J. Chaudhary, Mechanistic elucidation of germination potential and growth of wheat inoculated with exopolysaccharide and ACC-deaminase producing Bacillus strains under induced salinity stress، Ecotoxicology and Environmental Safety, (2019), 183:109466.

54) T. Atouei, A. A. Pourbabaee, M. Shorafa, Alleviation of Salinity Stress on Some Growth Parameters of Wheat by Exopolysaccharide-Producing Bacteria Maryam. Iran J Sci Technol Trans Sci., (2019), 43:2725–2733

55) R. Radhakrishnan, K H. Baek, Physiological and biochemical perspectives of non-salt tolerant plants during bacterial interaction against soil salinity. Plant Physiology and Biochemistr, (2017)، 116: 116-126.

56) M. S. Afridi, Amna, Sumaira, T. Mahmood, A. Salam, T. Mukhtar, S. Mehmood , Z. Ali Khatoon, M. Bibi, M. T. Javed, T. J. Sultan, H. J. Chaudhary, Induction of tolerance to salinity in wheat genotypes by plant growth promoting endophytes: involvement of ACC deaminase and antioxidant enzymes. Plant Physiol. Biochem., (2019), 139: 569–577.

57) A. Akcin, E. Yalcin, Effect of salinity stress on chlorophyll, carotenoid content,and proline in Salicornia prostrata Pall. and Suaeda prostrata Pall.subsp. prostrata (Amaranthaceae). Braz. J. Bot., (2016), 39(1):101–106.