ANALYSIS OF EXOPOLYSACCHARIDE OF IG-1 (*CITROBACTER FREUNDII*) ISOLATED FROM EARTHWORM UNDER DIFFERENT PHYSIOLOGICAL CONDITIONS

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خلاصہ

موجودہ ریسرچ میں کینچوے کی اندرونی پرت سے الگ کیے گئے جراثیمی اسٹرین گے سٹرو بیکٹر فرینڈاءی[آئی جی۔1](icitrobacter freund1-)(جن کو شکلیاتی، سالمیاتی،اور حیاتیاتی، کیمیائ خصوصیات کی بناپر پہلے ہی پرکھ لیا گیا تھا) کو بیرون خلوی کثیر شکری پرت(EPS)بنانے کی صلاحیت کے لیے جانچا گیا اس تہ پر مختلف فعالیاتی حالات (جیسے کہ درجہ حرارت، بڑھوتری میڈیا اور تحریک کے اثرات کا کیفیاتی اور مقداری جاءزہ لیا گیا۔بیکٹریا کو کیفیاتی تجزیہ کے لیے کانگو ریڈ اگر کے ذریعے چھانا گیا اور گھاڑے بھورے رنگ کی کالونی کو مثبت نتیجے کے طور پر اخذ کیا گیا۔ جراثیم کی بیرون خلوی کثیر شکری پرت پر مختلف درجہ حرارت 37,4اور 42°C)، بڑھوتری میڈیا(BL, 9M)اور تحریک کا اثر دیکھنے کے لیے ان کو بالترتیب مطلوبہ حالات میں رکھ دیا

(گیا بیرون خلوی کثیر شکری پرت کو علیحدہ نکالنے کے بعد اس کے اجزا جیسا کہ لحمیات اور نشاستہ کا جاءزہ لیا گیا۔ بالعموم یہ دیکھا گیا کہ بڑھوتری میڈیم لبی، حالت جمود اور درجہ حرارت 37°C بیرون خلوی کثیر شکری پرت کی زیادہ مقدار بنانے کے موجب تھے۔اسی طرح کاربجان لحمیات اور نشاستے کے اجزا میں بھی دیکھا گیا۔اسی بیکٹیریل کلچر کے پودوں کی افزاءش پر نمک کی موجودگی میں اثرات دیکھنے کے لیے بیجوں کو ڈبویا گیا۔بیکٹیریل کلچر نے نمک کی موجودگی میں بیجوں کی نمود کو 24٪ تک بہتر بنا دیا۔بیکٹیریل کلچر نے تناجڑ اور پودے کی لمبائ میں بھی اضافہ کیا۔جڑ کے عمودی تراش کا خوردبین کے ذریعے معاءنہ کیا گیا۔جڑ کے تراش کو سوڈان 3اور پوٹاشیم آیوڈائڈ کے ساتھ رنگنے سے عمودی تراش درمیان سے سرخ اور گہرے بھورے رنگ کا تھا۔مندرجہ بالا بیکٹیریا کی موجودگی میں پودوں کی بہتر افزاءش دیکھی گی جو نمک کے اثرات کو کم کرنے می مددگار ہوگا

Abstract

The strain IG-1 (*Citrobacter freundii*) previously isolated (from the internal surface of earthworms) and characterized at morphological, biochemical and molecular level was used for both qualitative and quantitative analysis of exopolysaccharide (EPS) under different physiological conditions such as temperature, different media and agitation. *Citrobacter freundii* (IG-1) was screened for EPS production by using Congo red agar method and appearance of dark brown color was considered as positive result. The influence of different temperatures on production of bacterial EPS was determined by incubating cultures at 4ºC, 37ºC, 45ºC temperature and EPS was extracted. Exopolysaccharide content was also determined by using different growth media such as L-broth and M9 under shaking and non- shaking conditions. After extraction, EPS were quantified in terms of protein and carbohydrate contents. In general it was observed that higher amount of EPS was produced in LB media, in static conditions and at 37^{0}C respectively. Similar trend was observed for protein and carbohydrate content of EPS. Seeds were inoculated with cultures to observe plant growth potential of microbes. Seeds were also subjected to salt stress. Inoculation with IG-1 improved (24%) seed germination in salt stress as compare to non-inoculated control. Bacterial inoculation significantly improved the shoot length in the presence of salt stress. Similar pattern was recorded for root length and seedling length. Microscopic examination of cross section of roots was done. Root sections stained with Sudan III appear red from center while Potassium iodide stained roots appeared dark from center.

Introduction

It is considered that the largest component of the animal biomass are earthworms which are also known as ecosystem engineers (Raja *et al.*, 2017). Earthworms impart many beneficial effects on overall soil quality through its burrowing and feeding activities, thus influencing the rate of crop production (Duiker and Stehouwer, 2017). It is suggested that the synergistic relation between microorganisms and earthworm results in amendment of soil such as introduction of nutrients into soil putrefaction of organic material (Azadeh and Zarabi, 2014).

Different environmental factors such as high temperatures, salinity, dryness of soil, presence of excessive water in soil have reduced the crop productivity, salinity is the top most factors which reduce the fertility of land and effect the crop quality (Jan *et al.,* 2017). Inoculum of EPS producing bacteria in plants enhanced tolerance to water stress and improved soil structure and salinity (Naseem and Bano, 2014). Inoculation of sunflower in drought conditions, with EPS producing bacterial strain enhanced root tissues (Naseem and Bano, 2014). Bacteria not only show tolerance against stress conditions, but can also provide plant with some tolerance against harsh conditions of salinity etc.).

Polysaccharides produced by microbes have ability to accumulate on soil particles and form soil aggregates (Qurashi *et al.,* 2012). Inoculums of bacteria which produce exopolysaccharide are reported to show enhanced tolerance against water and salt stress and also improve soil structure. It was reported that inoculation of plant growth promoting rhizobium bacteria along with growth of *Bacillus subtilis* and *Arthrobacter sp.* can diminish the harsh effect of salinity on growth of wheat and can also enhance soluble sugars and dry biomass (Upadhyay *et al*., 2011). *Azospirillium* was isolated from saline and non-saline environment and its effect was checked on growth or production of wheat grown in saline soil; and it was observed that it enhanced the resistance of wheat against saline environment; and also enhance dry weight of shoot remarkably and also grain production (Nia *et al.,* 2012).

The understanding of the EPS produced by bacterial strains can be useful for the study of ecology and biotechnology. Keeping these factors in mind, the present work was aimed to check the influence of different physiological parameters on production of EPS by *Citrobacter freundii* (IG-1). This bacterium was previously isolated from the internal surface of earthworm. EPS role can be further investigated in improving soil aggregation and fertility.

Materials and Methods

The strain IG-1 (*Citrobacter freundii*) previously isolated (from the internal surface of earthworms) was and characterized at morphological, biochemical and molecular level, was used for both qualitative and quantitative analysis of EPS under different physiological conditions such as temperature, media etc.

Qualitative Analysis of EPS: *Citrobacter freundii* (IG-1) was screened for EPS production by congo red agar (CRA) method, following the modifications described by Ferreira *et al*., 2014, however, a little change was made by using 20 % glucose instead of sucrose (Mariana *et al*., 2009). Strains were streaked over BHI agar and incubated at 37^0C for 24 hours. Following incubation, change in colony color from brown to black was considered as positive.

EPS Extraction: Extraction of exopolysaccharide (EPS) from bacterial strain *Citrobacter freundii* was done by using method of De Vuyst *et al*.1998 following modifications described by Qurashi *et al*., 2012. To study the effect of varying temperature (4ºC, 37ºC, 45ºC) on bacterial EPS, cultures were incubated at respective temperature conditions and EPS was extracted. Exopolysaccharide content was also determined by using different growth media such as L-broth and M9 under shaking and non- shaking conditions. Fresh weight and dry weight of EPS was also measured and expressed in terms of SI (System international) unit mg per mL. After extraction, EPS were quantified in terms of protein following (Lowery *et al.,* 1951) and carbohydrate contents following phenol-sulfuric acid method described by (Dubois *et al*., 1956). Protein and glucose content was expressed as mg per gram fresh weight of EPS (Qurashi *et al.,* 2012).

Plant-Microbe Interaction: Experiment was performed to investigate the effect of IG-1 (*Citrobacter freundii*) on plant growth. For this purpose, wheat seeds Var (Lasani 08). were used. Surface sterilization of seeds was done with 0.1 % $HgCl_2$ solution for 2-3 minutes. Then, it was rinsed with sterile distilled water repeatedly for 2-3 times. Seeds were inoculated with bacterial cell culture suspension in sterile water (OD was adjusted to 0.3) for 20 minutes and finally sown on Petri plates layered with moist cotton and filter paper. Seeds were also subjected to salt stress, by providing 8 mL of 100 mM of NaCl solution instead of distilled water. For control plates 8 mL of sterile distilled water was used. Plates were placed in dark for 3 days at room temperature. Watering was done with autoclaved distill water to avoid moisture loss. Plates were transferred to light after three days of germination and harvesting was done to check differnet plant growth parameters i.e., germination of seeds (%), shoot length of seedlings (cm), root length of seedlings (cm), seedling length (cm), fresh weight of seeds (g) following Afrasayab *et al*., 2010.

Microscopy of Plant Roots: Roots were isolated from the seeds with the help of sterile blade and cut down into thin circular sections. Cutting was done in water containing petri plate. After cutting, root sections were dipped in 70 % ethanol for 5 minutes. Then transferred into 1N HCl and kept in oven at 60ºC for 6-8 minutes. Rinse the sections with water.

Staining For Lipids: To analyze lipids root sections were dipped in Sudan III for 1 minute. Then, rinsed with water and observed under 10 X and 100 X.

Staining For Starch: To analyze starch, root sections were dipped in 2% potassium iodide solution for 15 seconds and then rinsed with water. Stained root sections were observed under 10 X and 100 X.

Results

Qualitative Analysis For Exopolysaccharides (EPS): Results for congo red agar method was observed, dark brown colonies were observed showing positive results for EPS production. For EPS extraction, bacterial growth was recorded in LB and M9 media, under different temperature and agitation conditions. Using different media, it was observed that bacterial growth was 6.9 % higher in L-broth as compared to M9 media (Fig.1.). In L-broth medium, bacterial growth was 3.96 % higher in medium at non-shaking conditions at 37ºC as compared to media kept in shaking conditions (60 rpm, 37ºC). Similarly, for M9 media relatively high growth was recorded at non-shaking media (37ºC) as compared to media kept at shaking conditions (60 rpm, 37ºC) (Fig.1.). Strain IG1 incubated at 37ºC showed 41 % increase in bacterial growth in L-Broth medium as compared to growth recorded at 4ºC. Similarly, this increase was 86 % when compared with temperature 45ºC (Fig.1.).

Quantification OF EPS: Amount of EPS was determined by taking fresh weight of EPS for all set parameters including different media, agitation and temperature conditions. Fresh weight of EPS was taken for EPS extracted from cultures grown on different media and It was recorded that amount of EPS was 20 % higher in LB media as compared to culture grown in M9 media (Fig.2.). It was observed that amount of EPS in LB media under static conditions was increased by 42 %when it was compared with shaking conditions. Similarly, amount of EPS was 37 % higher for M9 media in non-shaking conditions when it is compared with shaking conditions (Fig.2.). Strain IG1 incubated at 37ºC showed 74 % increase in EPS production in L-Broth medium as compared to growth recorded at 4ºC. Similarly, this increase was 86 % when compared with temperature 45ºC (Fig.2.).

Quantification of EPS In Terms of Protein: Comparative estimation of protein was done in L-broth and M9 media at shaking and non-shaking conditions. Results showed that the protein content of the EPS obtained from bacterial culture grown on L-broth was 34 % higher as compared to the protein content of EPS obtained from culture grown on M9 media (Fig.3.). Protein content of EPS obtained from L-broth culture was higher in shaking conditions by 6 % as compared to non-shaking conditions. While in case of M9 medium, in non-shaking conditions it was 18 % higher than shaking condition (Fig.3.). Protein content of EPS obtained from culture grown on 37^0C was 27 % higher than culture grown on 4^0C and 34 % more than the culture grown at 45^0C (Fig.3.).

Glucose Estimation By Phenol-Sulfuric Acid Method

Media: Glucose content of EPS obtained from bacterial culture grown on L-broth culture was 15 % higher in bacterial culture grown on M9 medium (Fig.4.). Glucose content of EPS obtained from L-broth culture was 11 % more in non-shaking conditions as compared to shaking conditions. While in M9 media it was more than 3 % in shaking conditions as compared to EPS obtain in non-shaking conditions (Fig.4.). Glucose content of EPS obtained from culture grown on 37^0C was 19 % higher than culture grown on 4^0C and 26 % more than the culture grown at 45^0C (Fig.4.).

Plant-Microbe Interaction:

Seed Germination: It was found that non-inoculated plants showed improved growth as compared to inoculated seeds. In the presence of salt stress seed germination was greatly (37%) reduced in non-inoculated control. Inoculation with IG-1 in salt stress improved (24%) seed germination as compare to non-inoculated control (Fig.5).

It was found that shoot length of 15 days old seedlings was 56 % reduced in the presence of salt stress then when seeds were not inoculated. However, bacterial inoculation significantly improved the shoot length in the presence of salt stress. Similar pattern was recorded for root length and seedling length. Salinity showed 22 % reduction in root length in non-inoculated control while inoculation significantly improved the length in the presence of salt stress (Fig.5).

Fresh Weight: Fresh weight of non-inoculated seedlings was 31 % reduced at 100 mM salt stress while more than 100 % (122 %) increment in fresh weight of seedlings was recorded in inoculated seeds under same stress conditions (Fig.6).

Estimation of Protein and Glucose Content of Seeds It was observed that protein content of inoculated seeds was 25 % more than the protein content of non-inoculated seeds. Inoculation with IG-1 in salt stress improved (35 %) protein content as compare to non-inoculated control (Fig.7).

It was observed that inoculated seeds showed 48 % increment in glucose content as compared to non-inoculated seeds. Inoculation with IG-1 in salt stress improved (32 %) glucose content as compare to non-inoculated control (Fig.7).

Microscopy: For microscopic examination of plant, plant roots of different condition including control, inoculated and non-inoculated seeds under salt stress as well, were stained with Sudan III for lipid staining and potassium iodide for determination of starch contents of root respectively. Results showed that Sudan III stained root section appear red from center (Fig.8) of root cross section showing larger cells most probably of endodermis. This might showed the presence of microbes. Potassium iodide stained roots appeared dark (Fig.8) from center and corners as well showing absence of starch in germinating seedlings.

Discussion

A previously isolated strain IG-1 (*Citrobacter freundii)* was studied for EPS production under different physiological conditions. This strain was previously isolated from earthworm internal surfaces. *C. freundii* is considered as opportunistic pathogen which is the member of family Enterobacteriaceae (Badger *et al.,* 1999).It has been indicated previously that this bacteria encodes resistance to antibiotics and toxic compounds as well as genes for production of exopolysaccharide and biofilm synthesis (Akbar *et al.,* 2015). Thus these microbes seem to have great ecological significance.

EPS production was checked both qualitatively and quantitatively. Phenotypic evaluation of exopolysaccharide was done using Congo Red Agar medium (Mariana *et al,* 2009) supplemented with 20 % of glucose concentrations. Although Congo red method have less precision, but it is economical and have easy protocol. On the basis of Congo red assay colonies can be evaluated on visual basis (Liberto *et al.,* 2007; Hassan *et al.,* 2011). The results were recorded as dark brown colonies. The appearance of colonies after 24 hours of culture incubation resulted in dark brown colonies suggested by Arciola *et al.* (2002). It is suggested in previous findings that concentration of a carbon source can influence the gene expression of intercellular polysaccharide adhesion (PIA) which affect the biofilm formation in *Staphylococcus* (Rachid *et al.,* 2000; Baldassarri et al., 2001; Dobinsky *et al*., 2003). However, most of the researchers do not consider this method as a reliable screening source for biofilm formation (Knobloch *et al.,* 2002; Hassan *et al.,* 2011). Although not a reliable method but the variable composition of media can increase the reliability of this method for EPS screening.

Different growth media (L-Broth, M9 media), agitating and non-agitating conditions and different temperature ranges were tested to check the amount of EPS produced. Before EPS extraction, cell growth was determined spectrophotometrically (METASH. Model: UV-5100B. Made in Shanghai.) by measuring OD_{600nm}. In general it was observed that cell growth was higher in LB medium as compared to M9 media which is a minimal medium. These results are in line with the results of (Li *et al*., 2014) who suggested the higher growth of *E.coli* BL21 in rich media (LB, Terrific broth) as compared to minimal media. The higher growth in rich media (such as in LB or TB) could be possibly due to the presence of already synthesized precursor molecules for example amino acids, hence cells do not need to synthesize them and have opportunity to use them for the synthesis of macromolecules, which are essential for cell growth. Hence, cells can show higher growth rate in rich media as compared to defined media (Li *et al.,* 2014).

It was observed that higher growth was observed in non-shaking conditions as compared to shaking conditions. It is suggested in previous studies by (Rodriguez-Tudela *et al.,* 2000) the growth of *C. neoformans (non-fermentive yeast)was higher in shaking conditions as compared to static conditions. Cryptococcus neoformans* is aerobic non-fermentative yeast. Agitation provide good amount of oxygen which enhance the growth rate of *Cryptococcus neoformans* (Rodríguez-Tudela *et al.,* 2000). These results are contradictory to the previous studies as agitation does not influence the growth of IG-1 culture. This could be due to the reason that *Citrobacter freundii* is a facultative anaerobe.

As cultures were incubated at different temperatures (4^0C, 37^0C and 45^0C), in general it revealed that highest growth rate was observed at 37^0C. As strain IG-1 is isolated from earthworm internal surface so it truly reflects its own growth conditions.

Analysis of total soluble protein content of the extracted EPS showed that high protein content was present in LB as compared to M9 medium. Similar trend was observed for amount of carbohydrate, as it was high in L-broth as compared to M9. These results are in line with results of previous findings of (Jang *et al*., 2016) showing that alkyl hydro peroxide reductase (AhpC) mutant cells *ahpC* grown in rich media are suffering more oxidative stress as compared to minimal medium and it actually resulted in more EPS production. It can be assumed that in LB medium more oxidative stress led the IG1 *Citrobacter* sp. to produce more EPS. These results were similar with the results of Torino *et al.,* (2005) who studied EPS production in *Lactobacillus*

Helveticas ATCC 15807 using chemically defined media and demonstrate that cell growth and EPS production was high in rich media. This could be possibly due to the reason that L-broth is a rich medium which may support the growth of bacteria more effectively. This explanation is consistent with the research work of kimmel *et al.,* (1997) who describe that EPS production is associated with cell growth.

It was observed that carbohydrates contents were higher than proteins, which is similar to the results of Mostefaoui *et al.,* (2014) whose results showed that carbohydrate content of EPS was higher than protein content. Carbohydrates are the major constituent which comprises almost 20 % of the dissolved organic matter, with polysaccharides, which contain large amount of such compounds (Benner *et al.,* 1992).The amount of polysaccharide decreases along with depth, showing that these compounds are labile (Benner and Pakulski, 1994). The ratio of monosaccharide such as glucose is usually low (less than 50 mM) (Borch *et al.,* 1997; Skoog *et al*., 1999). However, on the surface of the ocean, huge fluxes can support the growth of bacteria more than 30 % (Carlson, 2002). Phytoplanktons and other organisms can produce carbohydrates by releasing actively or passively (Elifantz *et al.,* 2005). Nutrient rich regions such as upwelling zones and coastal regions show increased production and high levels of polysaccharides and EPS (Underwood *et al.,* 2004).

Optimization of conditions such as aeration and agitation act as an important tool to control the growth of cell and production of scleroglucan. These factors are responsible for proper mixing of culture and adequate transfer of mass and heat, which enhance nutrient and oxygen transportation from medium to cells. Thus influencing the rate of release of metabolites, like biopolymers from cells to the environment (Giavasis *et al.,* 2005).As Schilling *et al.,* (1999) suggested that stirring at elevated rates resulted in production of low molecular weight (MW) scleroglucan as compared to the one produced at moderate stirring. Moderate agitation and high rate of aeration produce scleroglucan with maximum molecular weight. Even though moderate agitation is critical for proper mixing, but if it exceeds, it may cause drastic effects on culture and quality of EPS. However, aeration plays an important role in good mixing without disturbing the culture and polysaccharide properties (Giavasis *et al*., 2005). Moreover, special care is required to prevent foaming at elevated agitation. However, it is observed that some antifoaming agents have ability to enhance production of EPS while others can inhibit (Stasinopoulos and Seviour, 1990; Farina *et al.,* 1998; Yang *et al.,* 2000; Hsieh *et al.,* 2006).

In submerged culture, fungal morphology (pelleted in contrast with dispersed filamentous growth) can be influenced by a number of factors such as size of inoculums, composition of media, centrifugation of fermenter and specifically rate of agitation (Papagianni, 2004; Fazenda *et al.,* 2008).It was reported earlier that pelleted culture in *Sclerotium* sp. may result in production of high amount of β-glucan as compared to diffused mycelia cultures (Gibbs *et al.,* 2000; Papagianni, 2004; Wucherpfennig *et al.,* 2010; Schmid *et al.,* 2011; Seviour *et al.,* 2011). Previous studies on correlation between EPS production, mycelial morphology and rate of aeration have done for *Cordyceps militaris*, resulted in observation that lack of dissolved oxygen due to low aeration results in autolysis of pellet. Nevertheless, aeration at elevated levels was not considered beneficial (Castillo *et al.,* 2015).These results were contradictory to previous studies as no significant effect of agitation was observed on the growth rate of cells and EPS production. Amount of EPS was higher in non-shaking as compared to shaking conditions.

EPS production was observed at different temperatures. It was observed that maximum amount of EPS produce at 37^0C. These results are similar to Polak-Berecka *et al.,* (2014) who described that EPS production was maximum at 37^0C. This could be possibly due to the maximum growth rate at 37^0C, as it is evident from different researches that EPS production is directly related with growth temperature (Polak-Berecka *et al.,* 2014). EPS production was generally associated with growth conditions however, other parameter still influence its production. This is in line with findings of other study done by (Nichols *et al.,* 2005) where EPS yield was higher at -2, 10°C as compared to 20°C which is the optimum temperature for growth of many psychrotolerant strains. Since our strain IG-1 is isolated from earthworm internal surface so it truly reflects its own growth conditions. High temperature (42°C) reduced the amount of EPS production (Zisu and Shah, 2003) as findings of our studies where less EPS at 45°C was reported.

Seeds were inoculated and their growth was tested under different salinity levels. Growth in terms of germination, length and weight parameters was significantly improved as compared to non-inoculated treatment. Same results were recorded as in previous studies of Qurashi and Sabri, 2012 where EPS forming strains improved growth of *Cicer arietinum* under salinity of 100 mM stress. Plant exudates from roots act as chemo attractant and help the bacterial strains to move toward a root (Davey and O'Toole, 2000). Estimation of protein and glucose content of seeds revealed that inoculated seeds showed significant increase in values of protein and glucose content as compared to non-inoculated seed at 100 mM stress. Similar results were mentioned in previous studies of Qurashi and Sabri, 2012, where inoculation resulted in increase in value of protein and sugar components as compared to non-inoculated control plants. It is suggested in previous studies by Mohamed and Ismail, 2011 that assimilation of these components (osmolytes) helps in adjustment of osmotic pressure in stress bearing tissues of plant. As salinity increase, alteration in values of protein, sugar or lipids suggest osmoregulation (Chen and Murata, 2011: Aly et al., 2012). In addition to this, polysaccharides also play a significant role in attachment of bacterial strains to surfaces of plants (Danhorn and Fuqua 2007; Saravanan et

al., 2008). The bacterial biofilm on plant roots tend to colonize roots of legume plants. The Sudan test detected many different microorganisms (protists, also some diatoms), identified by red staining of their cell membranes. These organisms could be found in the soil, and may be attached along with the root hair surfaces and could also be found in all the intercellular spaces of the root .The root hairs are present in a large number and could vary in size - from short to long. Histochemical tests using transverse sections of the roots were done, and Sudan test was used for detection of total lipids (Oliveira *et al*., 2013).

EPS produced by strain IG-1 seems to be significant as it have ability to tolerate different physiological parameters. It seems that this EPS from strain IG-1 can facilitate it in plant growth promotion.

Acknowledgment

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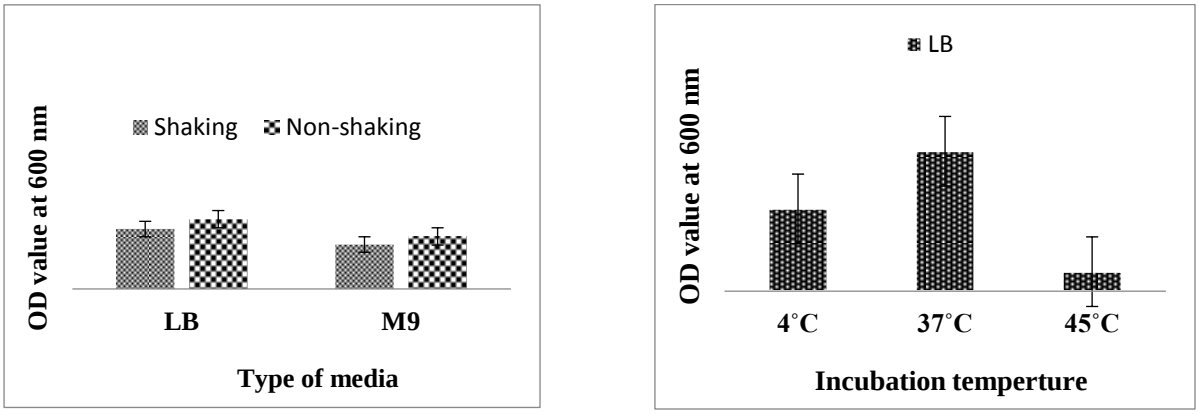


Fig.1: Bacterial growth in different media and varying temperature.

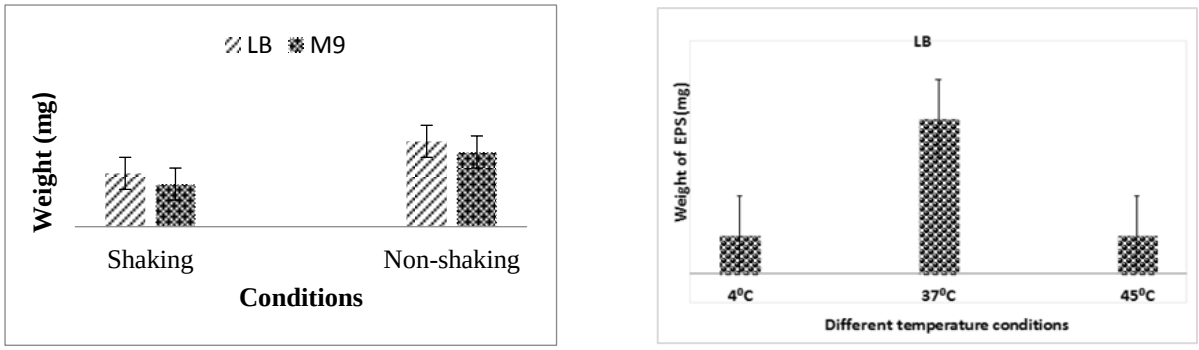


Fig.2: Bacterial EPS fresh weight of EPS (grams per gram fresh weight of bacterial cells) in different media and varying temperature.

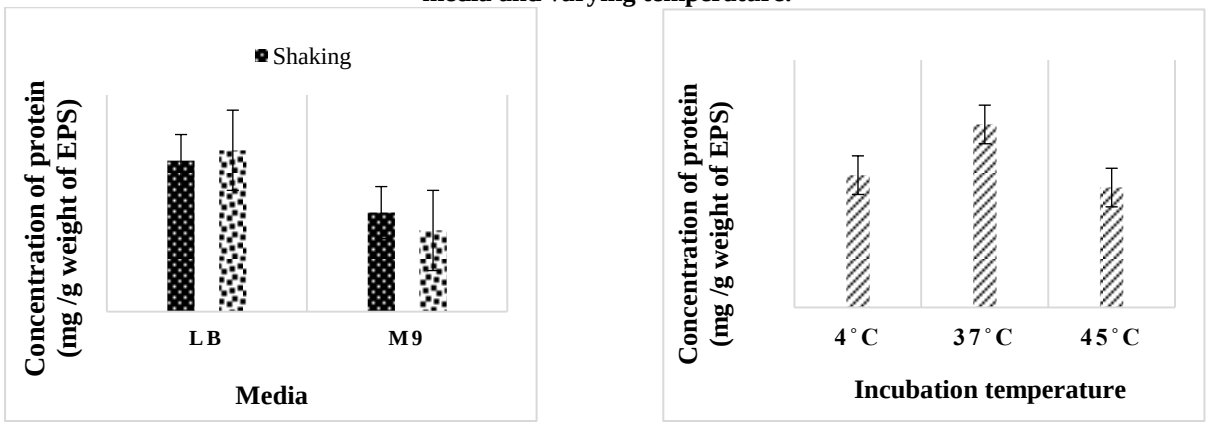


Fig.3: Protein contents of EPS produced at different media and varying temperature.

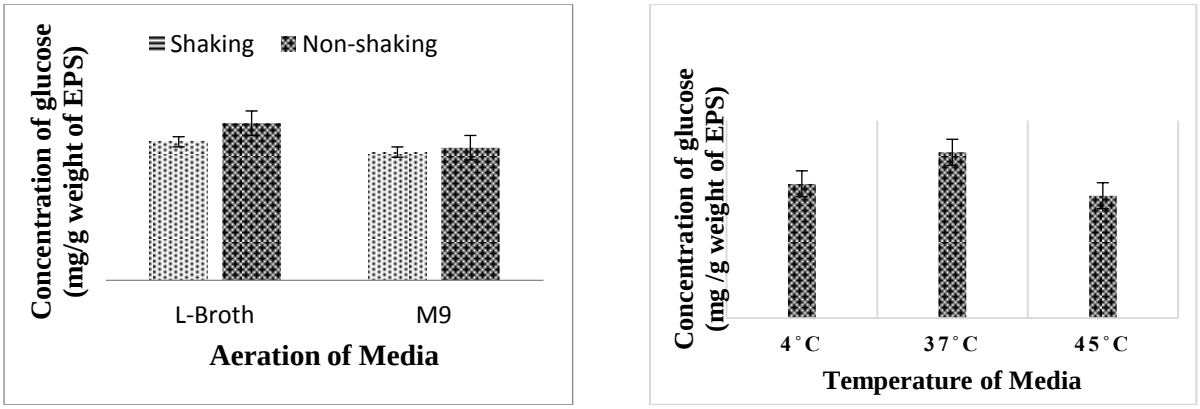


Fig.4. Glucose contents of EPS produced at different media and varying temperature.

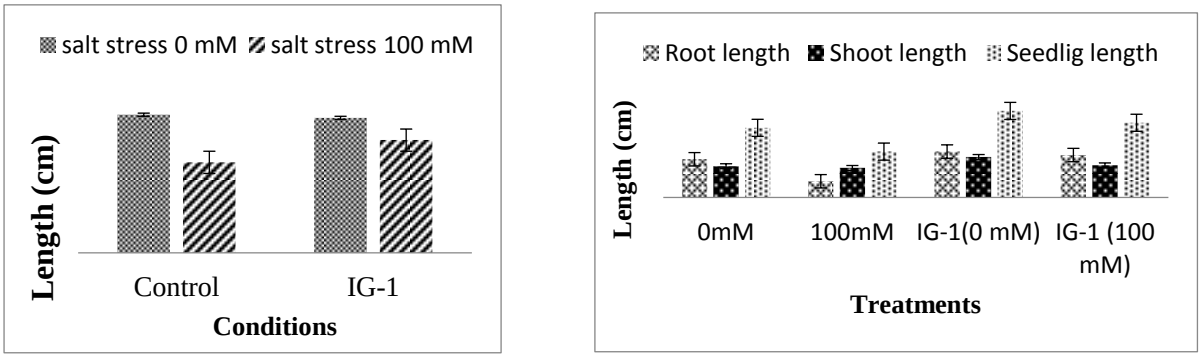


Fig.5. Plant growth recorded in terms of length (cm). Acronyms: 0mM = No salt stress 100mM = 100mM Nacl, IG-1(0mL) = No salt stress + IG-1, IG-1(100mM)= 100mM salt stress (Nacl)+IG-1

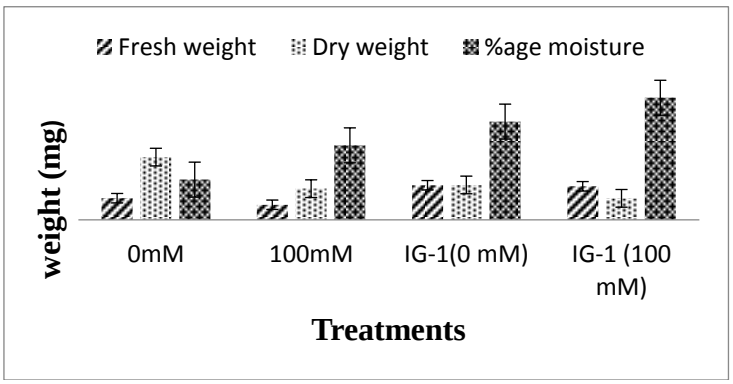


Fig.6. Plant growth recorded in terms of fresh weight, dry weight and %age moisture content .

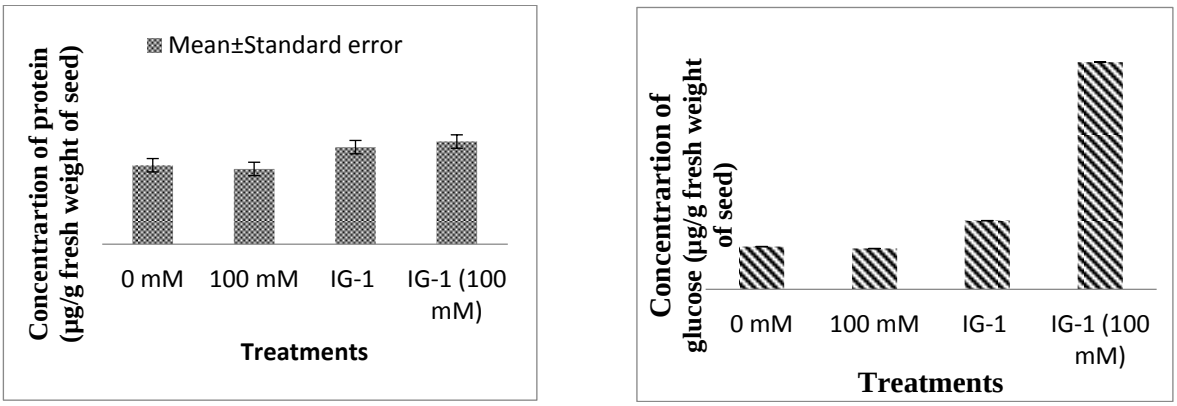


Fig.6. Graph showing comparison between values of (mean± standard error) of (a) protein Contents of fresh seeds (b) glucose value of fresh seeds.

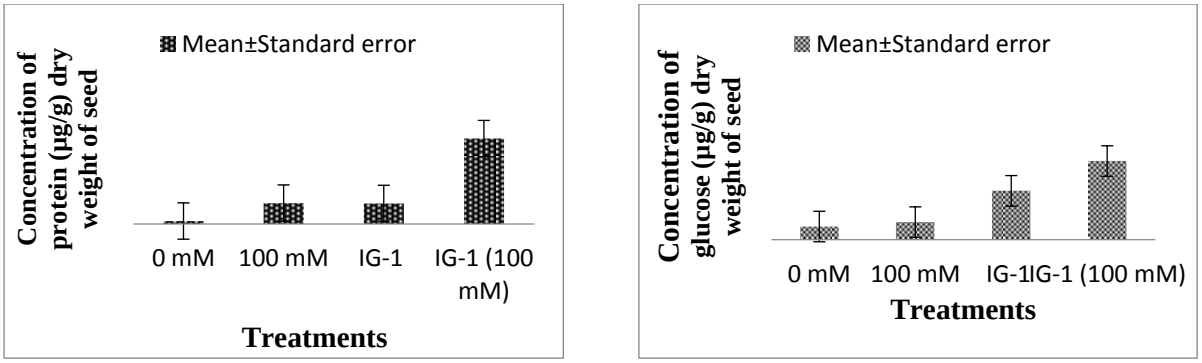
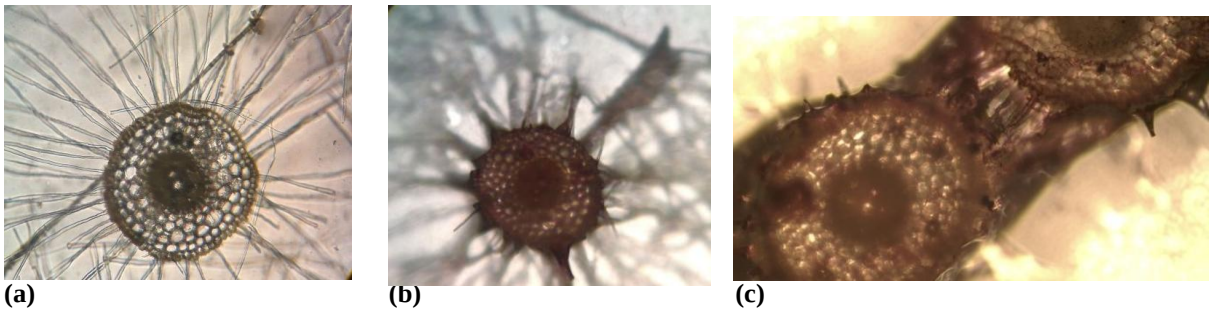


Fig.7. Plant growth in terms of protein and glucose contents of seedlings.



(a) **(b)** **(c)**

Fig.8. Microscopic view of culture inoculated plant root cross section stained with (a) potassium iodide and (b) Sudan III showing centrally located red zone of endodermis (c) Cross section of plant roots of control plate, stained with Sudan III.

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