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Research Article

# Optimization of Fermentation Conditions Required for Exopolysaccharide (EPS) Production by *Bacillus altitudinis* Strain EPBAS.1 Obtained from Brewery Wastewater Sludge

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***Abstract***— Bacteria exopolysaccharides (EPS) are long-chain high-molecular weight polysaccharides synthesize in bacteria and exported into the external environment and their production is influenced by several key cultural and fermentation conditions, which this study aimed to determine the optimum specific for *Bacillus altitudinis* strain EPBAS.1 obtained from brewery wastewater sludge for maximal production. Brewery wastewater sludge samples were collected and screened for EPS-producing bacteria using standard microbiological methods. The isolates were grown in broth culture for 48h, centrifuged at 4000 rpm for 30 minutes, the EPS extracted by ethanol precipitation to determine its yield, and the best EPS-producer identified molecularly and used for optimization study using one-factor at a time method. A total of 85 bacterial isolates were screened, 29 showed EPS production ability, while isolate EPBS.1 produced statistically ($P<0.05$) higher quantity (0.58±0.04g/100ml) of EPS, and was identified to the species level as *Bacillus altitudinis* strain EPBAS.1. From the optimization study, higher EPS yield (0.65±0.03g/100ml) was obtained with sucrose than other carbon sources. Organic nitrogen, with yeast extract as the most preferred, gave higher yield (0.68±0.01g/100ml) than inorganic nitrogen. pH, temperature, incubation period, inoculum size, and $NaCl_2$ concentration gave optimal EPS yields at 7.0 (0.66±0.01g/100ml), 35$^oC$ (0.72±0.03g/100ml), 72h (0.69±0.01g/100ml), 4% (0.74±0.02g/100ml), and 5% (0.68±0.01g/100ml), respectively. The EPS extracts showed higher total carbohydrate (68.5±0.10%) than protein (16.2±0.31%) contents. Hence, the study reveals the optimal EPS production conditions for *Bacillus altitudinis* strain EPBAS.1 and recommends the use of sucrose, yeast extract, neutral pH, temperature at 35$^oC$, 72h incubation time, 4% inoculum size, and 5% $NaCl_2$ for optimal EPS production by the bacteria strain.

***Keywords***— Optimization, fermentation conditions, exopolysaccharides, *Bacillus altitudinis*, brewery wastewater sludge

## 1. Introduction

Bacteria exopolysaccharides (EPS) are long-chain high-molecular weight polysaccharides synthesize in bacteria and exported into the external environment [1]. It is made up of repeating monomeric units, such as monosaccharide, amino sugars, and uronic acids connected by glycosidic linkages, as well non-carbohydrate materials such as amino acids, nucleic acids, phospholipids, humic acids, functional groups and glycerol [2, 3]. Most bacteria naturally produce and export diverse types of polysaccharides as part of their normal metabolic activity and these polysaccharides may be grouped into three categories as (i) intracellular polysaccharides, which functions primarily as carbon and energy store in the cell, (ii) structural polysaccharides, which confer structural integrity to the bacterial cell wall, and (iii) extracellular polysaccharides which are exported outside the bacteria cells [4], [5]. However, in a hostile environment, bacteria may produce EPS as a means of protection against such environment [6].

Bacteria EPS have found outstanding applications in many areas, as conjugates for drugs in biomedical industries, thickening and stabilizing agent in the food and cosmetics industries, as well as emulsifiers and surfactants in bioremediation and flocculants in wastewater treatment [7, 8]. The use of EPS-producing microorganisms in the remediation of heavy metal-related environmental pollution is a growing field of biotechnology [5].

EPS-producing bacteria are found in diverse of environment, but environment having a medium with high carbon-nitrogen

 

ratio or high amount of organic substances have been claimed as suitable habitat for the growth of bacteria with high EPS-producing potentials [9]. However, several conditions which affect microbial growth in the environments or medium have been reported to have direct effect on exopolysaccharide production and such include microbial growth phase, carbon source, nitrogen source, oxygen availability, temperature, pH, presence of heavy metals, and salinity [10].

Though several studies have been carried out on bacterial EPS production in recent times, studies to discover novel bacteria strains for high production of EPS are still needed. Hence, this study was designed to isolate novel EPS-producing bacteria from brewery wastewater sludge and optimize the fermentation conditions required for the production of EPS.

## 2. Related Work

Many bacteria genera are endowed with inherent abilities to synthesize and export exopolysaccharides (EPS) to the external environment [11]. In recent years, there has been increasing interest in the search for novel EPS-producing bacteria and the optimization of medium conditions to enhance the production of EPS due to its diverse industrial applications and high commercial values. EPS production is affected by many factors, including the microbial species, medium composition and several environmental conditions [12].

In a study of the optimization of exopolysaccharide produced by *lactobacillus plantarum* R301 using single-factor and response surface optimization, [12] have reported 84.70% increase in EPS yield from 53.34 mg/L before optimization to 97.85 mg/L after optimization. In a study by [3] to determine the effect of carbon sources on EPS production by *Lysinibacillus macroides* using cheap agricultural wastes, bagasse was found a more suitable substrate with highest EPS production of 13.13±0.44 $gL^{-1}$.

Similar study have also been conducted on optimization of EPS production using cost-effective medium and the result obtained showed that sweet potato peels containing medium gave maximum EPS yield of 2.26 g/l compared to sucrose containing medium with yield of 1.25 g/l and highest production yield of 9.46 g/l from beans bran extract medium as compared to yeast extract medium (5.41 g/l) [13]. The effect of other parameters such as incubation period, agitation speed, inoculum size, pH, and the temperature was also studied and found to have the highest EPS production after an incubation time of 120h (15.10 $gL^{-1}$), agitation speed of 100 rpm (12.47 $gL^{-1}$), 10% inoculum size (13.43 $gL^{-1}$), pH 7 (11.47 $gL^{-1}$) and incubation temperature at 30°C (13.17 $gL^{-1}$). Ref. [14] had investigate the effects of the cultivation time, temperature, and pH value on the yield and composition of extracellular polymeric substances (EPS) from *Enterobacter* sp. Maximum EPS production was obtained when the cultivation time, temperature, and pH value were 24 h, 30 °C, and 8.0, respectively. During the first 24 h of cultivation, the yields of EPS increased with increasing cultivation time and reached maxima of 267.78mg/l, 63.85mg/l, and 20.43 mg/l. According to [15], in their screening for potential exopolysaccharide producers from *Lactobacillus* species isolated from locally fermented milk (*Nono*), EPS production and yield were influenced by the type of carbon and its concentration. EPS yield increased with increase in sugar concentration. However, each isolate had a preferred carbon source for EPS synthesis. In a study of the purification and characterization of novel exopolysaccharides produced from *Lactobacillus paraplantarum* KM1 isolated from human milk and its cytotoxicity, it has been revealed that low temperature has very little effect on the EPS activity as compared to the high temperature [16]. Similarly, with respect to pH variation, it was observed that EPS retained 75.3% of activity at pH 4.0, whereas 92.9% of its relative activity was retained at pH 9.0. In a study to optimize the production of EPS by marine *Bacillus subtilis* SH1, effect of medium type, incubation period and pH were studied using the one-factor at a time experiments and result showed 4-fold increase from 24g/l to 33.8 g/l of exopolysaccharides using yeast malt glucose medium at pH 9 and four days of incubation period [17].

Ref. [18] had carried out a study on medium optimization for exopolysaccharides production by *Bacillus Zhangzhouensis* BZ16 strain isolated from Khnifiss Lagoon and under optimal medium composition, EPS was produced at 12.37g/L, which was 6 times greater than the production yield achievable without optimizing conditions.

## 3. Methodology

### 3.1 Isolation and Screening of EPS-producing Bacteria

Brewery wastewater and sludge samples were collected from Champion Brewery Ltd., Uyo, Nigeria (longitudes 7°55'E – 7°56'E and latitudes 5°00'N – 5°01'N) using standard microbiological procedure. The samples were prepared by serial dilution in normal saline and 1ml aliquot from the dilution factor $10^{-6}$ was inoculated into sterile nutrient agar by pour plating technique, and incubated at room temperature for 24h. Colonies with characteristic mucoid and ropy appearance were isolated and purified [13]. The pure colonies were screened for EPS production by staining with Alcian blue 8GX as described by [19]. Colonies showing blue-black colouration were selected and preserved on agar slants at 4 ± 3 °C as EPS-producing isolates.

### 3.2 Tests for Growth and EPS Production Yields of the Isolates

This was carried out according to the methods described by [13], [19], with only a slight modification. Pure EPS-producing isolates were inoculated into 100 ml of trypticase soy broth (TSB) in 250 ml Erlenmeyer flasks and incubated on a rotary shaker (120 rpm) at room temperature for 48h. The cultures after incubation were centrifuged at 4000 rpm for 30 minutes to harvest the cells. The cells were washed twice with normal saline to remove every elements of broth, placed in *Petri* dishes and dried to constant weight in desiccators overnight at room temperature, and weighed. To determine EPS yields, the supernatant phase of the

 

centrifuged culture of each isolate was mixed with ice-cold absolute ethanol (3 volume of ice-cold ethanol to 1 volume of supernatant) and incubated at 4°C in the refrigerator for 24h to precipitate the EPS. After incubation, the mixtures were centrifuged at 4000 rpm for 30 min and the pellet containing slime EPS collected into Petri dishes, weighed and dried in desiccators overnight at room temperature. The dried EPS were weighed and preserved as crude EPS at $4^oC$ in the refrigerator for further studies.

### 3.3 Determination of Optimal Fermentation Conditions for EPS Production

Effect of fermentation conditions on EPS production was studied using one-factor at a time method [13]. [20]. One hundred millilitre (100ml) of production medium [containing (g/L) $KH_2PO_4$ 0.5, $MgSO_4 \cdot 7H_2O$ 0.2, NaCl 0.5, $CaCl_2 \cdot 2H_2O$ 0.5, $FeCl_3 \cdot 6H_2O$ 0.05, $MnCl_2 \cdot 4H_2O$ 0.001, $ZnSO_4 \cdot 7H2O$ 0.001, distilled water (1L), final pH 7.0] in 250ml flasks was inoculated with 1% of 24h broth culture (at 1 $O.D_{600nm}$ ) of the isolate and incubated on a rotary shaker (120 rpm) at room temperature for 48h. Growth and EPS yields were determined at each interval of the factors tested as earlier described. Varied levels of carbon sources (0%, 1%, 2%, 3%, 4% and 5%), nitrogen sources (0%, 1%, 2%, 3%, 4% and 5%), pH (4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0), Temperature ($25^oC$, $30^oC$, $35^oC$, $40^oC$, and $45^oC$), time (24h, 48h, 72h, 96h, and 120h), inoculum size (1%, 2%, 3%, 4%, and 5%), and $NaCl_2$ (1%, 2%, 3%, 4%, and 5%) were used.

### 3.4 Determination of Total Carbohydrate and Protein Contents of EPS Extract

Total carbohydrate and protein contents of the EPS produced by isolate EPBS.1 were determined using phenol-sulphuric acid method and bicinchoninic acid (BCA) method, respectively [21]. Exactly 1g of EPS extract was dissolved in 1.0 ml of distilled water in test tubes and 1ml of phenol (5% w/v) was added, mixed thoroughly and allowed to stand for 5 min. Then 5.0ml of concentrated sulphuric acid was added using adjustable pipette, mixed immediately and incubated at 30 $^oC$ on a water bath for 10 min and the optical density of the reaction mixture measured with spectrophotometer at 490nm to determine the total carbohydrates content using glucose as a standard.

For the total protein, 100µl of a solution of EPS extract was transferred into a clean test tube, 2.0 ml of bicinchoninic acid (BCA) reagent was added, mixed carefully and incubated on a water bath at 37 $^oC$ for 30 minutes. The intensity of the color formed was measured in a spectrophotometer at 562nm using bovine serum albumin as a standard.

### 3.5 Molecular Characterization and Identification of Isolate

Molecular identification of the EPS-producing isolate EPBS.1 was carried out using PCR amplification of the 16S rRNA and Sanger sequencing methods. Norgen DNA extraction kit (model 24700, Norgen, Canada) and procedure was used in the extraction of the genomic DNA of the isolate and amplified on a real-time thermocycler (ThermoFIsher, Model 9700, USA). The Sequence for the primer pairs used for the amplification of 16S rRNA conserved bacterial gene was 27FAGAGTTTGATCMTGGCTCAG and 1492RTACGGYTACCTTGTTACGACTT. The integrity of the PCR amplicons was visualized on a 1% agarose gel (CSL-AG500, Cleaver Scientific Ltd) stained with EZ-vision® Bluelight DNA Dye. The nucleotide sequences of 16S rRNA gene obtained were compared for similarity with existing 16S rRNA sequences on NCBI GeneBank database using BLASTN and a neighbour-joining phylogenetic tree based on the sequences constructed using MEGA 11.0 software [22].

### 3.6 Data Analysis

One-way ANOVA was carried out using IBM SPSS statistics v.23 at 5% significant level to compare the effect of the fermentation conditions on EPS production. Data were expressed as means ± SE and separated using the Duncan.

## 4. Results

### 4.1 Isolation, Screening, and EPS Production yield of the Bacteria Isolates

A total of 85 mucoid and ropy bacterial colonies were isolated from 155 brewery wastewater sludge samples and screened for EPS production. Twenty nine (29) of the isolates, coded EPBS.1 to EPBS.29, produced EPS at significantly ($P<0.05$) varied quantity which ranged between 0.08±0.00 g/100ml and 0.58±0.04 g/100ml. Isolate EPBS.1 produced the highest (0.58±0.04 g/100ml) quantity of EPS and was selected for optimization study. Table 1 shows the morphological characteristics of the EPS-producing bacteria isolate EPBS.1 obtained from the brewery wastewater samples. Microscopy confirms the isolate as Gram's positive rod with endospores and capsule.

**Table 1:** Morphological Characteristics of the EPS Producing Isolate EPBS.1

| | |
|---|---|
| Size | Large |
| Shape | Circular |
| Colour | Creamy |
| Elevation | Raised |
| Margin | Entire |
| Mucoid formation | + |
| Ropyiness | +++ |
| Gram's reaction | + Rods |
| Spore formation | Terminal endospore |
| Capsule formation | Capsulated |

### 4.2 Optimal EPS Production Conditions of Bacteria Isolate EPBS.1

**Effects of Carbon Sources on EPS Production:** The effects of carbon sources (glucose, fructose, galactose, sucrose, maltose, lactose, and corn-steep liquor) on EPS production yields of the bacteria isolate EPBS.1 is presented in figure 1. The result shows increase in EPS yield across the different carbon sources as their concentrations were raised from 1% to 5%. Highest yield of 0.65±0.03g/100ml was obtained with sucrose while the least EPS yield of 0.26±0.01g/100ml was obtained with lactose. In all, the yields obtained with the carbon sources were higher than the control (0.14±0.02

 

g/100ml) and varied significantly (P<0.05) among the different carbon sources.

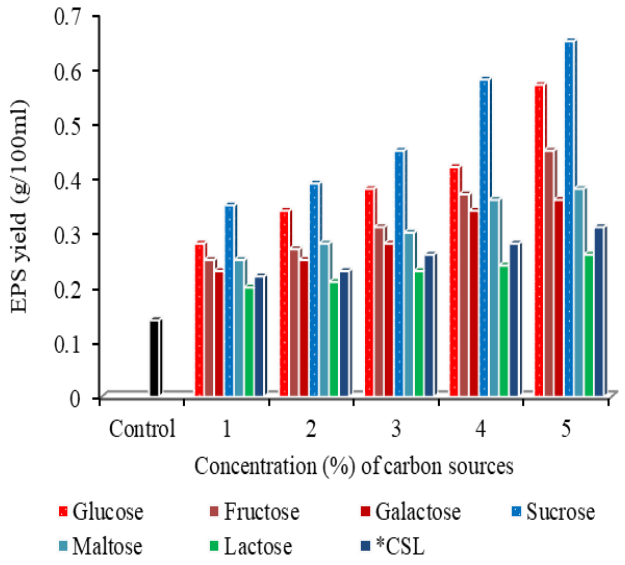


**Figure 1:** Effect of carbon sources on EPS production by bacteria isolate EPBS.1 obtained from brewery wastewater sludge (*CSL- corn steep liquor)

**Effects of Nitrogen Sources on EPS Production Yield:** The effect of organic nitrogen such as tryptone, yeast extract, peptone, beef extract and soya bean meal, and inorganic nitrogen such as $(NH_4)_2NO_3$ and $(NH_4)_2SO_4$ on EPS production by the bacteria isolate EPBS.1 is shown in Figure 2. Among the organic nitrogen sources, EPS production yield was highest (0.68±0.01g/100ml) with yeast extract and lowest (0.42±0.1g/100ml) with tryptone. Similarly, of the inorganic nitrogen sources, higher EPS yields of 0.40±0.2g/100ml was obtained with $(NH_4)_2SO_4$ than $KNO_3$ with the yield of 0.36±0.2g/100ml. The organic nitrogen sources supported higher EPS yield than the inorganic sources, but both yields were comparatively higher than the control (0.14±0.02 g/100ml).

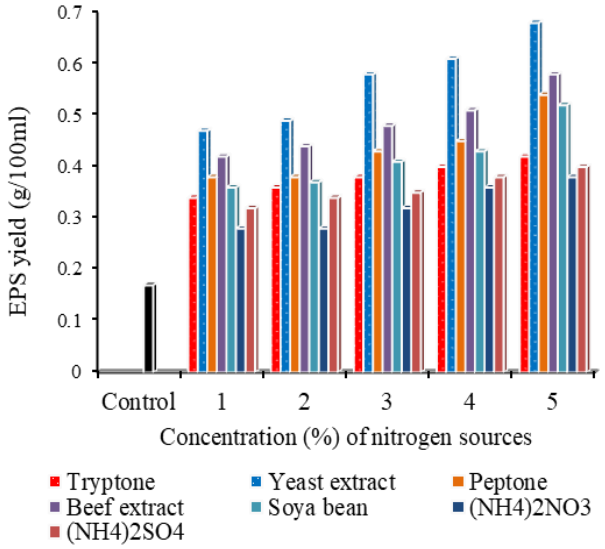


**Figure 2**: Effect of nitrogen sources on EPS production by bacteria isolate EPBS.1 obtained from brewery wastewater sludge

**Effects of pH on EPS Production Yield**: The results of the effect of different levels of pH (4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0) on EPS production by the bacteria isolate EPBS.1 is presented in Figure 3. The bacteria isolate EPBS.1 produced varied quantity of EPS at the different levels of pH. The yields increased significantly (P<0.05) from a minimum of 0.15±0.01g/100ml at pH 4.5 to an optimum of 0.66±0.00g/100ml at pH 7.0 and then decreased to 0.25±0.02g/100ml at pH 8.0.

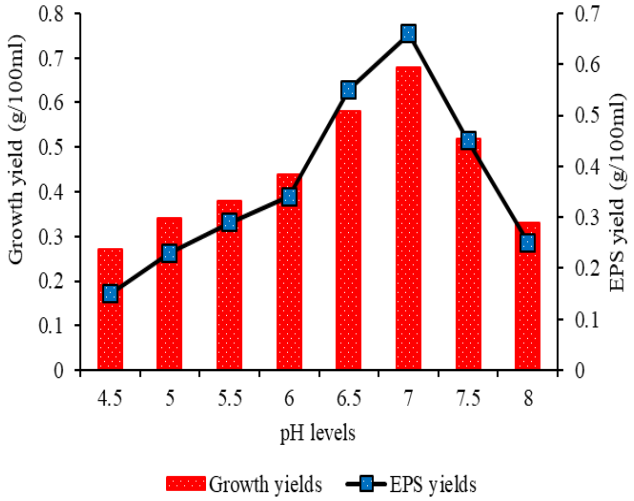


**Figure 3:** Effect of pH on EPS production by bacteria isolate EPBS.1 obtained from brewery wastewater sludge

**Effects of Temperature on EPS Production Yield:** The results of the effect of varying incubation temperature (25°C, 30°C, 35°C, 40°C, and 45°C) on EPS production by the bacteria isolate EPBS.1 is presented in Figure 4. EPS production by the bacteria isolate varied significantly (P<.05) at the different incubation temperature. The yield increased from a minimum of 0.61±0.02g/100ml at 25°C to an optimum of 0.72 ± 0.03g/100ml at 35°C and then decreased sharply at temperature above 35°C to 0.34±0.01g/100ml at 45°C.

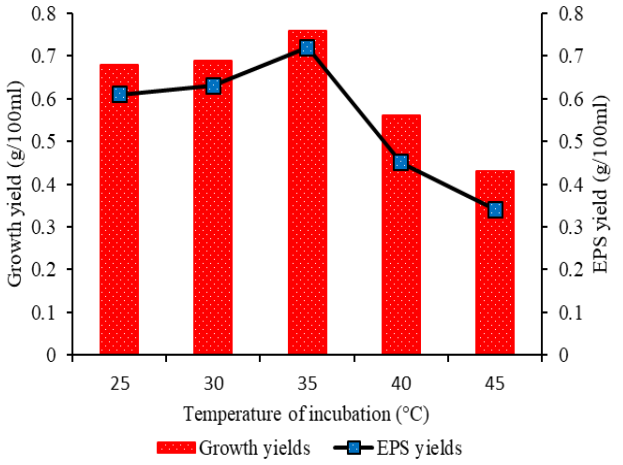


**Figure 4:** Effect of incubation temperature on EPS production by bacteria isolate EPBS.1 obtained from brewery wastewater sludge

 

**Effects of Incubation Time on EPS Production Yield:** Figure 5 shows how incubation time affect EPS production yield of the bacteria isolate EPBS.1. According to this result, EPS yield by the bacteria isolate EPBS.1 increased as the incubation time was increased from 24h with a minimum yield of 0.41±0.01g/100ml to 72h with a maximum yield of 0.69±0.01g/100ml and then dropped to 0.57±0.01g/100ml at the incubation time greater 72h.

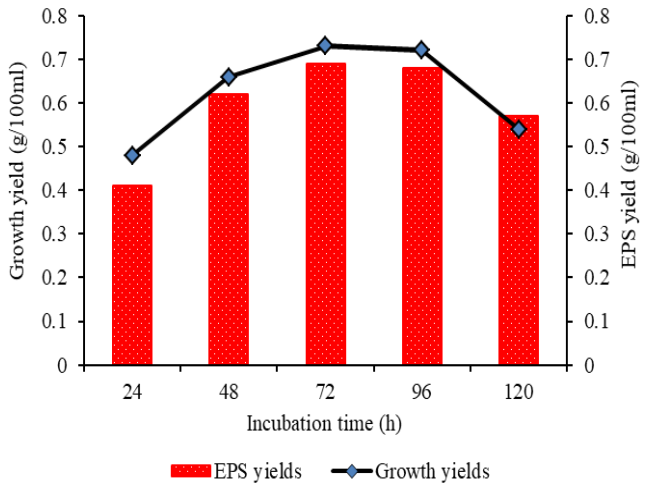


**Figure 5:** Effect of incubation time on EPS production by bacteria isolate EPBS.1 obtained from brewery wastewater sludge

**Effects of Inoculum Size on EPS Production Yield**: The results obtained regarding the effect of inoculum size on EPS production yield showed a significant ($P<0.05$) increase in the yield of EPS by the bacteria isolate EPBS.1 as the inoculum size was increased from 1% with a yield of 0.64±0.02g/100ml to 4% with a yield of 0.74±0.04g/100ml (Figure 6). The result recorded a decreased yield (0.66±0.01g/100ml) as the inoculum size was increased above 4%.

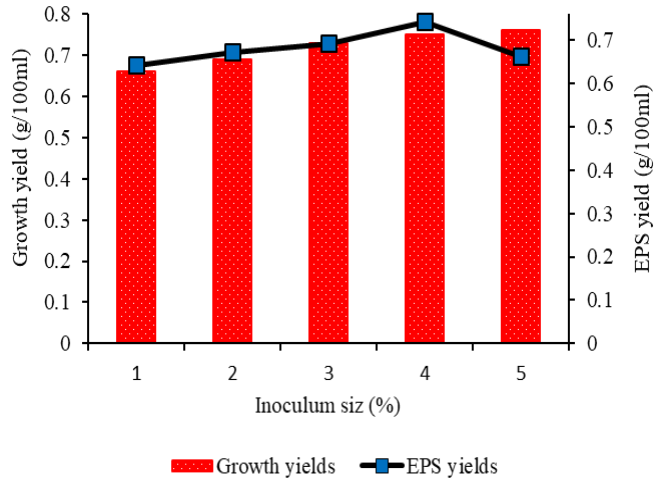


**Figure 6:** Effect of inoculum size on EPS production by bacteria isolate EPBS.1 obtained from brewery wastewater sludge

**Effects of $NaCl_2$ on EPS Production Yield:** The effect of $NaCl_2$ on EPS production by the bacteria isolate EPBS.1 is shown in figure 7. From the result, EPS yield by the isolate increased as the concentration of $NaCl_2$ was increased from 1% with a yield of 0.62±0.01g/100ml to 5% with a yield of 0.68±0.03g/100ml. However, the growth yield of the isolate decreased with further increase in $NaCl_2$ concentration beyond 3%.

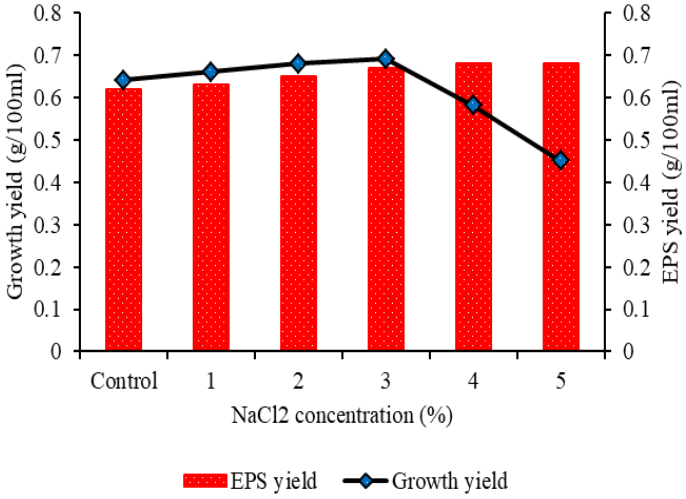


**Figure 7:** Effect of $NaCl_2$ on EPS production by bacteria isolate EPBS.1 obtained from brewery wastewater sludge

### 4.3 Total Carbohydrate and Protein Contents of the EPS Extract

The results of the analysis of total carbohydrate and protein contents of the EPS extract produced by the bacteria isolate EPBS.1 showed higher total carbohydrate of 68.5±0.10% and total protein content of 16.2±0.31% (Figure 8).

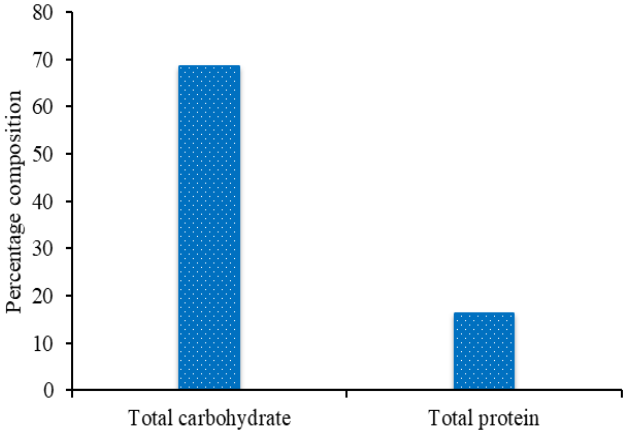


**Figure 8:** Total carbohydrate and protein composition of EPS produced by bacteria isolate EPBS.1 obtained from brewery wastewater sludge

### 4.4 Molecular Characteristics and Identity of the EPS-Producing Bacteria Isolate EPBS.1

The gel-image (Plate 1) of the PCR product showed a clear band of base pairs of the bacteria EPBS.1 and the marker (ladder). The BLASTN analysis of the 16S rRNA sequence of the bacteria isolate revealed a similarity of 97.83% with *Bacillus altitudinis* (ON514292.1) (Figure 9). The sequence was submitted to Genebank and a new accession number and

 

strain name assigned as PP216566 (*Bacillus altitudinis* strain EPBAS.1).

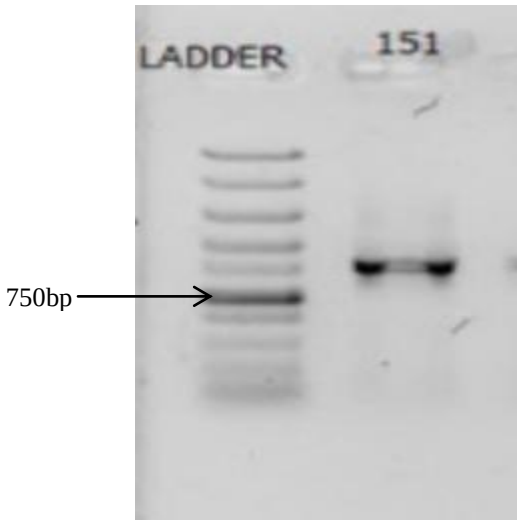


**Plate 1:** Gel-image of the PCR product of the EPS producing bacteria isolate EPBS.1

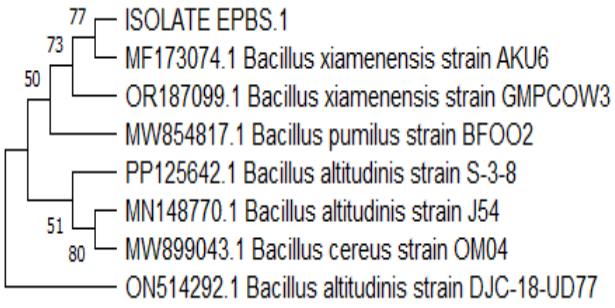


**Figure 9:** Phylogenetic tree and evolutionary relationship of the 16S rRNA nucleotide sequence of PP 216566.1 *Bacillu altitudinis* EPBAS 1 with closely related sequences available in GenBank

## 5. Discussion

EPS production by bacteria is dependent on several key cultural and fermentation conditions, such as carbon and nitrogen sources, pH, temperature, incubation time, inoculum size, and $NaCl_2$. Different strains of EPS-producing bacteria require certain levels of these conditions for optimal growth and EPS production, and a slight deviation in these conditions may reflect in the quality and quantity of EPS produced [3], [23]**.** Many strains of *Bacillus* species possesses a diversity of physiological properties which confer on them several adaptive advantages to live and survive in diverse natural environment. Hence, it is very important to determine the optimal levels of the fermentation conditions specific for *Bacillus altitudinis* to ensure maximal EPS production.

In this study, *Bacillus altitudinis* strain EPBAS.1 was isolated and confirmed by the molecular identification procedures. As a member of *Bacillus* genera, *Bacillus altitudinis* exhibit diverse physiological ability which enable its survival in different environment, including brewery wastewater sludge and is a known source of huge microbial metabolites with commercial and biotechnological values [24]. The test for EPS production has revealed the organism as outstanding EPS-producing bacteria and a promising source of this metabolite to meet industrial and biotechnology demands.

The result of the effects of carbon sources on EPS production revealed that the isolate (*Bacillus altitudinis* strain EPBAS.1) responded differently to the different carbon sources, suggesting a preferred carbon source. The EPS production yields differs significantly ($P<0.05$) among the carbon sources and increases as the concentrations of the carbon sources in the medium were increased. Sucrose was the most preferred carbon source which gave the highest EPS yields while lactose gave the least yield. Corn steep liquor (CSL) as a cheap agricultural carbon source gave higher EPS yields than xylose and lactose. This is in tandem with the reports from similar studies [24], [25], [26]. Moreover, [26] has linked these variations in carbon sources requirement by EPS-producing bacteria to the differences in their EPS biosynthesis regulatory pathways.

According to the results on the effects of nitrogen sources on EPS production, the EPS production yields of the bacteria strain varied significantly ($P<0.05$) with the different nitrogen sources and increases steadily as the concentration of the nitrogen sources were increased. Among the organic nitrogen, higher EPS yield was obtained with yeast extract, followed by beef extract and peptone, while tryptone gave the least EPS yield. These were statistically ($P<0.05$) higher than the yield obtained from the inorganic nitrogen sources. Similar results have been obtained in other studies [18], [25].

pH is one of the important fermentation factors which affect microbial metabolic functions , such as solubility and assimilation of nutrients, activity of enzymes, cell membrane integrity, and metabolites production [27]. The optimum pH for exopolysaccharide production varies among bacterial species and usually occurs near neutrality [28]. However, at extreme acidic or alkaline pH, the process of bacterial growth and biosynthesis activity of the enzymes associated with EPS production are severely decreased [14]. In this study, the optimum EPS production by *Bacillus altitudinis* strain EPBAS.1 was obtained at pH 7.0 and correlate with the findings reported by [3], [15].

Temperature is a critical factor affecting the biosynthesis processes of bacteria and its optimal level for EPS production is variable and strains-specific [28]. In this study, EPS production by the bacteria strain increased significantly ($P<0.05$) from 25$^{o}$C to reach its optimum at 35$^{o}$C. The decrease in EPS production yields at higher temperature may be caused by inhibition of enzyme activities. This result is in contrast to similar studies which have identified 30˚C as optimal temperature for EPS production [3], [15].

The optimum incubation period for EPS production varies with bacterial species. According to this result, the optimum EPS production yield by the bacterial strain was obtained at 72h and decreased at the incubation periods above the optimum. This is not surprising as EPS biosynthesis begins at a latter exponential growth phase and a decrease in the

 

incubation period may have a corresponding effect on growth and EPS production [23]. The decreased EPS yields at higher incubation period may be due to the accumulation of other metabolites, such as enzymes, which might degrade polysaccharides [23].

The production of EPS by the bacteria strain increased significantly ($P<0.05$) from the least inoculum size of 1% to its maximum at 4%, and then decreased at the inoculum size above this level. This result agrees with [15]**.** Moreover, [3] has reported highest EPS production at 10% inoculum size. Ref. [29] has reported that the optimum inoculum size for EPS production varies from 3% to 10%. According to [3]**,** however, if the inoculum size is increased beyond the optimum, crystallization of the medium may occur as a result of heavy biomass accumulation, causing a fall in EPS yield.

In a hostile environment, bacteria may produce EPS as a means of protection against such environment [6]. As shown in the result, EPS production yields increased as the concentration of $NaCl_2$ was increased. However, growth yield decreased at high salt level. This study agrees with the reports by [18]**.**

The result of the analysis of total carbohydrate and protein contents of the EPS extracts indicates high total carbohydrate than protein. This result correlates with similar studies which have reported higher carbohydrate composition than protein in their EPS extracts [17], [30], [31]

## 6. Conclusion and Future Scope

In this study, *Bacillus altitudinis* strain EPBAS.1 was isolated from brewery wastewater sludge and identified as a high EPS-producing strain among other bacteria isolates screened. This strain showed significantly higher yield of EPS under optimized fermentation conditions, such as sucrose as a carbon source, yeast as a nitrogen source, neutral pH (7.0), mesophilic temperature at 35$^{o}$C, 72h incubation time, 4% inoculum size, and 5% $NaCl_2$. Analysis of total carbohydrate and protein contents has indicated high carbohydrate content than protein, confirming the extract as EPS. This study therefore contributes to our understanding of EPS production by *Bacillus altitudinis* strain EPBAS.1, recommends that brewery wastewater sludge could be a rich source of novel EPS-producing bacteria if explored, and suggests that future study be focused on molecular methods to enhance the overproduction of EPS by *Bacillus altitudinis* and other EPS-producing bacteria strains.

**Data Availability**
Whole data not displayed in this paper, can only be provided on request when contacted.

**Conflict of Interest**
We declare no conflict of interest**.**

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**Authors' Contributions**
First Author collected the samples, carried out the laboratory work, and documented the results. Second and third authors supervised every steps of the work, reviewed, edited and approved the final version of the manuscript.

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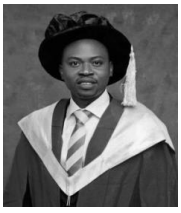

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