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SALT-INDUCED EXOPOLYSACCHARIDE PRODUCTION IN *AZOTOBACTER CHROOCOCCUM* STRAINS UNDER NaCl AND Na₂SO₄ STRESS CONDITIONS

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Abstract. Salinity is one of the major environmental factors limiting the growth and metabolic activity of beneficial soil microorganisms. The present study investigated the effects of NaCl and Na₂SO₄ on biomass accumulation and exopolysaccharide (EPS) production by *Azotobacter chroococcum* strains XH2018 and XH2020. The strains were cultivated in Ashby medium supplemented with 0.5%, 1.0%, and 2.0% concentrations of NaCl or Na₂SO₄. Biomass and EPS production were determined after cultivation under shaking conditions.

The results showed that both strains were able to grow under salt stress; however, their responses varied depending on the salt type and concentration. Strain XH2020 generally produced higher biomass than strain XH2018 under most experimental conditions. The highest biomass values were recorded at 2% NaCl and 0.5% Na₂SO₄, reaching 6.2 g/L in strain XH2020. Salt stress also significantly influenced EPS biosynthesis. Under NaCl treatment, moderate increases in EPS production were observed in both strains. In contrast, Na₂SO₄ strongly stimulated EPS synthesis, particularly in strain XH2020. The highest EPS yield (15.0 g/L) was obtained at 2% Na₂SO₄, whereas the maximum EPS production by strain XH2018 was 4.4 g/L under the same conditions. These findings indicate that sulfate-induced salt stress enhances EPS production more effectively than NaCl and that strain XH2020 possesses superior adaptive mechanisms for coping with saline environments. The high EPS-producing capacity of strain XH2020 under Na₂SO₄ stress highlights its potential for agricultural and biotechnological applications in saline environments.

Keywords: *Azotobacter chroococcum*, exopolysaccharides, EPS, salinity stress, NaCl, sodium sulfate, biomass production, alginate.

Introduction. Salinity stress is among the leading environmental challenges threatening sustainable crop production globally. By 2050, nearly 50% of the world's agricultural land is expected to be affected by salinization [5]. Salinity stress negatively influences plant functions at physiological, biochemical and molecular levels. It disrupts nutrient transport, alters hormone regulation, triggers the accumulation of reactive oxygen species and causes both ionic toxicity and osmotic imbalance, all of which contribute to stunted plant growth [6,7]. Salinization of agricultural land mainly occurs due to the accumulation of salts in the soil, especially sodium and chloride ions. Apart from Cl^- ions, sulfate ions $-\text{SO}_4$ also form salts in soils and create adverse conditions for plant growth and development [8]. Enhanced Na^+ accumulation restricts soil porosity, aeration and water conductivity. Additionally, the microbial diversity within and around plant roots is adversely impacted by soil salinity stress [6,9]. Numerous bacteria are found in the rhizosphere of plants and these plant growth-promoting rhizobacteria play a crucial role in mitigating soil stress, to plants may be able to adapt to salinity stress [9,10].

Recent surveys indicate that more than 80% of irrigated agricultural lands in Uzbekistan are affected by salinity [3]. Conventional agriculture production methods have proven ineffective under such conditions. Seeking alternative and supplementary biofertilizers to replace traditional NPK fertilizers is of paramount importance. In this respect, microbe-based biofertilizers and biomaterials have attracted considerable interest due to their biocompatibility and cost-effectiveness. Bacteria of the *Azotobacter* genus have been successfully applied as biofertilizers, since they fix molecular nitrogen and produce a wide variety of biologically active compounds [3,11]. *Azotobacter* can boost the immune response of plants against stress [11], but direct salt stress mitigation by enhanced Na^+ removal by their exocellular biopolymers has not been fundamentally studied.

Therefore, the aim of the present study was to evaluate the effects of NaCl and Na_2SO_4 at different concentrations on biomass accumulation and exopolysaccharide production by *Azotobacter chroococcum* strains XH2018 and XH2020. The findings provide new insights into the adaptive responses of these strains to saline stress and their potential application in saline agricultural environments.

2. Materials and Methods

2.1. Bacterial strains and cultivation conditions

Azotobacter chroococcum strains XH2018 and XH2020 obtained from the Culture Collection of Phytopathogenic and Unique Microorganisms of the Institute of Genetics and Experimental Plant Biology, Academy of Sciences of Uzbekistan, were used in this study. The 16S rRNA gene sequence of strain XH2018 is available in GenBank under accession number PQ862345.1. The strains were cultivated in Ashby broth containing (g/L): sucrose, 20.0; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2; KH_2PO_4 , 0.2; NaCl , 0.1; and CaCO_3 , 10.0. The initial pH of the medium was adjusted to 7.0 before sterilization.

2.2. Salt stress treatments

To evaluate the effect of salt stress on bacterial growth and exopolysaccharide production, NaCl and Na₂SO₄ were added separately to the culture medium at concentrations of 0.5%, 1.0%, and 2.0% (w/v). Cultures grown without additional salt served as controls.

2.3. Biomass production

The strains were cultivated in 100 mL of Ashby broth and incubated at 30°C on a rotary shaker at 150 rpm for 72 h. After cultivation, bacterial biomass was harvested by centrifugation at $10,000 \times g$ for 15 min, washed twice with distilled water, and dried to constant weight. Biomass production was expressed as g/L of culture medium.

2.4. EPS isolation and quantification

EPS production was carried out in Ashby medium under the same cultivation conditions. After incubation, bacterial cells were removed by centrifugation at $10,000 \times g$ for 15 min. The supernatant was mixed with three volumes of ice-cold 95% ethanol and kept for EPS precipitation. The precipitated EPS was collected by centrifugation at $10,000 \times g$ for 30 min, washed twice with 95% ethanol and once with absolute ethanol, and dried to constant weight. EPS yield was expressed as g/L of culture medium.

3. Results

3.1. Effect of salt stress on biomass production

The growth of *Azotobacter chroococcum* strains XH2018 and XH2020 was affected differently by NaCl and Na₂SO₄ treatments (Table 1, Figure 1). Under NaCl stress, biomass production of strain XH2018 increased from 3.6 g/L in the control to 4.9 g/L at 2% NaCl. In strain XH2020, biomass accumulation increased from 4.9 g/L in the control to 6.2 g/L at 2% NaCl. In the presence of Na₂SO₄, strain XH2018 produced the highest biomass at 1% Na₂SO₄ (5.6 g/L), while strain XH2020 reached a maximum biomass of 6.2 g/L at 0.5% Na₂SO₄. Overall, strain XH2020 maintained higher biomass production than strain XH2018 under most salt stress conditions, indicating greater tolerance to salinity.

Salt type	Concentration (%)	Biomass (g/L) Strain 2018	EPS (g/L) Strain 2018	Biomass (g/L) Strain 2020	EPS (g/L) Strain 2020
Control	0	3.6	0.6	4.9	1.15
NaCl	0.5	2.3	-	5.4	2.34
NaCl	1	4.6	1.3	5.6	1.849
NaCl	2	4.9	1.1	6.2	1.097
Control	0	4.1	0.07	5.9	1.313
Na ₂ SO ₄	0.5	5.2	-	6.2	0.9
Na ₂ SO ₄	1	5.6	-	5.7	3.2
Na ₂ SO ₄	2	5.4	4.4	5.9	15

Table 1. Effect of NaCl and Na₂SO₄ concentrations on biomass accumulation and EPS production of *Azotobacter chroococcum* strains 2018 and 2020

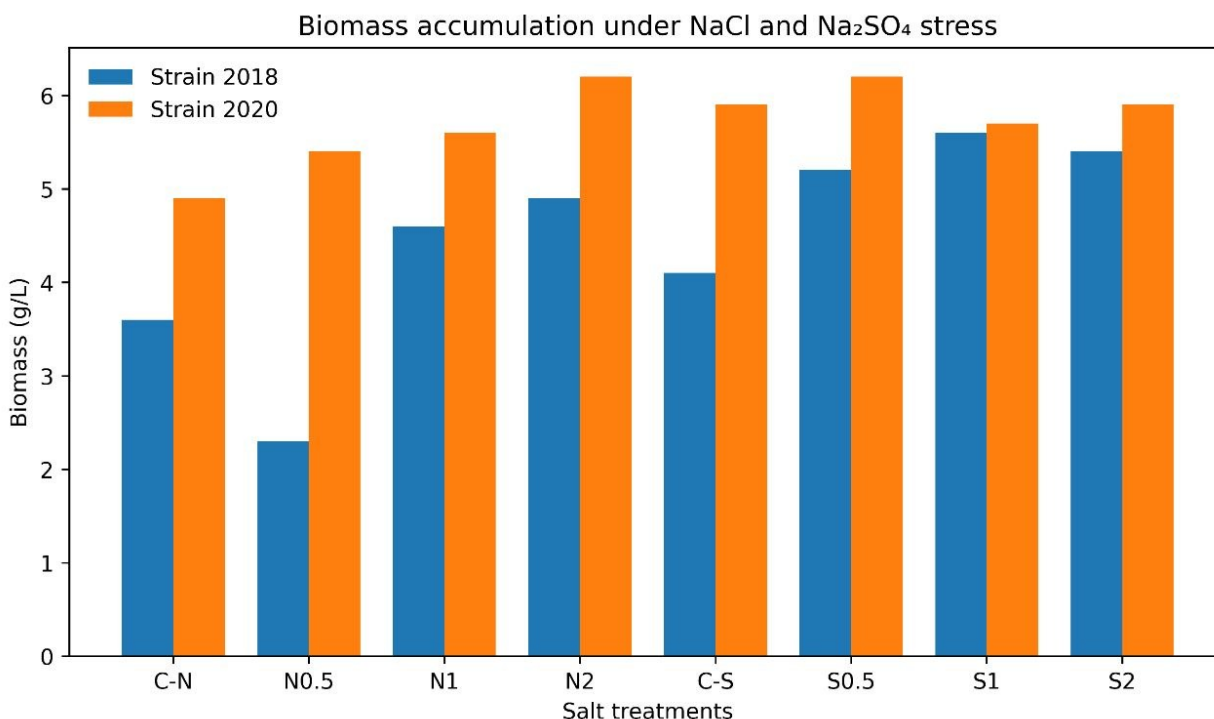


Figure 1. Biomass accumulation of *Azotobacter chroococcum* strains XH2018 and XH2020 under different concentrations of NaCl and Na₂SO₄. Biomass production was expressed as g/L of culture medium.

3.2. Effect of salt stress on EPS production

The synthesis of exopolysaccharides was strongly influenced by both salt type and concentration (Table 1, Figure 2). Under NaCl stress, EPS production by strain XH2018 increased from 0.6 g/L in the control to 1.3 g/L at 1% NaCl, followed by a slight decrease at 2% NaCl. In contrast, strain XH2020 produced the highest EPS level at 0.5% NaCl (2.34 g/L). Under Na₂SO₄ stress, marked differences between the two strains were observed. No detectable EPS production was recorded for strain XH2018 at 0.5% and 1% Na₂SO₄. However, EPS synthesis increased substantially at 2% Na₂SO₄, reaching 4.4 g/L. The highest EPS production among all treatments was observed in strain XH2020 cultivated in the presence of 2% Na₂SO₄, reaching 15.0 g/L. This value was considerably higher than those obtained under NaCl stress and control conditions. The enhanced EPS synthesis under sulfate-induced salt stress suggests activation of protective mechanisms that improve adaptation to high-salinity environments.

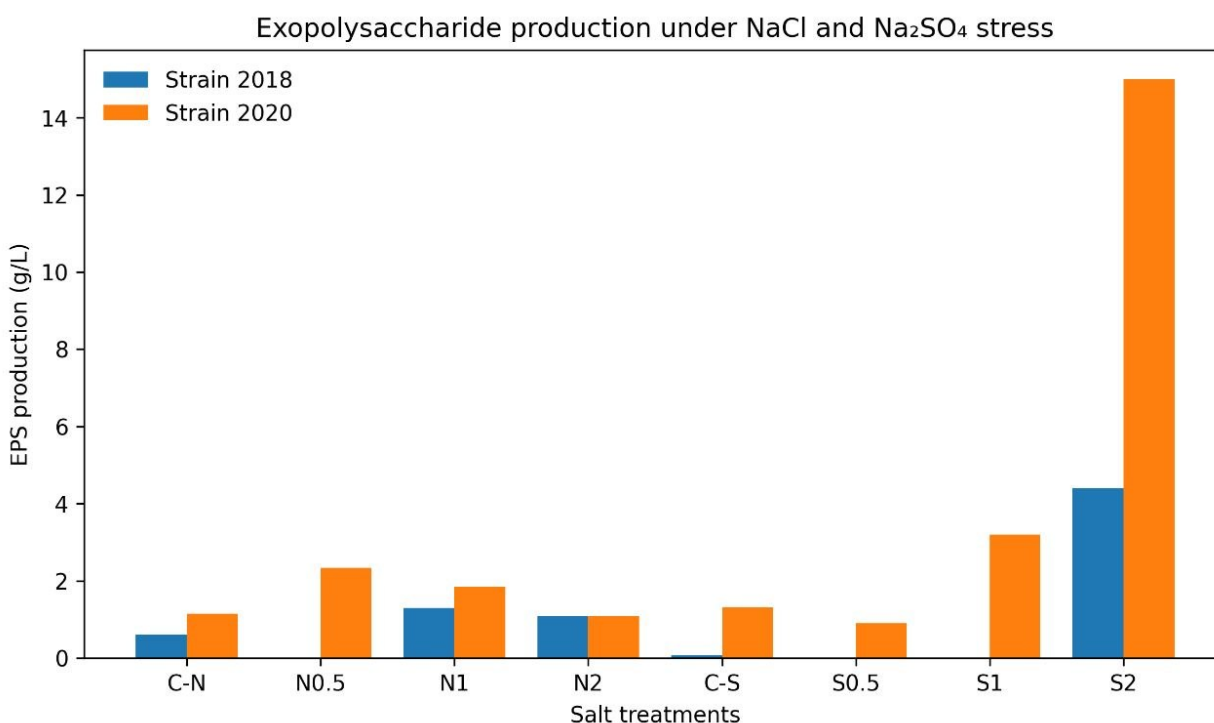


Figure 2. Effect of NaCl and Na₂SO₄ on exopolysaccharide (EPS) production by *Azotobacter chroococcum* strains XH2018 and XH2020.

4. Discussion

Salt stress significantly affected both biomass accumulation and EPS production in *Azotobacter chroococcum* strains XH2018 and XH2020. The obtained results demonstrated that the response of the strains depended on both the type and concentration of salt. In general, strain XH2020 exhibited higher biomass production than strain XH2018 under most experimental conditions, indicating a greater tolerance to saline environments. The observed increase in biomass under NaCl and Na₂SO₄ treatments suggests that both strains were capable of adapting to moderate salt stress. Similar observations have been reported for *A. chroococcum*, where physiological and metabolic adjustments allowed the bacteria to maintain growth under stressful environmental conditions [3]. The higher biomass values recorded for strain XH2020 indicate that this strain possesses more efficient mechanisms for coping with osmotic stress.

Exopolysaccharide production was strongly influenced by salt stress and showed a clear strain-dependent pattern. Under NaCl treatment, EPS production increased moderately in both strains. However, under Na₂SO₄ stress, particularly at a concentration of 2%, a substantial increase in EPS synthesis was observed. The highest EPS yield (15.0 g/L) was recorded for strain XH2020 cultivated in the presence of 2% Na₂SO₄. This result indicates that sulfate-induced osmotic stress stimulated EPS biosynthesis and may have activated protective mechanisms associated with extracellular polymer production.

According to Gan et al. (2024), salt-tolerant bacteria frequently respond to osmotic stress by increasing EPS production. These polymers create a protective extracellular matrix that reduces the harmful effects of high salt concentrations and improves cellular survival. Similarly,

Bhagat et al. (2021) reported that bacterial EPS contributes to stress tolerance by retaining water, stabilizing cell surfaces, and binding toxic ions in the surrounding environment. Therefore, the enhanced EPS synthesis observed in strain XH2020 may represent an adaptive strategy to maintain cellular homeostasis under elevated salinity. The marked difference between the two strains under Na_2SO_4 stress further suggests genetic and physiological variability in their stress-response mechanisms. While EPS production was not detected in strain XH2018 at 0.5% and 1% Na_2SO_4 , strain XH2020 continued to synthesize considerable amounts of EPS. This finding indicates that XH2020 possesses a more efficient regulatory system controlling exopolysaccharide biosynthesis under stress conditions. The results are also consistent with the findings of Cote and Krull (1988), who demonstrated that the extracellular polysaccharides of *A. chroococcum* are primarily composed of alginate-like polymers. Increased production of these polymers under saline conditions may enhance protection against osmotic imbalance and environmental stress. Furthermore, Rasulov and Pattaeva (2024) reported that alginate biosynthesis in *A. chroococcum* is closely associated with environmental adaptation and stress resistance.

Overall, the present study demonstrates that Na_2SO_4 exerted a stronger stimulatory effect on EPS production than NaCl , particularly in strain XH2020. The exceptional EPS yield obtained under 2% Na_2SO_4 highlights the potential of this strain as a promising producer of exopolysaccharides for agricultural and biotechnological applications. Further studies focusing on the molecular regulation of alginate biosynthesis genes under salt stress would provide deeper insight into the mechanisms responsible for enhanced EPS production.

5. Conclusion

The present study demonstrated that both NaCl and Na_2SO_4 affected biomass accumulation and exopolysaccharide production in *Azotobacter chroococcum* strains XH2018 and XH2020. The response of the strains was dependent on both the type and concentration of salt. In general, strain XH2020 exhibited greater tolerance to salinity, maintaining higher biomass production and synthesizing larger amounts of EPS than strain XH2018 under most experimental conditions.

Among the tested treatments, Na_2SO_4 had a stronger stimulatory effect on EPS biosynthesis than NaCl . The highest EPS production (15.0 g/L) was recorded in strain XH2020 at 2% Na_2SO_4 , indicating that sulfate-induced salt stress strongly promotes exopolysaccharide synthesis. The enhanced EPS production observed under saline conditions suggests an important role of extracellular polysaccharides in the adaptation of *A. chroococcum* to osmotic stress.

These findings highlight the potential of strain XH2020 as a promising producer of EPS under saline conditions and support its possible application in agriculture and biotechnology, particularly in salt-affected environments. Further studies are required to investigate the molecular mechanisms regulating EPS biosynthesis and the expression of alginate-associated genes under salt stress.

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