Original Research Article

Isolation and screening of biosurfactant producing lactic acid bacteria from traditional Indian cereal-based fermented food "Seera."

ABSTRACT

Aim: The current study aims to isolate and screen biosurfactant-producing lactic acid bacteria (LAB) from Seera, a traditional Indian fermented food from the state of Himachal Pradesh.

Study design: Manymicro-organisms have the tendency to produce biosurfactant that can act as a better replacement for synthetic surfactants as they impart many beneficial properties like antimicrobial, anti-adhesive,and anti-biofilm activity. Among biosurfactant producing micro-organisms, Lactic acid bacteria were preferedin the present study due to their non-pathogenic nature.

Place and Duration of Study:The study was conducted in Punjab University Research Centre, Department of Biotechnology,Goswami Ganesh Dutta Sanatan Dharma College (GGDSD) College, Sector-32, Chandigarh, India, between June 2019 and July 2021.

Methodology: Microbial isolates from Seerasample were isolated on MRS agar platesand screened for biosurfactant production by a battery of tests including drop collapse assay, hemolytic activity, and emulsifying activity. Strain with better biosurfactant activity and yield was selected and identified morphologically, biochemically and genetically.

Results: The strain S-2 with an emulsification index of 69.27± 0.08, surface activity of42.32±0.17,biomass yield *i.e* 3.7±0.03 g/L and biosurfactant yield i.e 1.2±0.02g/L was chosen for further characterization. Morphological characterization of the strain S-2 was carried out, and the strain was found to be gram-positive, coccus shaped, and lack endospore. Biochemical characterization and 16sRNA sequencing employingGenTool software confirmed the selected strain as *Pediococcuspentosacaeus*.

Conclusion: *Pediococcuspentosacaeus* has a high potential for biosurfactants production and can be explored for different commercial applications. It is also regarded as a safe microorganism as it does not pose any illness so far. Therefore, further research will be carried out to determine the chemical nature of the biosurfactants and evaluate their antibacterial, antiadhesive activities, and anti-biofilm

***Key words**: Biosurfactants, Fermented food, Lactic acid bacteria (LAB), Pediococcuspentosaceusand Seera*

1. INTRODUCTION

Different ethnic groups of India consumed variety of fermented foods that are produced by different micro-organisms or enzymes, resulting in beneficial biochemical changes that produce significant modifications to the food, conferring several health advantages[1]. Numerous microorganisms are responsible for food fermentation including *Acetobacter*,*Bacillus*, yeasts, and molds. One of the major classes of microbes involved in fermentation is Lactic acid bacteria (LAB) which is responsible for the fermentation of an enormous variety of dairy products (fermented milk, cheese, yoghurt), cereal, meat, vegetable products ,*etc* [2]. Lactic acid bacteria (LAB) including *Pediococcus, Enterococcus, Oenococcus, Lactococcus, Streptococcus, Lactobacillus*, *Leuconostoc, Weissellaetc*have been found to be prevalent in fermented

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foods. LAB are Generally Recognized as Safe (GRAS) according to the US Food and Drug Administration owing to their non-pathogenic nature [3].

Fermented foods are consumed in diverse forms, some are cereal-based while others are pulse-based, dairy-based, vegetables, and meat-based [4].The majority of traditional fermented foods (wheat/barley/rice/buckwheat) are cereal-based. However cereals have a low protein content and are deficient in amino acids, specifically lysine and tryptophan as well as contain anti-nutrients such as phytic acid, tannins, and polyphenols, making them inferior to other diets [5].

LAB play a vital role in fermentation of cereal by enhancing aroma, flavor, texture, organoleptic properties, and nutritional content (essential amino acids, fatty acids, micronutrients)as well as elimination of anti-nutritive factors like proteinase inhibitors, phytic acid and tannins from cereals to promote mineral bioavailability [6,7]. Some cereal-based fermented foods specific to Himachal Pradesh state of India, such as Bhaturu, Siddu, Chilra, Marchu, Manna, Gulgule, babroo, Dosha, Pinni/ Bagpinni, Seera*etc*. Bhatoou, Marchu, Siddu and Chilra are prepared from wheat and buckwheat flour as regular diet while Gulgule and babroo are prepared on religious and wedding occasions[8]. Himachal Pradesh residents prepare Seera, a cereal based heathy food that is prepared from the natural fermentation of wheat grains.

During the fermentation of seera by LAB,there is a considerable increase in vitamins (thiamine, riboflavin, nicotinic acid, and cyanocobalamin) and amino acids including methionine, phenylalanine, threonine, lysine, and leucine that offers multiple health benefits especially beneficial to postpartum mothers [9]. LAB further secretes variety of metabolites such as organic acids, hydrogen peroxide, carbon dioxide, surface-active molecule, diacetyl, broad-spectrum antimicrobials such as reuterin, and bacteriocins[10,11]. Diverse LAB species produce surface-active compounds or biosurfactants in order to adapt and thrive on a different substrate along with other functions [12].

Biosurfactants are amphiphilic molecules with polar (hydrophilic) and nonpolar (hydrophobic) moieties that reduce the surface tension at the oil–water interface, improving the solubility of water-immiscible substances. They serve as an eco-friendly alternative to synthetic surfactants which have a detrimental environmental impact due to their non-degradable nature[13].

They are broadly classified as low molecular-weight microbial compounds (lipopeptides, glycolipids, etc.) and high molecular-weight microbial compounds (polysaccharides, lipopolysaccharides proteins, or lipoproteins), with the ability to reduce surface and interfacial tension and stabilize emulsions[14]. Many microorganisms such *as Acinetobacter sp., Bacillus sp.,Candidaantartica, and Pseudomonas aeruginosa,etc*are known to synthesize biosurfactants. Among all bacteria, *Bacillus and Pseudomonas* are known to synthesize biosurfactants with high yield, but their application is limited owing to their pathogenic nature, so there is a need to explore non-pathogenic lactic acid bacteria for biosurfactant production. Several studies have shown that LAB strains produce biosurfactants, which are mostly a complex combination of proteinaceous substances, glycolipids, glycoproteins, or glycolipopeptides [15]. Probiotic biosurfactants impart effective antibacterial, anti-adhesive, anticancer, and antibiofilm properties. Furthermore, they have an advantage over traditional microbial surfactants since probiotics are an essential element of normal human microflora and their biosurfactants are non-toxic to humans, allowing them to be used safely and efficiently in the food and cosmetics industries. Moreover, biosurfactants due to their stability at extreme pH/ temperature, biodegradable nature, and low toxicity, are employed in various fields of bioremediation, food, pharmaceutical, agricultural, and cosmetics sectors [16]. The purpose of this study aimed to exploit the surface-active molecules *i.e* biosurfactant from LAB isolated from fermented food Seera.

2. MATERIAL AND METHODS

Sample preparation

10 grams of wheat (*Triticum aestivum*) grains were soaked in 150 ml of distilled water and allowed to ferment for 4 days in a sterile flask. Water was replaced at regular intervals and after fermentation grains were ground and incubated at room temperature for 1hour to allow the starch granules to settle. The surface water is discarded, and the residue was collected aseptically[17].

Isolation of lactic acid bacteria

A fermented Seera sample was homogenized and serially diluted with PBS solution (pH 7) aseptically.100µL of dilutions from10^{-5} to 10^{-7} were spread on sterile MRS (De Man, Rogosa, Sharpe) agar plates and incubated for 24 hours at 37°C under aerobic conditions. Different morphological colonies were picked and streaked multiple times to obtain pure colonies [18].

Selection of biosurfactant-producing isolates.

Hemolytic test

The screening of biosurfactant-producing Lactic acid bacteria (LAB) was carried out by streaking various isolates on a sheep blood agar plate (Himedia-MP1301) and incubating them at 37°C for 48 hours and observed for hemolytic activity[19].

Oil displacement method

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In this test25 mL of distilled water was poured into an empty petri dish, followed by the addition of 25 μL of motor oil in the center of the dish to form an oil layer, and then 25 μL of various isolates cell-free broth was added to the oil layer. If a biosurfactant is present in the supernatant that results in the displacement of oil by forming a clear zone [20].

Drop Collapse method

In drop collapse test, 10 μL of cell-free broth/supernatant was pipetted on thin film. The flattening or spreading of droplet was observed for 40 seconds. As a negative control, distilled water was used. The presence of biosurfactant in cell-free broth results in reducing force or interfacial tension between the liquid drop and the hydrophobic surface eventually resulting in the spreading and collapsing of droplets [21].

Emulsifying activity.

The biosurfactant's emulsifying activity was tested by vertically mixing 2 ml supernatant (cell-free broth) with 2 ml soyabean oil for 2 minutes and observing the height of the emulsion layer after 24 hours [22].

Emulsification index = % height of emulsified layer

Total height of the column

Emulsification activity is proportional to the Emulsification index.

Surface tension

Drop count method was used to measure thesurface tension of the sample. For this a dried beaker with 40–50 drops of the sample was preweighed, and about 5-8 drops per minute were added. The beaker was then weighed again, and the process was repeated in triplicates. After calculating the average mass of each drop, the surface tension of the liquid containing the biosurfactant was calculated [23].

Biosurfactant production

The production of biosurfactants was carried out for the strains that exhibited the presence of biosurfactant in their supernatant after a series of screening assays. 2 ml of overnight culture was inoculated in 150 ml of sterile modified MRS broth without tween 80 and incubated at 37°C for 48 hours under static conditions. After 48 hrs culture was centrifuged at 9000 rpm for 15 minutes to obtain cell-free supernatant (CFS). Cell pellet was suspended in Phosphate Buffer Saline (PBS) and agitated gently for 2 hours to allow adhered biosurfactants to release from the pellet. It was again centrifuged at 9000 rpm for 15 minutes to recover the adhered biosurfactant [24].

Biomass determination

To determine biomass, 20 ml of culture broth was centrifuged at 8000 rpm for 15 minutes in pre-weighed centrifuge vials. The cell pellet was dried in an oven at 80°C for 24 hours after two washing cycles, and the biomass weight was determined [25].

Biosurfactant extraction and yield estimation

The supernatant (CFS) was acidified to pH 2 with 2N HCl and stored at 4°C overnight. After three extractions with chloroform/methanol, the organic fraction was vacuum evaporated (2:1). Acetone was employed to recover the biosurfactant, which was then dried. The biosurfactant was collected and yield was quantified [26].

Identification and characterization of selected LAB

Among biosurfactant-producing isolates, the isolate with the highest biosurfactant activity and yield was chosen and identified morphologically, biochemically and genetically.

Morphological and biochemical characterization.

According to Bergey's Manual of Determinative Bacteriology, the selected strain was identified on the basis of morphological andbiochemicalcharacterization[27].Gram staining, spore formation, and motility were utilized for morphological identification of LAB isolates while for biochemical identification, Indole production, nitrate reduction, methyl red, Voges-Proskauer citrate, H_2S production, and carbohydrate utilization assay were performed [28].

Growth curve

1ml of a 24 hourold culture was inoculated in 150 ml of autoclaved modified MRS broth (without tween 80) and incubated at 37°C for up to 72 hours under static condition.By measuring optical density at 600 nm after regular interval bacterial growth curve was determined by plotting O.D versus time graph [29].

DNA isolation

DNA extraction was carried out as per the method reported byLeehouts*et al.* [30]. 5 ml of culture was centrifuged after overnight growth. The pellet was rinsed with distilled water before being suspended in 1 ml of lysis solution containing 5 mg of lysozyme and of mutanolysin (30 U/ ml). The mixture was incubated at 37°C for 30 minutes before adding 20 μL of

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proteinase K (20 mg/ml) and 50 µL of 10% sodium dodecyl sulfate and then incubated at 60°C for 50 minutes. The lysate was extracted five times with phenol-chloroform-isoamyl alcohol (25:24:1) and twice with chloroform-isoamyl alcohol (24:1). The DNA was precipitated with 2 volumes of 96 percent ethanol (-20°C) after 3 M sodium acetate (0.1 volume) was added. The DNA was dissolved in 150 µL of TE (10 mM Tris [pH 8],1 mM EDTA) with 20 µg of RNase. DNA concentration was determined using a spectrophotometer at an absorbance of 260nm.

16S rRNA Sequencing and phylogenetic analysis

The 16S rDNA gene was amplified according to the method described by Chong *et al.* [31]technique using 16s forward primers 27F (5′- GGATGAGCCCGGCCTA -3′) and 16s reverse primer (5′- CGGTGTACAAGGCCCGG -3′).

NO.	Primer	Sequence(5`à 3`)	Tm (°C)	GC- Content 1
1	16s Forward	GGATGAGCCCGCGGCCTA	57	72.22%
2	16s Reverse	CGGTGTGTACAAGGCCCGG	58	68.42%

Table no 1. Details of PCR primers

Initial Denaturation	3 minutes at 94°C	30 Cycles
Denaturation	1 minute at 94°C	
Annealing	1 minute 50°C	
Extension	2 minutes at 72°C	
Final Extension	7minutes at 72°C	

Table no 2. Cycling condition for PCR

The amplification product was purified using the Gene Flow Gel Extraction PCR purification kit. The purified PCR product was sent to BioKart Pvt. Ltd. in Bengaluru, India, for 16S rRNA gene sequencing and BLAST analysis was performed to compare the acquired gene sequence with sequences that were already present in the NCBI Genbank database. Based on the greatest identity score, sequences with the highest similarity were chosen from the database and a phylogenetic tree was constructed [32].

3. RESULTS AND DISCUSSION

Preliminary screening of biosurfactant producing strain

LAB isolated from freshly prepared Seera sample was screened for biosurfactant production. Only oxidase and catalase-negative strains were chosen as these are two important characteristics of the biochemical traits of lactic acid bacteria. Seven out of thirteen isolates tested negative for catalase and oxidase, and they were marked as S1, S2, S3, S4, S5, S6, and S7.These isolates were inoculated in a modified MRS medium (without Tween 80) for 24 hours at 37° C and cell-free broth (supernatant) from various cultures were further tested for biosurfactant production using a battery of tests such as Hemolytic activity, Drop collapse assay, Oil displacement technique, surface tension and Emulsification method, as shown in table 3.

Isolate	Hemolytic activity	Drop collapse	Oil displacement method	Emulsification activity	Surface activity (mN/m)
S-1	γ	+	11.2±0.05	36.56±0.03	69.35±0.02
S-2	γ	+++	40.3±0.06	63.27±0.08	42.32±0.17
S-3	β	-	00.00	31.04±0.06	67.53±0.15
S-4	γ	-	00.00	13.64±0.05	70.36±0.13
S-5	β	-	05.5±0.03	19.33±0.12	68.57±0.08
S-6	β	++	23.4±0.50	54.24±0.05	59.24±0.03
S-7	α	++	27.2±0.44	49.36±0.07	52.74±0.05
(- = Negative, +++ = complete collapse after 1 min, ++ = collapse after 2 mins, + = collapse after 4 mins)					

Table 3. Preliminary screening for biosurfactant production by isolated strains.

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Fig 1. (Hemolytic assay for biosurfactant production)

As illustrated in table 1, out of seven isolates only strains S-3, S-5 and S-6 exhibited β- hemolytic activity. The hemolytic assay for strain S 2 is exhibited in Fig 1, distinct zone was not observed by the microbial isolates on sheep blood agar plate. It showed the strain had not any hemolytic activity.
Many biosurfactant-producing strains exhibited hemolytic activity due to lysis of erythrocyte according to Puphan*et al.*,[33]. However, there are certain strains that produce biosurfactants without showing any hemolytic activity [34]. The method is not selective since many lytic enzymes are known to produce clear zones by lysing RBC. Secondly, hydrophobic substrates cannot be employed as the sole carbon source in blood agar assay. Furthermore, various surfactants have varying diffusion properties, which might impede the production of a clean zone. As a result, this assay produces a large number of false negative and false positive outcomes hence, hemolytic test alone cannot predict the biosurfactant producing nature of micro-organism. This assay should be employed with other surface activity assessment-based methods for preliminary screening of biosurfactant [35].

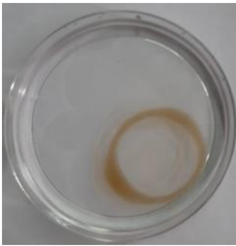

Fig 2. (Oil displacement test)

In the oil displacement test, only cell-free supernatant of isolate S-2 showed maximum oil displacement i.e40.3±0.06whileS-6 and S-7 showed displacement of 23.4±0.50 and27.2±0.44 respectively. Distilled water was used as negative control while SDS was used as positive control. The presence of biosurfactant in the supernatant was indicated in the oil displacement test by isolate S-2 in Fig 2.
The oil spreading approach is a reliable method for detecting biosurfactant synthesis by various microorganisms [36,37]. The presence of a biosurfactant decreases surface (liquid-air) and interfacial (liquid-liquid) tensions, hence oil was displaced at the interface between the two immiscible fluids (oil and water). The repulsive forces that exist between immiscible are diminished, enabling them to mix and interact more freely. As a result, the oil was pushed off the surface of the water, resulting in the formation of a clear zone [38]. Our findings matched with the study of (Ghasemi*et al.* in terms of lactic acid screening [39].In this assay, the oil displacement area is proportional to the biosurfactant concentration present in the cell-free supernatant [40].

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Fig 3. Drop collapse test of supernatant of different isolates

In the drop collapse assay, distilled water denoted by 0 act as negative control and Modified MRS broth (without tween 80) act as a control. Figure 3. Depicts the differences in the diameter of droplets from different isolates on thin parafilm in a drop collapse test. Isolates S-2, S-6, and S-7 indicated a significant increase in drop diameter. Flattening of droplets occurred within 60 seconds for S-2 strain.
In drop collapse method,if no surfactants are present in the liquid, the polar water molecules are repelled off the hydrophobic surface, and the droplets stay stable whereas presence of surfactants diminish the force or interfacial tension between the liquid drop and the hydrophobic surface, causing the droplets to spread or even collapse. Drop

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stability is affected by surfactant concentration and is related to surface and interfacial tension. Our results were in accordance with the study reported by [41] for the drop collapse test for screening biosurfactant synthesizing micro-organisms.

Emulsification activity implies the existence of a biosurfactant in the supernatant as a result of emulsifying two immiscible liquids into a semi-stable mixture. As illustrated in Table 3, maximum emulsification activity was observed for isolate S-2 i.e63.27±0.08 among all isolates. This assay provides an indication of the existence of biosurfactants. The emulsification index correlates with the amount of biosurfactant. According to our study the emulsification activity of biosurfactant producing strains S-2, S-6 and S-7 also lies within the range reported byAkintokun*et al.* that was from 42.5 to 74.4% for biosurfactant producing strains[42].
During biosurfactant screening assay,maximum reduction in surface tension was reported by strain S-2 from 72.21±0.03 to 42.32±0.17 followed by S-7 and S-6 isolates. According to the study reported by Rodrigues *et al.*showed that LABhas tendency to reduce surface tension from72 to 39 mN/m for *Streptococcus thermophilus* and 72 to 37 mN/m for *Lactobacillus fermentum*[43]. Based on the different screening test strains S-2, S-6 and S-7 were selected further biomass and biosurfactant production.

Biomass and biosurfactant Determination

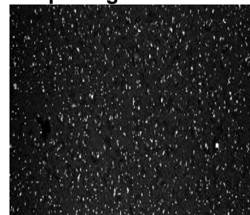
Isolates	Biomass determination (g/L)	Biosurfactant determination (g/L)
S-6	2.2 ±0.04	0.4 ±0.01
S-2	3.7 ±0.03	1.2 ±0.02
S-7	2.7 ±0.01	0.7 ±0.02

Table 4. Biomass and biosurfactant yield of isolates S-6, S-2 and S-7

Among three isolates, the biomass and biosurfactant yield of strain S-2 was comparatively higher. Biomass yield of strainS-2 was found to be 3.7 ± 0.03 g/L whereas biosurfactant yield was 1.2±0.02 g/L, hence selected for further characterization.
According to the study reported by Ghasemi*et al.,*biosurfactant yield from lactic acid bacteria *Pediococcusdextrinicus* was found to be 0.7g/L [44], whereas Souza E *et al.* reported biosurfactant yield of *Lactobacillus lactis* to be 0.1–4.6 g/L[46] . Variation in biosurfactant yield was caused due to distinct nature of the biosurfactant-producing strains and the different sources from which strains were isolated. Many additional factors impacted the yield, such as changes in growing conditions including temperature, pH, and medium composition. According to many studies, it was found that biosurfactants producing lactic-acid were mainly isolated from fermented batters, fruits, vegetables, and milk products[45,46]

Characterization and identification of selected LAB

Morphological identification



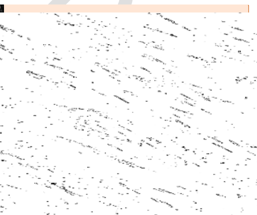


Fig 4. Negative staining **Fig 5. Gram staining**

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Morphological characteristics	Colour	Motility	Cell shape (Negative staining)	Gram staining	Endospore
Isolate S-2	Off white	-	Coccus	+ve	-

Table 5. Morphological characterization of strain S-2

Strain S-2 was selected and studied for morphological characteristics such as gram stain, morphology, spore production, and motilityusing the LAB procedures outlined by Kozaki*et al.* [47]. As depicted in table no. 5 and in Fig 4&5, Strain S-2 was found to be Gram-positive, cocci-shaped, non-motile, catalase-negative, and lack Endospore. The morphological findings were matched with the studies reported by Todorov *et al.,*&Cai *et al*[48,49].

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Growth curve

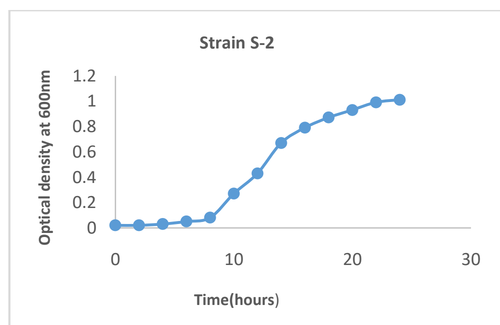

Fig 6. Growth curve of strain S-2

The synthesis of biosurfactants starts during the late exponential phases of micro-organism growth. As a result, it is necessary to correlate the growth curve of isolates with the synthesis of biosurfactant. As indicated in figure 6, there was a lag phase up to 9 hours, after that an exponential phase started and last till 24 hrs, then microbial cell growth attained the stationery phase that last for 48hrs after that decline phase started. According to our study maximum yield of biosurfactant occurred after 48hrs of the growth.

Biochemical test	Results
Catalase	-
Oxidase	-
MR	+
VP	+
Indole	+
Citrate	-
Nitrate Reduction	-
H_2S	-
Arginine hydrolysis	+

Table 6. Biochemical results of S-2 strain.

Different biochemical tests were performed for the strain S-2 and the findings are reported in Table 6. Catalase, oxidase, citrate, nitrate reduction, and H_2S activities were absent while selected strain S-2 was found to be positive for MR, VP, indole, and arginine hydrolysis. The results of biochemical tests were also in accordance with the study reported by (Vidhyasagar*et.al.* [50]

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1	Lactose	+	11	L-arabinose	+	22	Arabitol	+	32	D – Arabinose	+
2	Xylose	+	12	Mannose	+	23	Erythritol	+	33	Citrate utilization	-
3	Maltose	+	13	Insulin	+	24	α- methyl-D – glucoside	+	35	Sorbose	+
4	Fructose	+	14	Sodium gluconate	+	25	Rhamnose	+			
5	Dextrose	+	15	Glycerol	+	26	Cellobiose	+			
6	Galactose	+	17	Dulcitol	+	27	Melezitose	+			
7	Raffinose	+	18	Inositol	+	28	α- methyl-D – mannoside	+			
8	Trehalose	+	19	Sorbitol	+	29	Xylitol	+			
9	Melibiose	+	20	Mannitol	+	30	ONPG	-			
10	Sucrose	+	21	Adonitol	+	31	Esculin hydrolysis	+			

Table 7. Sugar utilization test for strain S-2

This test was performed usingHimediacarbokit, it was found that the S-2 strain has the ability to ferment all carbohydrates except ONPG and citrate as illustrated in table 7.Fermentation of many sugars *i.e*glucose, sucrose, galactose, mannose, xylose, fructose, and maltose,was in accordance to study described by Albano*et al.* [51] such as fermentation of but did not match with the fermentation pattern of arabinose, glycerol, sorbitol, and CMC (carboxy methyl cellulose). Our findings were also in accordance with the study reported by Cai *et al.*[52] except for sorbitol and Rhamnose. The differences in the pattern of sugar fermentation were due to the isolation of strains from diverse origins with different growth conditions.

PCR amplified 16s rRNA

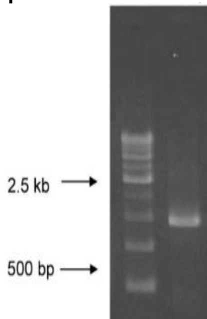

Fig 7. Lane Description: Lane 1: Ladder; Lane 2: Sample

Phylogenetic analysis

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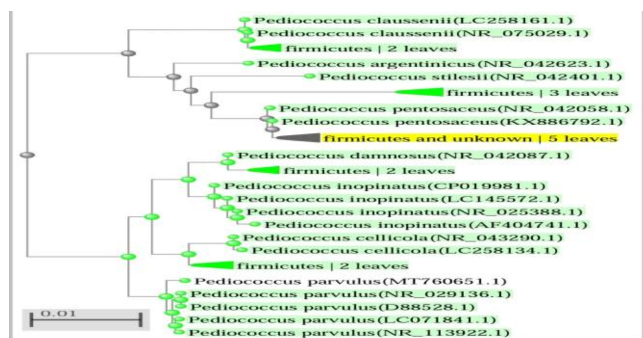

Fig 8. Phylogenetic analysis of *Pediococcuspentosaceus* based on 16S rRNA genes

The 16S rDNA nucleotide sequence of the selected strain S-2 was compared with the sequences which were already present in the Gen Bank database, it was found that the isolated strain closely related with *Pediococcus*genus. The BLAST analysis revealed that the genus *Pediococcus*had 99.86 percent identity with *Pediococcuspentosaceus*, and figure7 depicts a phylogenetic study of the 16S rDNA sequence. In a phylogenetic tree, the length of a branch represents the degree of genetic change of 0.01.The selected strain was found to be closest homologue to *Pediococcuspentosaceus* strain (KX886792.1) and the next closest homologue was found to be *Pediococcuspentosaceus* strain (NR 04058.1).16S rDNA sequence of strain S-2 *i.ePediococcuspentosaceus* consisting of 1396 nucleotides, was submitted to GenBank (National Center for Biotechnology Information, USA) and received the accession number OM843219.According to different studies*Pediococcuspentosaceus* was also isolated from different food sources like idli, fermented vegetables, sausages, fermented pickles, cheese, *etc*[53,54].

CONCLUSION

Fermented food products are versatile source of Lactic acid bacteria that produce variety of metabolites.In the present study biosurfactant-producing LAB strains were isolated from cereal-based fermented food, Seera. Strain S-2 with the highest emulsification index of 63.27±0.08, surface activity of 42.32±0.17and biosurfactant yield of 1.2g/L was chosen among other isolates. Selected strain S-2 was found to be coccus-shaped, gram-positive, non-motile, and non-spore-forming micro-organism that had tendency to utilize different sugars. The biochemical characterstics and 16sRNA sequencing confirmed the selected strain as *Pediococcuspentosaceus.* Many studies reported it as a safe microorganism as it does not pose any illness so far. Therefore, further research will be carried out to optimize the conditions for enhancement of biosurfactant yield,determination of chemical nature of the biosurfactants and evaluate its antibacterial, antiadhesive activities and anti-biofilm potential so it can be utilized in different sectors including pharmaceutical, cosmetics and food industries.

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