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Isolation and characterization of salt tolerant bacteria from saline soils of Bangladesh

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Abstract

Salinity is an important abiotic stress that limits the productivity of crops growing on the salt affected areas because excess salt concentration in the soil has detrimental effect on growth and development of plants. Beneficial microorganisms having the inimitable characteristics like tolerance to soil salinity, synthesis of plant growth hormones, facilitating nutrient uptake, bio-control ability and beneficial interaction with plants could be vital to address the problem. An experiment was carried out with the objectives of isolating and characterizing saline tolerant bacteria for utilizing as a tool for bioremediation. Soil samples were collected from three saline affected districts of Bangladesh viz. Khulna, Satkhira and Bhola. The highest bacterial population was found in Satkhira followed by Khulna and the lowest was found in Bhola. Eighteen (18) bacterial isolates viz. BU B1, BU B2, BU B3, BU B4, BU B5, BU B6, BU B7, BU B8, BU B9, BU S1, BU S2, BU S3, BU S4, BU S5, BU S6, BU S7, BU K1 and BU K2 were identified according to the colony color and shape. All the isolated bacteria showed positive response to produce IAA. Isolates BU S4, BU B7 and BU S1 showed highest IAA production ability. Among the 18 isolates, 12 were Gram positive and showed negative reaction on KOH test and the rest 6 isolates were Gram negative and showed positive reaction on KOH test. The isolates BU B1, BU B4, BU B6, BU S6, BU K1 and BU K2 were slow growing bacteria and the rest were fast grower. Biochemical tests indicate that 13 isolates were positive for catalase and P solubilization test. Whereas, 11 isolates could degrade the cellulose. For screening of bacterial isolates against NaCl tolerance, the isolates were cultured on NA medium having different salt concentrations. Experimental results reveal that all the isolates could tolerate 4.0% NaCl concentration except BU B6. Ten isolates showed the ability to tolerate NaCl up to 8.0%. The isolates BU B7 and BU S4 showed highest salinity tolerance along with better response to different biochemical characteristics. Therefore, these isolates may become promising for the bioremediation of soil salinity in the saline affected areas of Bangladesh.

Keywords: Salinity, bacteria, salinity tolerance, bioremediation.

Introduction

Soil salinity is considered as a vital abiotic stress that negatively influence the agricultural production all over the world. Research report indicated that 20% of the cultivable land and 33% of the irrigated farming lands of the world are saline affected (Shrivastava and Kumar, 2015). In Bangladesh, the coastal region comprises of 19 districts that covers around 32% of the country and accommodates about 35 million people (Huq and Rabbani, 2011). The salt-affected area in Bangladesh is increasing gradually due to the influence of

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cyclonic storms, leading to salt water intrusion in the non-saline crop lands (SRDI, 2010). Higher soil salinity level affects the growth of most of the field crops and thus hampers crop productivity significantly.

To fulfill the excess food requirement of the increasing population, it is vital to find out the possible ways of utilizing saline soils for better agricultural production (Haque, 2006). Utilization of salt-tolerant bacteria to face the negative influence of soil salinity on crop production is getting popularity in many parts of the world. Research findings reveal that utilization of plant growth promoting bacteria (PGPB) is one of the important strategies to alleviate salinity induced plant stress (Yao et al., 2010). These beneficial microorganisms facilitate growth of the plants through a number of mechanisms (Nia et al., 2012; Solaiman et al., 2012; Ramadoss et al., 2013; Alam et al., 2015) including phytohormone production, nitrogen fixation, nutrient solubilization, disease suppression, siderophore production etc. Therefore, the halotolerant and halophilic organisms are important for maintenance of soil health and nutrition recycling in saline environment. It is hypothesized that the isolation and characterization of saline tolerant bacteria and their application would have immense potential for increased crop productivity in saline affected areas. Considering the above-mentioned facts, the present research work was undertaken with the objectives of isolating and characterizing saline resistant bacteria and to screen promising bacteria against soil salinity for better crop production in coastal saline areas.

Material and Methods

The research study was carried out at the Laboratory of Soil Microbiology under the Department of Soil Science of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh.

Collection of soil samples

Soil samples were collected from salt affected regions of Bangladesh viz. Bhola, Satkhira and Khulna. Randomly collected soil samples were carefully labeled and put in an ice box and transported to the research laboratory and preserved in a refrigerator at -4°C temperature for culturing the bacteria. A portion of each sample was transferred to the laboratory, air dried at room temperature and then sieved using a 2 mm sized sieve and used for subsequent physical and chemical analysis.

Analysis of collected soil samples

The mechanical analysis of soil was performed following hydrometer method (Bouyoucos, 1962) using standard hydrometer (ASTM No. 152H). Cation exchange capacity (CEC) of the collected samples were estimated following ammonium acetate (1N) extraction method (Jackson, 1973). Organic carbon of the soil samples was determined by Walkley and Black wet digestion method (Nelson and Sommers, 1982). Electrical conductivity of saturated paste extract (EC) was determined by the procedure as described by Rhoades et al. (1999). Exchangeable cations like K, Ca, Mg and Na were estimated by 1N ammonium acetate extraction method as prescribed by Soil Survey Staff (2011).

Isolation of bacteria

From each soil sample, 1g of soil was suspended in 9 ml of double distilled water and vortex for 3 minutes. The resulting suspensions were serially diluted to 10^{-10}. An amount of 0.1ml dilution fluid from each dilution tube was taken and transferred separately into freshly prepared Nutrient Agar (NA) media and then incubated at 37°C for bacterial growth. pH of the NA culture media was adjusted to 7. Bacterial growth was monitored on a daily basis and single colonies were picked up with sterile tooth pick and sub-cultured over the pre-solidified NA media. All the subsequent *in vitro* plate assay analyses were done in triplicate until the colonies were more purified. After bacterial growth on NA media, the pure cultures were sub-cultured in NA slants. The slants were incubated at 37°C to get vigorous bacterial growth and therefore preserved in 20.0% glycerol eppendorf tubes at -4°C (Saha and Santra, 2014).

Enumeration of bacteria from saline soil was done by using serial dilutions through drop plate count method. Nutrient agar (NA) (MacFaddin, 2000) media was used for the counting of bacterial populations. After 3 days of incubation, bacterial population was counted as CFU (colony forming unit) that developed on the particular agar plates.

CFU or viable cells / gm of dry soil was calculated by the following formula-

$$= \frac{\text{Mean plate count} \times \text{Dilution factor}}{\text{Amount of dilution}}$$

Morphological and biochemical tests

The morphology of the bacterial colony was observed on NA plates. Individual colonies of each of the isolates were examined based on morphological features including shape, margin, elevation, surface and color (Aneja, 2003). Gram staining tests were performed following standard protocols. Bacterial isolates were also

grown in glucose peptone agar, NA media containing bromothymol blue (BTB), NA media containing Congo red.

Catalase test

Different isolates were flooded with 3.0% freshly prepared hydrogen peroxide (H_2O_2) to perform the catalase test. The effervescence indicated catalase activity that is formation of air bubbles within 10 seconds indicates positive result for catalase test. Negative catalase test was confirmed by the formation of no bubbles or few scattered bubbles.

Phosphate solubilizing assay

Assessment of P-solubilization was carried out following the method as described by Sharma et al. (2012). The cultures of bacterial isolates were spot inoculated on the plates containing Pikovaskya's medium. The plates were incubated in an incubator at 28°C for 4-5 days. Phosphate solubilization was ensured by the formation of clear zone around the bacterial colonies.

Determination of cellulose activity

Cellulose degradation test was done to confirm whether the isolates could degrade the cellulose or not. The test was performed on Jensen's agar plates containing 0.1% carboxymethyl cellulose (CMC). About 10 µl liquid culture of the bacterial isolates were spot inoculated. The plates were incubated for 24 hours and then the colonies were streaked and washed off with sterile water and discarded. Examination plates were then stained with 0.1% congo red for the period of 30 minutes and subsequently rinsed with 1M NaCl. Cellulose degradation activity of the isolates was confirmed by the formation of clear hallo zone around the colony.

Determination of indole acetic acid (IAA) production

Indole acetic acid production test was carried out by the inoculation of desired bacterial isolates in Jensen's broth media. The media containing bacterial isolates were incubated at 29 ±2^{0}C for 48h according to Bric et al. (1991). From the inoculated broth media, 1 ml was transferred into freshly prepared 50ml Jensen's broth culture having 2mg ml^{-1} of tryptophan. The culture was then incubated at 29±1^{0}C for 72h. About 2ml of culture was then centrifuged for 7 min at 7000 rpm. After completion of the centrifugation, 1 ml of the supernatant was mixed with 2ml of Salkowsky's reagent (2.0% of 0.5M $FeCl_3$ in 35.0% perchloric acid) according to Gordon and Weber (1951). Color absorbance was measured by a spectrophotometer at 530nm. Standard curve for Indole Acetic Acid was prepared from pure IAA as 0, 5, 10, 15, 20, 25, 30, 35, 40 and 45µg ml^{-1} of IAA. The concentration of IAA was obtained from the standard graph. Supernatants collected from un-inoculated test tubes were used as control, where no visible color was found.

Growth of the isolates at 37°C temperature

Bacterial isolates were cultured (nutrient agar media) in the growth chamber at 37^{0}C to find out whether the bacterial isolates could survive high temperature or not.

Screening of bacterial isolates against NaCl tolerance

The isolates were tested for their sodium chloride (NaCl) tolerance by growth in nutrient agar plates containing various concentrations of NaCl (0%, 2.0%, 4.0%, 6.0% and 8.0%).

Results and Discussion

Bio physico-chemical properties of the collected soil samples

The soil sample collected from Khulna had organic carbon 1.0%, electrical conductivity 7.09 ds/m and cation exchange capacity 15.50 cmol/kg (Table 1). The soil of Satkhira had organic carbon 0.73%, electrical conductivity 6.68 ds/m and cation exchange capacity 15.33 cmol/kg. On the other hand, soil sample collected from Bhola had organic carbon 0.95%, electrical conductivity 12.29 ds/m and cation exchange capacity 12.34 cmol/kg. Soil texture of the Khulna soil was silt loam, while the texture of Satkhira and Bhola soils were sandy loam. Exchangeable cations (Na, K, Ca and Mg) also vary from one location to another. Bhola soils demonstrate higher exchangeable cations as compared to Khulna and Satkhira soils. Results obtained from the present study (Table 1) reveal that bacterial populations also differ from one place to another. Higher bacterial population was enumerated in Satkhira soil (6.7× 10^5 cfu g^{-1} soil) which was very close to Khulna soil (6.5× 10^5 cfu g^{-1} soil). On the contrary, lowest bacterial population was counted in Bhola soil (1.31× 10^5 cfu g^{-1} soil). It seems that bacterial population in saline soil is related with the EC value. Increasing the EC value might decrease the bacterial population in saline soil. In the present study Bhola soil had the highest EC value and consequently showed lowest bacterial population. This finding implies that soil salinity adversely influences the bacterial population. Our findings are in harmony with many other findings (Moradi et al., 2011; Ma and Gong, 2013) that illustrated the negative influence of soil salinity on microbial abundance.

Table 1. Properties of the collected soil samples

Sample ID	EC (ds/m)	CEC (cmol/kg)	OC (%)	Exchangeable cations (cmol/kg)				Texture	Bacterial population (cfu g^{-1} soil)
				Na	K	Ca	Mg		
K (Khulna)	7.09	15.50	1.00	0.79	0.62	5.67	4.00	Silt Loam	6.5×10^5
S (Satkhira)	6.68	15.33	0.73	0.86	0.74	6.06	5.70	Sandy Loam	6.7×10^5
B (Bhola)	12.29	12.34	0.95	0.93	1.20	9.38	6.23	Sandy Loam	1.31×10^5

Isolation of bacteria from saline soils

A total of 18 bacterial isolates viz. BU B1, BU B2, BU B3, BU B4, BU B5, BU B6, BU B7, BU B8, BU B9, BU S1, BU S2, BU S3, BU S4, BU S5, BU S6, BU S7, BU K1 and BU K2 were isolated from 3 soil samples that were collected from salt affected areas of Bangladesh (Table 2). Out of the 18 bacterial isolates, 9 isolates were obtained from Bhola soil, 7 isolates from Satkhira soil and remaining 2 isolates were isolated from Khulna soil.

Table 2. Isolates with their codes and location of collections

Serial No.	Location of collected soil sample	Isolate code	Serial No.	Location of collected soil sample	Isolate code
1	Bhola	BU B1	10	Satkhira	BU S1
2	Bhola	BU B2	11	Satkhira	BU S2
3	Bhola	BU B3	12	Satkhira	BU S3
4	Bhola	BU B4	13	Satkhira	BU S4
5	Bhola	BU B5	14	Satkhira	BU S5
6	Bhola	BU B6	15	Satkhira	BU S6
7	Bhola	BU B7	16	Satkhira	BU S7
8	Bhola	BU B8	17	Khulna	BU K1
9	Bhola	BU B9	18	Khulna	BU K2

Characterization of the bacterial isolates

Morphological characteristics of the isolates

The isolates were characterized based on their morphological features such as shape, margin, elevation, surface, color. All the isolates have different morphological characters in the nutrient agar medium. All of the isolates were sticky in nature. On nutrient agar (NA) media, 3 isolates (BU B2, BU B4 and BU S1) out of 18 isolates produced round and flat colonies, having smooth margin with smooth shiny surface (Table 3). Colony color of the isolates vary from one isolate to another. In the present study, creamy colored (4 isolates), off-white (4 isolates), yolk yellowish colored (3 isolates), orange colored (2 isolates), greenish yellow colored (2 isolates), brownish colored (2 isolates) and whitish (1 isolate) colored colonies were observed. The results of the study indicate that all the isolates were able to grow in the nutrient agar medium with variable morphological features (Table 3 and Figure 1). Our findings are in harmony with the findings of Hossain et al. (2021) where they isolated bacteria from soil having different morphological features which indicates the diversified presence of bacterial community in soil.

Table 3. Morphological characteristics of the bacterial isolates

Isolates	Colony shape	Elevation	Surface	Margin	Color
BU B1	Round	Raised	Smooth shiny	Smooth	Yolk yellowish
BU B2	Round	Flat	Smooth shiny	Smooth	Creamy
BU B3	Round	Raised	Smooth shiny	Smooth	Off white
BU B4	Round	Flat	Smooth shiny	Smooth	Orange
BU B5	Round	Raised	Smooth shiny	Smooth	Creamy
BU B6	Round	Raised	Smooth shiny	Smooth	Creamy
BU B7	Round	Raised	Smooth shiny	Smooth	Greenish yellow
BU B8	Round	Raised	Smooth shiny	Smooth	Brownish
BU B9	Round	Raised	Smooth shiny	Smooth	Brownish
BU S1	Round	Flat	Smooth shiny	Smooth	Creamy
BU S2	Round	Raised	Smooth shiny	Smooth	Whitish
BU S3	Round	Raised	Smooth shiny	Smooth	Orange
BU S4	Round	Raised	Smooth shiny	Smooth	Greenish yellow
BU S5	Round	Raised	Smooth shiny	Smooth	Yolk yellowish
BU S6	Round	Raised	Smooth shiny	Smooth	Off white
BU S7	Round	Raised	Smooth shiny	Smooth	Yolk yellowish
BU K1	Round	Raised	Smooth shiny	Smooth	Off white
BU K2	Round	Raised	Smooth shiny	Smooth	Off white

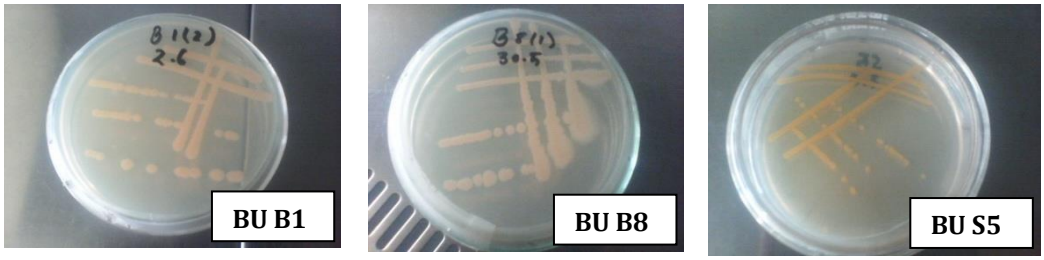


Figure 1. Pure culture of different bacterial isolates

Biochemical characteristics of the isolates

Gram staining and KOH string test

Result presented in Table 4 reveal that 6 isolates were Gram negative in reaction, whereas remaining 12 isolates were Gram positive in reaction. Red colored cells under the microscope were considered as Gram negative and violet-colored cells were considered as Gram positive. The KOH test also confirmed that 6 isolates were Gram negative as the KOH test was positive while 12 isolates were Gram positive as the KOH test was negative (Table 4).

Table 4. Gram staining, KOH, Catalase activity, cellulose degradation and phosphate solubilization test of the bacterial isolates

Isolates	Gram reaction	KOH test	Catalase Test	Phosphate Solubilization Test	Cellulose Degradation Test
BU B1	+ve	-ve	+	+	+
BU B2	+ve	-ve	+	+	+
BU B3	+ve	-ve	-	+ +	-
BU B4	+ve	-ve	+	-	-
BU B5	-ve	+ve	+ +	+	-
BU B6	+ve	-ve	+ +	+	+ +
BU B7	+ve	-ve	+ +	+ +	+ +
BU B8	+ve	-ve	+	-	-
BU B9	-ve	+ve	-	+	+ +
BU S1	+ve	-ve	+ +	+	+ +
BU S2	-ve	+ve	+	+	+
BU S3	-ve	+ve	+ +	-	-
BU S4	-ve	+ve	+ +	+ +	+ +
BU S5	+ve	-ve	+	+	-
BU S6	+ve	-ve	-	-	-
BU S7	-ve	+ve	+ +	+ +	+
BU K1	+ve	-ve	-	-	+
BU K2	+ve	-ve	-	+	+

(-) ve = Negative test, (+) ve = Positive test, Note: (-) =No ability, (+) = Weak and (++) = High

Catalase test

Catalase activity test was carried out with 18 bacterial isolates to find out whether the isolates could decompose the added H_2O_2 or not. Results of the present study (Table 4 and Figure 2) demonstrate that among 18 isolates, 13 were catalase positive as implied by the formation of bubbles upon addition of hydrogen peroxide to the cultures and 5 isolates were catalase negative as they formed no bubbles (Reiner, 2010). Catalase test positive bacterial isolates are highly resistant to harsh environmental conditions as well as have the ability to resist chemical and mechanical stresses (Glick et al., 1999). Since most of the bacterial isolates under the present were found as catalase positive, therefore it implies the possibilities of utilizing these isolates to alleviate salt stresses for better crop production in coastal areas.

Figure 2. Catalase test of different bacterial isolates

Phosphate solubilizing assay

Experimental results confirm that out of 18 bacterial isolates, 13 bacterial isolates were positive for P-solubilization, while rest of the isolates were found negative for P-solubilization test (Table 4 and Figure 3). Phosphorus is an essential and primary plant nutrient. In both acidic and alkaline soils, phosphorus fixation is a common phenomenon which makes the soluble P into insoluble form, thus the efficiency of applied P become low (Mahdi et al., 2012). Therefore, bacterial isolates having P solubilizing ability would play vital role in enhancing the availability of this crucial nutrient for the plant uptake (Richardson, 2001). The most important microbiological way through which insoluble phosphorus compounds are solubilized is by the production of organic acids by the P solubilizing microorganisms.

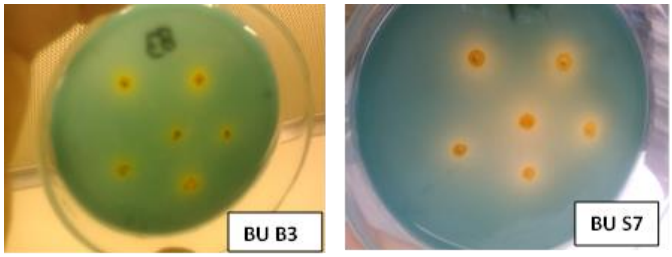


Figure 3. Phosphate solubilization assay by the bacterial isolates

Cellulose degradation activity test

Results presented in Table 4 and Figure 4 show that 11 bacterial isolates (BU B1, BU B2, BU B6, BU B7, BU B9, BU S1, BU S2, BU S4, BU S7, BU K1 and BU K2) out of 18 were positive for cellulose degradation activity. The remaining 7 isolates viz. BU B3, BU B4, BU B5, BU B8, BU S3, BU S5 and BU S6 were identified as negative for cellulose degradation activity. Biodegradation of various organic wastes through efficient microorganisms is an excellent approach (Rahman et al., 2020) which is getting popularity during the last few years. Under normal conditions, compost preparation takes longer period of time due to the slow decomposition of the organic residues (Bui et al., 2014). Thus, bacterial isolates having cellulose degrading activity might play important role in preparing agricultural compost in a useful and ecofriendly way.

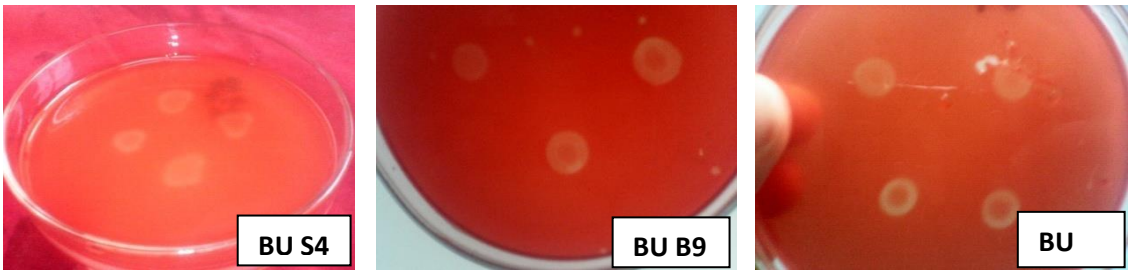
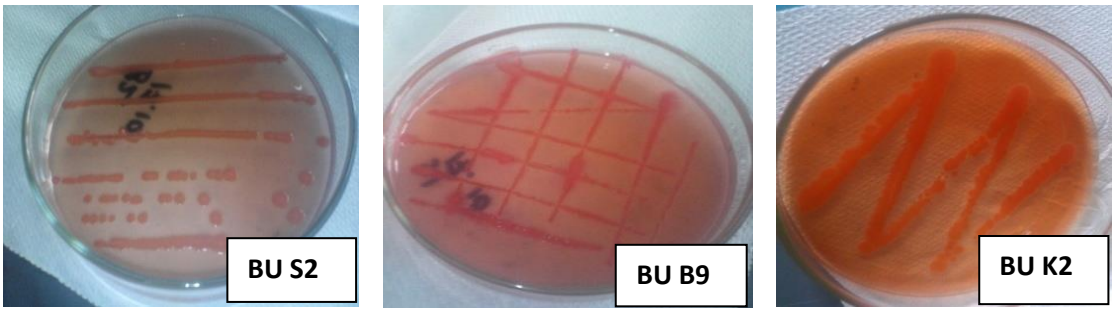


Figure 4 Cellulose degradation activity test of the bacterial isolates

Growth on Congo red dye

In general soil bacteria absorbs congo red dye highly as compared to *Rhizobium.* Results presented in Table 5 show that all the bacterial isolates absorbed the dye from Congo red NA agar media. Among 18 isolates, BU B2, BU B3, BU B5, BU B9, BU S1, BU S2, BU S3, BU S5, BU S6, BU K1 and BU K2 absorbed the dye strongly from Congo red NA agar media (Figure 5). In contrast, BU B1, BU B4, BU B7, BU B8, BU S4 and BU S7 absorbed the dye moderately and the isolate BU B6 show slight absorbance of the dye from Congo red NA agar media.



Figue 5 Growth of bacterial isolates on Congo red NA agar media

Table 5. Growth on Congo red NA agar media, growth on peptone glucose agar, Bromothymol blue test and Growth at 37^{0}C of the bacterial isolates

Isolates	Growth on Congo red NA agar (absorption of dye)	Growth on glucose peptone agar media	Bromothymol blue test			Growth at 37^{0}C
			Growth rate	Color	Result	
BU B1	Moderate	Moderate	Slow	Blue	Alkaline	+ve
BU B2	High	High	Fast	Yellow	Acidic	+ve
BU B3	High	High	Fast	Yellow	Acidic	+ve
BU B4	Moderate	Moderate	Slow	Blue	Alkaline	+ve
BU B5	High	High	Fast	Yellow	Acidic	+ve
BU B6	Slight	High	Slow	Blue	Alkaline	+ve
BU B7	Moderate	High	Fast	Yellow	Acidic	+ve
BU B8	Moderate	High	Fast	Yellow	Acidic	+ve
BU B9	High	High	Fast	Yellow	Acidic	+ve
BU S1	High	High	Fast	Yellow	Acidic	+ve
BU S2	High	High	Fast	Yellow	Acidic	+ve
BU S3	High	High	Fast	Yellow	Acidic	+ve
BU S4	Moderate	High	Fast	Yellow	Acidic	+ve
BU S5	High	High	Fast	Yellow	Acidic	+ve
BU S6	High	Moderate	Slow	Blue	Alkaline	+ve
BU S7	Moderate	High	Fast	Yellow	Acidic	+ve
BU K1	High	High	Slow	Blue	Alkaline	+ve
BU K2	High	High	Slow	Blue	Alkaline	+ve

Growth on glucose peptone agar media

Study results indicate that most of the bacterial isolates grow strongly on glucose peptone agar medium (Table 5 and Figure 6). Out of 18 bacterial isolates, 15 isolates showed high growth on this medium. Remaining 3 isolates BU B1, BU B4 and BU S6 showed moderate growth on glucose peptone agar medium (Table 5). The glucose peptone agar media is usually recommended for cultivation of wide variety of microorganisms. It has been reported that the growth of *Rhizobium* bacteria is normally poor whereas the other bacteria grow well on this media (Upadhayay et al., 2015). In the present study, as most of the bacterial isolates grew well on this media indicating the presence of diversified group of bacteria other than *Rhizobium*.

Figure 6. Growth of bacterial isolates on glucose peptone agar media

Acid/ alkali production in YEM agar medium containing Bromothymol blue

In the present study, bacterial isolates BU B2, BU B3, BU B5, BU B7, BU B8, BU B9 isolated from Bhola soil and BU S1, BU S2, BU S3, BU S4, BU S5, BU S7 isolated from Satkhira soil demonstrated acidic reaction during their one week of growth (Table 5). These isolates turned green color of the medium to yellow (Figure 7). On the other hand, the isolates BU B1, BU B4, BU B6, BU S6, BU K1 and BU K2 showed alkali reaction (Table 5). Alkali producing isolates turned green colored medium to blue. The isolates that produced acid indicating fast growing nature and the isolates that produced alkali indicating slow growing nature.

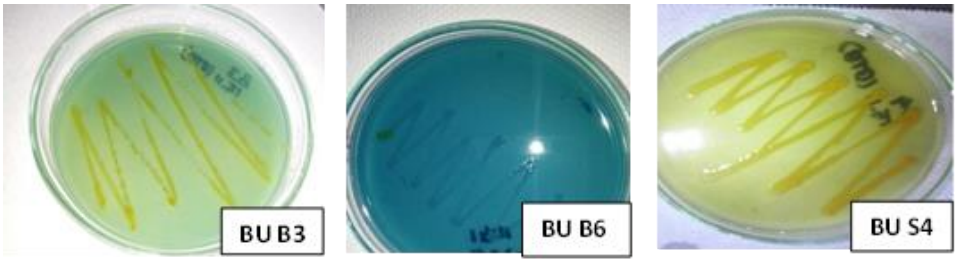


Figure 7. Growth of the bacterial isolates on NA agar medium containing BTB

Indole acetic acid production

Results presented in Figure 8 show that all the 18 isolates were capable of producing indole-acetic-acid (IAA) in variable quantity, indicating a substantial variability among the isolates in terms of IAA production. All isolates were positive for IAA production but among those, seven isolates BU B6, BU B7, BU S1, BU S2, BU S4, BU S6 and BU S7 were selected as potential IAA producers. Among the bacterial isolates, IAA production varied from 12.5 to 115µg/ml. The maximum IAA production was obtained from the isolate BU S4 (115µg/ml) which was followed by BU B7 (114µg/ml) and BU S1 (110µg/ml) (Figure 8).

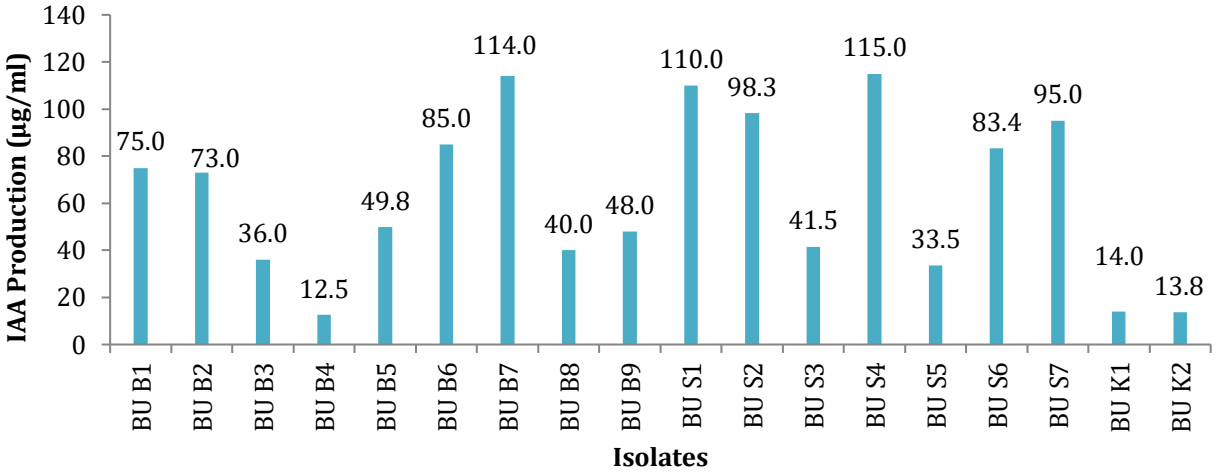


Figure 8. Indole acetic acid production (µg/ml) by different bacterial isolates

However, the lowest IAA production (12.5µg/ml) was found in case of isolate BU B4 (Figure 8). Joseph et al. (2007) showed that bacteria specially *Bacillus*, *Pseudomonas*, *Azotobacter* and *Rhizobium* could produce IAA. Due to the significant influence of IAA producing bacteria on plant growth promotion, production of IAA is considered as one of the vital tools for the screening of beneficial microbes (Wahyudi et al., 2011) The amount of IAA determined in the present study seems relatively higher as compared to the findings reported by Suliasih and Widawati (2020) but are in line with the findings of Hossain et al. (2021). Bacterial isolates that produced considerable amount of IAA might play effective role in plant growth enhancement under saline condition.

Growth of the isolates at 37^{0}C

In the growth chamber, all the bacterial isolates were able to grow in the NA growth medium at 37^{0}C (Table 5). Our findings indicate that all the isolates could tolerate high temperature. As the isolates were collected from harsh environment (saline soil), therefore they might tolerate other environmental stresses like high temperature.

Screening of bacterial isolates against NaCl tolerance

All the bacterial isolates were able to tolerate NaCl up to 2.0% (Table 6 and Figure 9). At 4.0% NaCl level, all the isolates were grown except the isolate BU B6 (Table 6). There was variation in the growth of the isolated bacteria at 6.0% and 8.0% NaCl level (Table 6). At 8.0% NaCl concentration, 9 isolates showed higher growth, 1 isolate demonstrate moderate growth and rest 8 isolates were failed to grow in the nutrient agar plate (Table 6 and Figure 10).

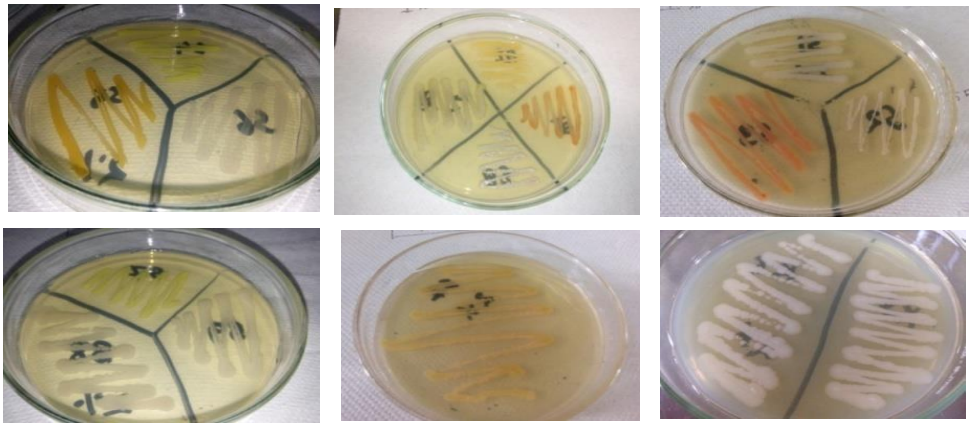


Figure 9. Growth of the bacterial isolates at 2.0% NaCl concentration

Table 6. Growth performance of the isolated bacteria in different NaCl concentration

Isolates	NaCl concentration				
	0%	2.0%	4.0%	6.0%	8.0%
BU B1	+ +	+ +	+ +	+ +	+ +
BU B2	+ +	+ +	+ +	+ +	+ +
BU B3	+ +	+ +	+	+	-
BU B4	+ +	+ +	+	-	-
BU B5	+ +	+ +	+ +	+ +	+ +
BU B6	+ +	+ +	-	-	-
BU B7	+ +	+ +	+ +	+ +	+ +
BU B8	+ +	+ +	+ +	+ +	+ +
BU B9	+ +	+ +	+ +	+ +	+ +
BU S1	+ +	+ +	+ +	+ +	-
BU S2	+ +	+ +	+ +	+	-
BU S3	+ +	+ +	+ +	+ +	+ +
BU S4	+ +	+ +	+ +	+ +	+ +
BU S5	+ +	+ +	+ +	+ +	+
BU S6	+ +	+ +	+	-	-
BU S7	+ +	+ +	+ +	+ +	+ +
BU K1	+ +	+ +	+ +	-	-
BU K2	+ +	+ +	+ +	-	-

Note: (-) =No growth, (+) = Moderate growth and (++) = High growth

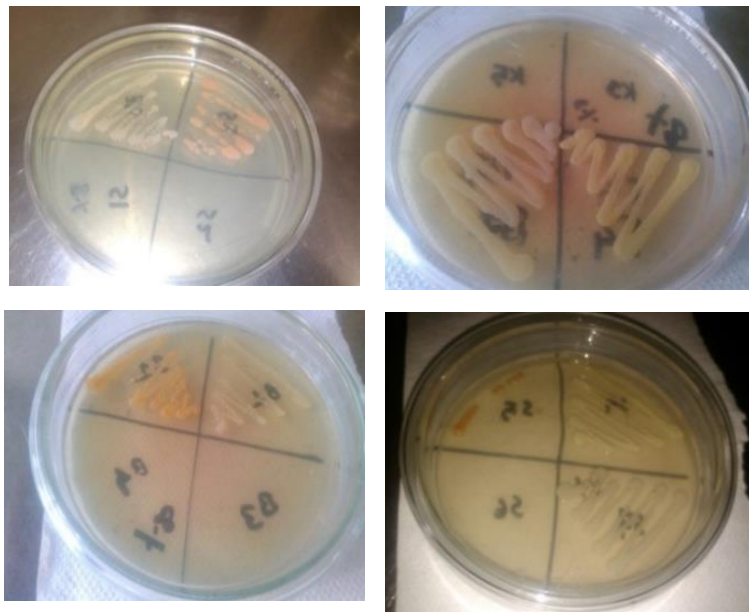


Figure 10. Growth of the bacterial isolates at 8.0% NaCl concentration

Results of the study reveal the possibility of using the high salt tolerant bacteria as effective biofertilizer to enhance the crop production in the saline areas.

Conclusion

A total of 18 isolates were isolated from saline soils of Bangladesh. All the isolated bacteria showed positive response to produce IAA but the Isolates BU S4, BU B7 and BU S1 showed highest IAA production ability. The isolates BU B7 and BU S4 showed better results in all biochemical tests including catalase test, cellulose degradation and P solubilization. All the bacterial isolates were found as salt tolerant against 4.0% NaCl concentration, except isolate BU B6. Ten (10) isolates showed tolerance up to 8.0% NaCl concentration of which BU B7 and BU S4 performed better for different biochemical characteristics. The isolates BU B7 and BU S4 might be recommended as effective bioremediation agent against soil salinity for better crop production in the coastal regions.

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