

# Media Optimization for Exopolysaccharide Producing *Klebsiella pneumoniae* KU215681 under Varying Cultural Conditions

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**Abstract** The soil samples were collected from Western Ghats. It was processed as per microbiological procedure. The observations in terms of morphological, physiological, biochemical and genetical were made for identifications of selected exopolysaccharides producing bacteria. The selected isolate was confirmed as *Klebsiella pneumoniae* by 16S rRNA sequences. It was taken up for further studies to ascertain the various cultural conditions for exopolysaccharide production. The EPS (exopolysaccharides) producing ability was evaluated by nutritional defined media. The data obtained reveals that the pH, temperature, carbon, nitrogen, aminoacids surfactants, metal ions conditions had the most significant influences. Among the various nutritional sources of interactive effects the maximum EPS production was recorded at pH 7.00 ( $1.58 \pm 0.0096$ ), Temperature at 37°C, Jaggery ( $1.37 \pm 0.003$ ), Tryptone ( $1.56 \pm 0.012$ ), Ferric chloride ( $1.98 \pm 0.02$ ), Glutamine ( $2.13 \pm 0.067$ ), Polyethylene glycol ( $0.79 \pm 0.043$ ).

**Keywords** EPS, Media Optimization, 16S rRNA, *Klebsiella pneumoniae*

## 1. Introduction

The past few decades, several microbes have been investigated for the production of exopolysaccharides using whey or lactose as fermentation substrate [1]. Xanthan and gellan gums are examples of fighting fit acknowledged bacterial exopolysaccharides that have significant industrial importance. Apart from bacteria, attempts have been made to produce exopolysaccharides from submerged fungi cultures [2]. Over the past three decades, attempts have been made to improve the utilization of whey by using it as a fermentation substrate for producing value added products such as ethanol and microbial exopolysaccharides. Research on producing commercial bacterial gums using whey or lactose based

media, with the goal of improving whey utilization, have been done with little success. Isolate lactose metabolizing exopolysaccharide producing microorganisms have been made by under optimized conditions, *Klebsiella oxytoca* produced copious amounts of exopolysaccharide, 15 g/l, that had very high apparent viscosity. It has been reported that the location of genes for exopolysaccharide production in this *K. oxytoca* are plasmid encoded [3] thus prompting favorable recombinant DNA technology manipulation with lactic acid bacteria. This is paramount because several researches have shown that lactic acid producing bacteria produces very low exopolysaccharides yields [4, 5].

In the natural environment, exopolysaccharide (EPSs) is generally hetero polymeric (made of different monomeric units), non-sugar components like uronic acid, methyl esters, sulphates, pyruvates, proteins, nucleic acids and lipids. EPS also contain divalent metal cations that act as ionic bridges linking adjacent polysaccharide chains. Many microorganisms like bacteria, fungi and actinomycetes are produce high molecular weight, hydrated polymeric compounds called exopolysaccharides (EPS) during their lifecycle. Many bacterial cultures produce different types of EPS during its life-cycle. For example, most bacteria produce capsular form of EPS during the exponential growth phase and slime type EPS during the stationary growth phase [6]. EPS may exist as capsules, sheaths, slimes (loosely attached to the cell wall), apical pads or mesh like fibrils in the natural environment. Capsules are tightly bound to the cell wall by non-covalent linkages whereas sheaths are linear EPS containing structures surrounding chain of cells. Slime layer is a less organised form of capsule or sheath that diffuses into the surrounding environment. The presence of side linkages and organic molecules influence the overall charge, stability, binding capacity, rheology and solubility of the polymer [7].

Most microorganisms produce exopolysaccharide either for attachment to substratum (adhesion), formation of micro-consortium/biofilms or binding to other particulate matter (cohesion or aggregation). EPS produced for

attachment by microorganisms may influence biofouling by conditioning the substratum. Other functions like gliding motility, protection against osmotic shock, predation, desiccation and detoxification of toxic compounds, nutrient sequestering, chelation of metals, horizontal transfer of genetic material have also been attributed to microbial EPS [8, 9]. The purpose of this research was to determine the properties of the exopolysaccharides produced by *Klebsiella pneumoniae* ku 215681, so that its media optimization for EPS production can be ascertained.

## 2. Material and Methods

Soil sample was collected from Western Ghats near Tamilnadu (North *Latitude*, 11° 00' and 12° 00' N, East *Longitude*, 77° 28' and 78° 50') and Kerala (77° 15' and 77° 36' East latitude & 8° 03' and 8°35' North *Latitude*). Isolates were obtained by serial dilution plating on nutrient agar medium. Total of 10 different colonies were isolated and the exopolysaccharide producing bacteria were screened based on their morphological characters, mucous and ropy appearances.

### 2.1. Identification and Biochemical Characterization of Bacterium

#### 2.1.1. Motility Test

The motility test was carried out by hanging drop method. A loop full of overnight bacterial cell was transferred on the center of the coverslip. The cavity slide was placed over the coverslip and turned to prepare a hanging drop. It was examined under light microscope and the non-motility of bacterial cells was observed.

#### 2.1.2. Indole Production Test

Indole production test is used to test whether the organism can oxidize tryptophan for the synthesis of indole compounds. The isolate was inoculated into appropriate test tubes and one tube serves as control. Incubate tubes for 24 hours at 37°C. Culture producing a cherry red color following addition of Kovac's reagent is indole positive. The absence of red coloration indicates a negative reaction.

#### 2.1.3. Methyl Red Test

The methyl red test is employed to detect the ability of microorganisms to oxidize glucose with the production of high concentration of acid and acid products. The isolate was inoculated into test tubes containing MR-VP broth and one tube serves as control. Tubes were incubated for 24 hours at 37°C. The methyl red indicator in the pH range of 4 will turn red, which is indicative of a positive test. If the indicators turn yellow, it is a negative test.

#### 2.1.4. Voges-Proskauer Test

This test is used to differentiate the capacity of the

microorganisms to produce some non-acidic (or) neutral end product such as acetyl methyl carbinol (or) 2,3butanol. The isolate was inoculated into test tubes containing MR-VP broth and one tube serves as control incubate tubes for 24 hours at 37°C. Development of deep rose color in the culture 15 minutes following addition of Barrit's reagent is the indication of positive result. The absence of rose colouration is a negative result.

#### 2.1.5. Citrate Utilization Test

The culture was inoculated in Simmon's citrate agar and incubated at 37°C for 24 hours. This medium contains bromothymol blue (green at acidic pH around 6.0 and changes to blue at alkaline pH around 7.6). Uninoculated simmon's citrate agar has a pH of 6.9, with light green colour. Growth of bacteria in the media leads to the development of a Prussian blue color.

#### 2.1.6. Catalase Test

The isolate was transferred to the center of the slide and 1-2 drops of hydrogen peroxide was added. Catalase positive organisms were indicated by strong bubble formation by breaking H<sub>2</sub>O<sub>2</sub>. Catalase negative organism was indicated by no bubble formation.

#### 2.1.7. Oxidase Test

A clean glass slide was taken and a drop of culture suspension was placed on it. To the culture few drops of p-amino dimethylaniline oxalate was added. Oxidase positive microorganisms were indicated by blue color formation. Oxidase negative organisms were indicated by no color formation.

#### 2.1.8. Hydrogen Sulfide Production

Experimental organisms were inoculated into appropriately labelled tubes by means of a stab and steak inoculations. The tubes without organism served as control. All the cultures were incubated for 24 hours at 37°C. Development of black color in the medium was positive for H<sub>2</sub>S production. Absence of the black color was evidence of a negative reaction.

#### 2.1.9. Nitrate Reduction Test

The culture was inoculated into nitrate broth and incubated at 37°C for 24 hours. After incubation few drops of naphthalamine and sulphanic acid was added and mixed well. Positive result is indicated by red color formation.

#### 2.1.10. Urease Test

Urea base medium was prepared and sterilized. The urea was added after sterilization and slant was prepared. The loop full of inoculum was streaked on the urease slants and they detected for urease production by utilization of urea with respect to production by utilization of urea with respect to production of alkaline conditions indicated by color change.

## 2.2. Identification of Bacteria by 16S rRNA Gene Sequencing Analysis

### 2.2.1. Genomic DNA Isolation from Isolate

The isolated bacterial strain was grown in 25ml LB broth overnight at 35°C. The culture was spin at 5000rpm for 5min. The pellet was resuspended in 400µl of sucrose TE buffer (Tris EDTA). Lysozyme was added to a final concentration of 8mg/ml and incubated for 1 hour at 35°C. To this tube, 100µl of 0.5M EDTA (pH 8.0), 60µl of SDS and 3µl of proteinase-K (20 mg/ml) were added and incubated at 55°C. After incubation, they were centrifuged at 7000 rpm for 3min and then the supernatant were extracted twice with phenol: chloroform (1:10 and again with chloroform: isoamyl alcohol (24:1). It was precipitated with ethanol. The DNA pellet was resuspended in sterile buffer.

### 2.2.2. Amplification of 16S rRNA Gene Sequence

Bacterial 16S rDNA was amplified from the extracted genomic DNA using the following universal eubacterial 16S rRNA primers: forward primer 5' AGAGTTTGATCCTGGCTCAG 3' and reverse primer 5' ACGGCTACCTTGTTACGACTT 3'. Polymerase chain reaction was performed in a typical reaction mixture was 2µl of template DNA and 1.5µl of forward primer, 1.5µl of reverse primer, 10µl of 2X PCR master mixes and 5µl of nuclease free water for 20µl reaction. The reaction was performed with an initial denaturation at 94°C for 2 minutes. 30 cycles of denaturation at 94°C for 45 seconds, annealing at 56° for 1 minute, extension at 72°C for 1 minute 30 seconds followed by final extension at 72°C for 5 minutes and hold at 4°C. The amplification of 16S rRNA gene was confirmed by running the amplification product in 1% agarose gel electrophoresis.

### 2.2.3. Bacterial EPS Quantification

After 72 hours of incubation, basal medium were centrifuged at 5000rpm for 20 min. The EPS was then precipitated from the supernatant by addition of equal amount of carbinol. The mixture were agitated with addition of methanol to prevent local high concentration of the precipitate and left over night at 4°C and centrifuged at 7000 rpm for 20 mins. After centrifugation the precipitate was collected in a petri plate and dried at 60°C [10].

## 2.3. Media Optimization of Physiological Factors for EPS

### 2.3.1. Effect of Different pH

Different pH (3, 4, 5, 6, 7, 8 and 9) were adjusted into the production medium to determine the effect of pH on bacterial growth for EPS production. Growth of the organism was determined by optical density measured at 600 nm for media optimization of physiological factors for EPS[11].

### 2.3.2. Effect of Different Temperature

Different temperatures (10, 20, 30, 40, 50 and 60°C) were used to prepare production medium to determine the effect of temperature on bacterial growth and EPS production. Growth of the organism was determined by optical density measured at 600 nm for media optimization of physiological factors for EPS [11].

### 2.3.3. Effect of Different Incubation Time

In the production medium different incubation time (24, 48, 72, 96 and 120) hours were incubated to determine the effect of incubation time on bacterial growth and EPS production. Growth of the organism was determined by optical density measured at 600 nm for media optimization of physiological factors for EPS [11].

### 2.3.4. Effects of Different Carbon Sources

Different carbon sources at 1% concentration (Dextrose, Jaggery, Sucrose, Maltose, Lactose and Molasses) were introduced to the production medium to determine the effect of carbon dose on EPS production. Growth of the organism was determined by optical density measured at 600 nm for media optimization of physiological factors for EPS [11].

### 2.3.5. Effects of Nitrogen Sources

Different nitrogen sources at 0.5% concentration (Urea, Tryptone, Glycine, Ammonium sulphate, Ammonium chloride and Ammonium carbonate) were introduced into the production medium individually to determine the effect of nitrogen source on microbial growth and EPS production. Growth of the organism was determined by optical density measured at 600 nm for media optimization of physiological factors for EPS.

### 2.3.6. Effects of Metal Ions

Different metal ions at 0.02% concentration (Mercuric choride, Manganese sulphate, Ferric chloride, Zinc sulphate, Copper sulphate, Calcium chloride, Disodium hydrogen phosphate, Mercuric oxide and ferrous sulphate) were introduced into the production medium individually to determine the effect of metal ions on microbial growth and EPS production. Growth of the organism was determined by optical density measured at 600 nm for media optimization of physiological factors for EPS.

### 2.3.7. Effects of Amino Acids

Different amino acids at 0.2% concentration (Glycine, Glutamine, Cysteine, Alanine, and Methionine) were introduced into the production medium individually to determine the effect of amino acids on microbial growth and EPS production. Growth of the organism was determined by optical density measured at 600 nm for media optimization of physiological factors for EPS [12].

### 2.3.8. Effects of Surfactants

Different surfactants at 0.2% concentration (SDS, PEG, Tween 80 and Triton X 100) were introduced into the

production medium individually to determine the effect of surfactants on microbial growth and EPS production. Growth of the organism was determined by optical density measured at 600 nm for media optimization of physiological factors for EPS [13].

### 3. Results

#### 3.1. Isolation and Selection of Selected Bacterium

Soil samples were taken from Western Ghats area for this study. The samples were serially diluted and plated for screening of efficient EPS producing microorganism. After 24 hrs of incubation, the plates were kept at 37°C. Selected strain which exhibited EPS production on basal medium after further confirmation was used for further studies. The selected strain was identified by various physical, biochemical and molecular characters. This strain exhibited Gram negative, rod shaped and non-motile bacteria. According to Bergey's manual of Determinative Bacteriology, the selected microbes were identified as *Klebsiella* sp. All the carbon utilizing and biochemical studies were performed and results were presented (Table. 1.).

**Table 1.** Morphological and biochemical characteristics of *Klebsiella* sp.

Colony morphology	Observation
Gram staining	Gram negative, rod
Motility	Non Motile
Biochemical Characters	
Indole production test	Negative
Methyl red	Negative
VogesProskauer	Negative
Citrate utilisation test	Positive
Catalase test	Positive
Oxidase test	Negative
Nitrate utilisation test	Negative
Hydrogen sulphide production	Negative

#### 3.2. Identification of Strains based on 16S rRNA Sequencing

The 16S rRNA gene of the *Klebsiella* sp. was amplified using Polymerase Chain Reaction (PCR) with the help of 16S rRNA Universal primers. The sequences were compared against 16S rRNA sequences available in the RDP database (<http://11rdp.me.msu.edu/>). The sequence analysis revealed that the strains were phylogenetically closely related to the genus *Klebsiella*. Blast analysis of the 16S rRNA sequence of isolate revealed that the selected isolates showed maximum similarity of 98% with *Klebsiella pneumoniae*

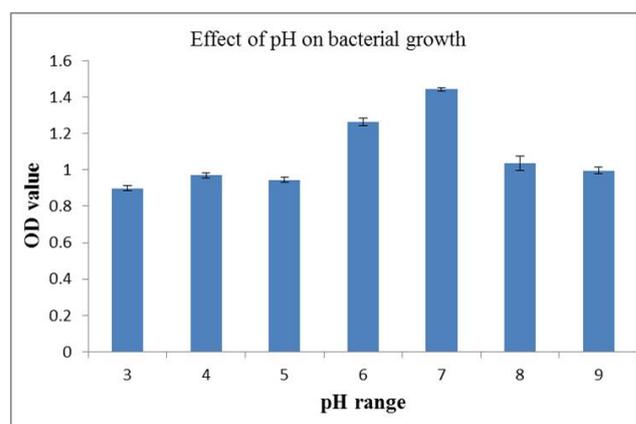
The Phylogenetic relationship was obtained using neighbor joining by pair wise comparison among the 16S rRNA gene sequence of selected isolates with species. The dendrogram was constructed for their Phylogenetic relationship and it revealed that the isolate

*Klebsiellapneumoniae* was distinctly placed under separate clusters. The 16S rRNA gene sequences of the isolates had been submitted to the NCBI Genbank and accession number *Klebsiella pneumoniae ku215681*.

#### 3.3. Media Optimization of Cultural Conditions for Eps Production by *Klebsiella pneumoniae ku215681*

##### 3.3.1. Effect of pH

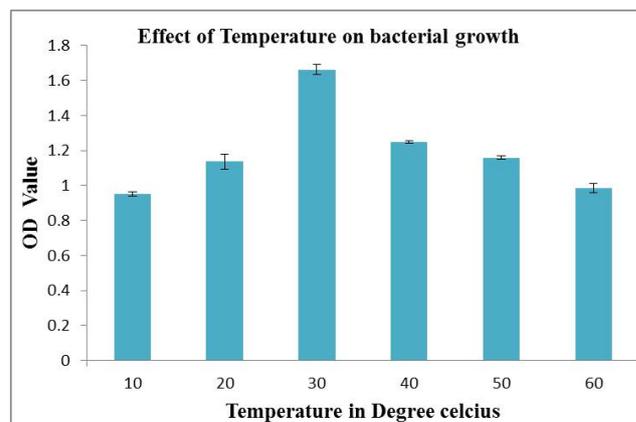
EPS production by the isolate isolated from Western Ghats soil was adjusted using various cultural conditions. The EPS production was assayed after 72 hours of incubation at 30°C under various pH. Maximum EPS production was recorded at pH 7.00 ( $1.58 \pm 0.0096$ ) next to that maximum EPS production was observed at pH 6.00 ( $1.245 \pm 0.021$ ). Minimum EPS production was recorded at pH 3.00 ( $0.879 \pm 0.0141$ ) (Fig.1).



**Figure 1.** Effect of pH on EPS production

##### 3.3.2. Effect of Temperature

Among the various temperature tested the maximum EPS production was obtained at 30°C ( $1.661 \pm 0.028$ ), followed by this at 40°C ( $1.249 \pm 0.007$ ) was the second best temperature on EPS production. On the other hand, the minimum amount of EPS production was observed at 10°C ( $0.952 \pm 0.125$  OD) (Fig.2).



**Figure 2.** Effect of temperature on EPS production

### 3.3.3. Effect of Carbon Source

The effect of carbon source on EPS production by *Klebsiella pneumoniae* KU215681 after 72 hours incubation at 30°C. Here the maximum EPS production was observed in Jaggery ( $1.37 \pm 0.003$ ) supplemented medium. The minimum EPS production was observed in Lactose ( $1.008 \pm 0.005$ ) provided medium (Fig. 3).

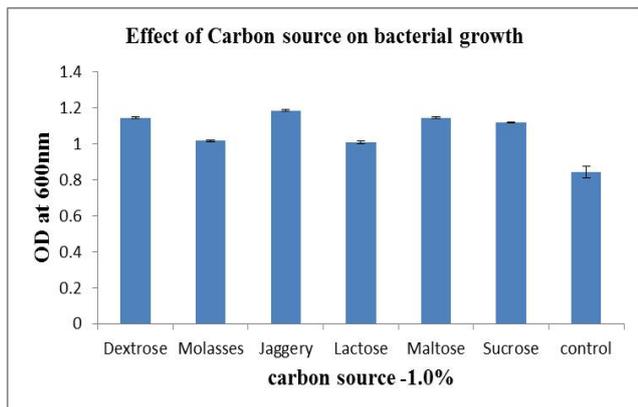


Figure 3. Effect of carbon source

### 3.3.4. Effect of Nitrogen Source

The effect of different nitrogen sources on EPS production after 72 hours of incubation period at 30°C showed maximum amount of EPS production on Tryptone ( $1.56 \pm 0.012$ ) supplemented medium and minimum amount of EPS production in urea ( $0.125 \pm 0.017$ )(Fig.4).

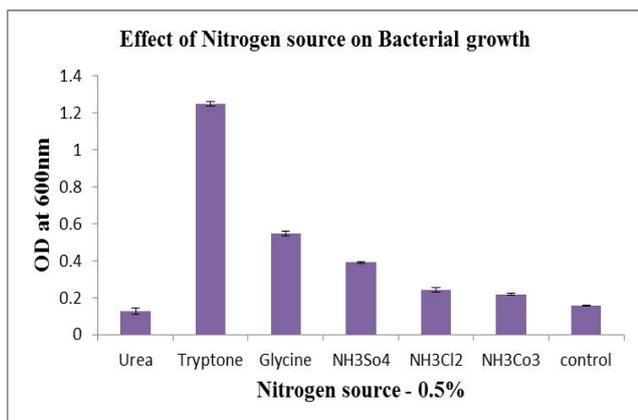


Figure 4. Effect of nitrogen source

### 3.3.5. Effect of Metal Ions

Among the tested metal ions, the maximum amount of EPS production was observed in Ferric chloride ( $1.98 \pm 0.022$ ) supplemented medium. Followed by this, magnesium oxide was the second best metal ions on EPS production ( $1.137 \pm 0.013$ ), whereas the minimum amount of EPS production was observed in zinc sulphate ( $0.793 \pm 0.004$ )(Fig.5).

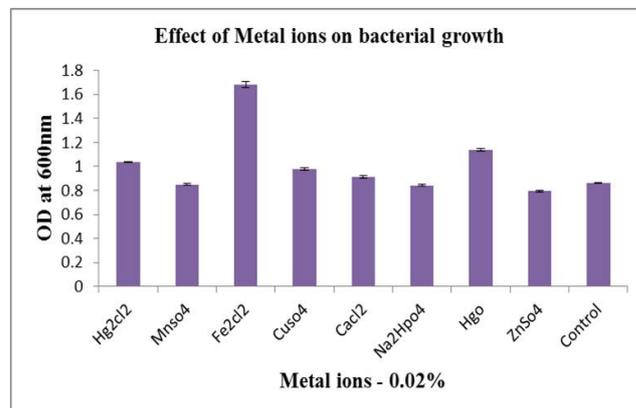


Figure 5. Effect of metal ions on EPS production

### 3.3.6. Effect of Amino Acids

The effect of various aminoacids on EPS production after 72 hours of incubation period at 30°C showed maximum amount of EPS production observed in Glutamine ( $2.13 \pm 0.067$ ) supplemented medium. Followed by this Alanine ( $2.036 \pm 0.023$ ) was second best aminoacids in EPS production, whereas the minimum amount of EPS productin was observed in Cysteine ( $1.065 \pm 0.041$ )(Fig.6).The glutamine also significant effect for EPS by the selected organism.

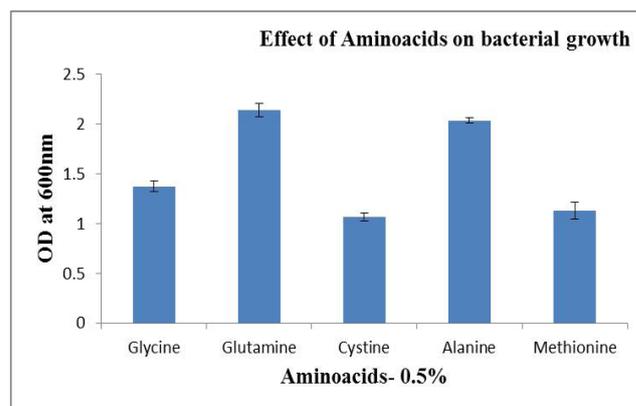


Figure 6. Effect of aminoacids

### 3.3.7. Effect of Surfactants

The effects of different kinds of surfactants was tested on EPS production after 72 hours of incubation period at 30°C. Among the tested surfactants, the maximum amount of EPS production was observed in polyethyleneglycol ( $0.79 \pm 0.043$ ) supplemented medium. Followed by this Tween-80 ( $0.667 \pm 0.033$ ) was second best surfactant in EPS production, whereas the minimum amount of EPS production was observed in SDS ( $0.0633 \pm 0.0313$ ).By the presence of PEG in the medium,the EPS producytion was higher than others(Fig.7).

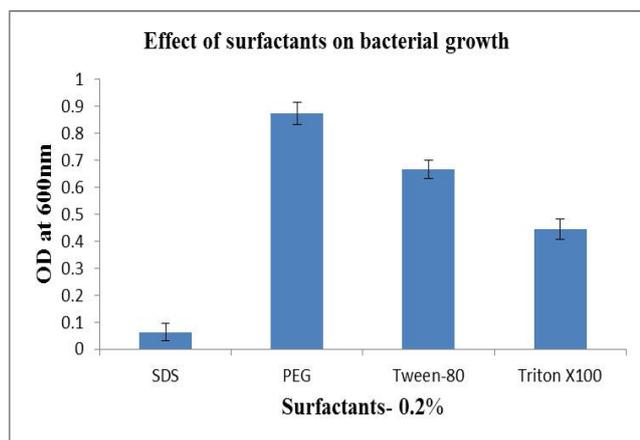


Figure 7. Effect of surfactants

## 4. Discussion

In the present study, the 16S rRNA gene sequences of the isolates had been submitted to the NCBI Genbank and accession number *Klebsiella pneumoniae ku215681*. Similarly, the B3 -72 strains of *Geobacillus thermodenitrificans*, isolated from a shallow, marine vent of Vulcano Island. The screened exopolysaccharide producing bacteria from french spoiled cidars allowed to isolate a ropy bacterium belonging to the *B. Licheniformis* species. Strains of *B. Licheniformis* are also common in foods including natural agricultural cereals [14, 15].

In the present study, the maximum EPS production was recorded at pH 7.00 ( $1.58 \pm 0.0096$ ) and Minimum EPS production was recorded at pH 3.00 ( $0.879 \pm 0.0141$ ). Likewise Liu *et al* [8] to observe that the optimal culture media were determined at pH 7.0. The optimal culture medium constituents were determined as follows: 30 g/L sucrose, 3.0 g/L soybean meal, 0.25 g/L  $MgSO_4$ , 1.5 g/L  $K_2HPO_4$ , 0.5 g/L  $KH_2PO_4$ , 0.03 g/L  $ZnSO_4$  and 0.01 g/L  $FeSO_4$ . The optimum parameters for the liquid fermentation were as follows: temperature, 25°C; cultivation time, 6days; initial pH, 8.0; volume of medium, 150 mL; and rotary speed, 180 rpm. GREP content and dry cell weight in optimized conditions were  $540.1 \pm 15.9$  mg/L and  $8.2 \pm 0.3$  g/L, respectively. GREP content under the optimized conditions was 2.5 times than that under the basic culture medium and initial conditions.

In the present study, the maximum EPS production was obtained at 30°C ( $1.661 \pm 0.028$ ). Similarly Sivakumar *et al.* [16] stated that the different temperature (25–50 °C), pH (5.0–7.5) and  $NaCl_2$  concentrations (0–4%) were analyzed in MRS broth. The optimal temperature for cell growth and EPS production were 35 °C with the corresponding cell growth ( $OD-1.333 \pm 0.02$ ,  $1.335 \pm 0.05$  and  $1.358 \pm 0.02$ ) and EPS (g/L) production ( $7.8 \pm 0.29$ ,  $7.9 \pm 0.34$  and  $8.1 \pm 0.27$ ) reported the maximum EPS production by *Lactobacillus plantarum* MTCC 9510 at 35°C.

In the present study, the carbon source on EPS production

by *Klebsiella pneumoniae* KU215681 after 72 hours incubation at 30°C. Here the maximum EPS production was observed in Jaggery ( $1.37 \pm 0.003$ ) supplemented medium. Vijayabaskaret *al.* [17] reported that maximum EPS production by *L. plantarum* MTCC 9510 was observed in presence of lactose ( $40 \text{ g L}^{-1}$ ). Growth and EPS production by lactic acid bacteria was also enhanced by nitrogen sources. Shankar *et al.* [13]. The effect of nitrogen sources on EPS production by *S. phocae* showed that yeast extract was most effective than other tested nitrogen sources. This may be due to the presence of larger quantities of free amino acids, short peptides and more growth factors in yeast extract. Among the various concentration, yeast extract at  $20 \text{ g L}^{-1}$  showed maximum EPS ( $12.14 \pm 0.31 \text{ g L}^{-1}$ ) production. Yeast extract was reported to be the most efficient nitrogen source, which greatly enhanced the EPS production by *L. plantarum* MTCC 9510 observed maximum EPS production was high in the presence of yeast extract with *Paenibacillus polymyca* EJS-3 [16, 17].

In the present study, the effect of different nitrogen sources on EPS production after 72 hours of incubation period at 30°C showed maximum amount of EPS production on Tryptone ( $1.56 \pm 0.012$ ) supplemented medium. Correspondingly, the effect of carbon sources on cell growth and EPS production by *S. phocae* was investigated in MRS broth. Among the carbons sources lactose ( $15 \text{ g L}^{-1}$ ) was found to be best for EPS production. Growth and EPS production by lactic acid bacteria was also enhanced by nitrogen sources [18]. The effect of nitrogen sources on EPS production by *S. phocae* showed that yeast extract was most effective than other tested nitrogen sources. This may be due to the presence of larger quantities of free amino acids, short peptides and more growth factors in yeast extract. Among the various concentration, yeast extract at  $20 \text{ g L}^{-1}$  showed maximum EPS ( $12.14 \pm 0.31 \text{ g L}^{-1}$ ) production (Fig.4) Yeast extract was reported to be the most efficient nitrogen source, which greatly enhanced the EPS production by *L. plantarum* MTCC 9510 observed maximum EPS production was high in the presence of yeast extract by *Paenibacillus polymyca* EJS-3 [16, 17].

In the present study, the maximum amount of EPS production was observed in Ferric chloride ( $1.98 \pm 0.022$ ) supplemented medium. Similarly Sivakumar *et al.* [12] stated that the crude invertase was pre-incubated at 30°C for 30 minutes at different concentration of the metal ions prior to standard invertase activity assay with sucrose. Maximum invertase activity of 92.74% was recorded at calcium chloride. Minimum invertase activity of 23.25% was recorded at potassium chloride. Partial inhibition of the crude invertase was in the order of  $KCl > MnSO_4 > ZnSO_4 > NiSO_4 > MgSO_4 > CoCl_2$

In the present study, the effect of various aminoacids on EPS production after 72 hours of incubation period at 30°C showed maximum amount of EPS production observed in Glutamine ( $2.13 \pm 0.067$ ) supplemented medium. In the same way Chen *et al.* [15] demonstrated that the effect of amino acids on invertase production by *Saccharomyces*

*cerevisiae* MTCC 170 was studied. Maximum amount of invertase production of  $0.26 \pm 0.005$  IU/ml was recorded in methionine and minimum invertase production of  $0.03 \pm 0.002$  IU/ml was recorded in L-lysine by *Saccharomyces cerevisiae* MTCC 170.

Among the tested surfactants maximum amount of EPS production was observed in polyethyleneglycol ( $0.79 \pm 0.043$ ) supplemented medium. Shankar *et al.* [13] investigated the effect of surfactants on invertase activity by *Saccharomyces cerevisiae* MK. They determined the effect of various metal ions such as Triton X-100, Tween-20, SDS, polyethyleneglycol and Tween-80 on the effect of microbial growth and EPS production. They recorded maximum invertase activity of 35.88% at poly ethylene glycol (1%) in *Saccharomyces cerevisiae* MK invertase which was in accordance with the present study.

## 5. Conclusions

A pure bacterial strain capable of producing EPS bacterial strain was isolated and identified as *Klebsiella pneumoniae ku215681* by carbohydrate fermentation profile and sequence analysis of 16S rRNA. The important parameters had significant positive effects on the EPS production in different nutrients and cultivation conditions.

## Conflict of Interest Statement

The authors declare that they have no conflicting interests.

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