RESEARCH PAPER

Extraction and Biochemical Characterization of Exopolysaccharides Produced from Lactococcus Lactis Strain Isolated from Fermented Dairy Products

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ABSTRACT
Exopolysaccharides (EPSs) are high–molecular-weight, complex carbohydrate biopolymers secreted extracellularly by various microorganisms, particularly lactic acid bacteria (LAB). LAB are well recognized for their remarkable capacity to synthesize diverse types of EPSs, which have attracted significant scientific and industrial interest. These LAB-derived EPSs exhibit numerous health-promoting properties and are extensively utilized in the food and pharmaceutical sectors. Several optimized methods have been developed for the production, extraction, purification, and characterization of LAB-produced EPSs and molecular docking studies. The simplest preliminary assessment of EPS production involves examining colony morphology, where ropy or mucoid textures indicate EPS formation. Ethanol precipitation is a common and effective technique for extracting EPSs from cell-free supernatants. Quantification of total carbohydrate content is typically performed using the phenol–sulfuric acid method. The structural and functional properties of EPSs are characterized through a variety of analytical techniques. Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance (NMR), and X-ray diffraction (XRD) are used to elucidate structural features. Surface morphology is examined using scanning electron microscopy (SEM) and the screened bioactive compound constituents against multiple therapeutic targets, namely BRAF, EGFR2, HDAC8, PPARA, VEGFR2, and RET, with binding energies ranging from weak to strong interactions. Overall, research on EPS from LAB involves isolating EPS-producing strains from probiotic products and their characterization.

Keywords: Lactic acid bacteria, Exopolysaccharides, Fermented Dairy Products

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1. INTRODUCTION

Exopolysaccharides (EPSs) are high-molecular-weight polysaccharides that are exuded outside the cell by many microorganisms. The term "exopolysaccharide" was first introduced by Sutherland in 1972. LAB are widely recognized for producing diverse EPSs, and these compounds have attracted significant scientific attention due to their functional and industrial relevance [1]. In microbial systems, EPSs contribute to biofilm formation, where they act as structural scaffolds that protect cells and facilitate attachment to surfaces [2]. They also help microorganisms tolerate harsh environmental stresses such as extreme temperature, osmotic imbalance, salinity, and desiccation.

Based on their sugar composition, EPSs are generally classified into two categories: homopolysaccharides (HoEPSs), which contain only a single type of monomer, and heteropolysaccharides (HeEPSs), which are made of repeating units comprising two or more different monomers [3].

EPSs are valued for being biocompatible, biodegradable, and generally regarded as safe [4]. Importantly, each bacterial strain produces structurally unique EPS with distinct biological properties. These EPS can be used in industrial and biomedical applications [5]. In LAB, EPS production has been linked to a wide spectrum of activities, including antimicrobial, antioxidant, anti-biofilm, anti-inflammatory, anti-obesity, anti-diabetic, and antitumor effects, as well as applications in emulsification, gelling, edible coatings, wound healing, and water/oil retention [6]. Recent studies also emphasize the health-improving potential of EPS, such as cholesterol-lowering, immune modulation, and prebiotic functions.

In addition to experimental characterization, computational approaches such as molecular docking have emerged as powerful tools to predict the interaction of

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EPS-derived compounds with biological targets. Molecular docking enables the identification of binding affinities and interaction mechanisms between bioactive molecules and specific proteins, thereby providing insights into their therapeutic potential [13]. This approach has been increasingly applied to evaluate the antimicrobial, anti-inflammatory, and enzyme inhibitory properties of microbial metabolites, including EPS derivatives [14].

Considering these promising attributes, the present study was designed to isolate EPS-producing probiotic LAB strains, evaluate their probiotic potential, extract and purify EPS, characterize its structural features using multiple analytical techniques, and further investigate its biological interactions through molecular docking analysis.

2. MATERIALS AND METHODS

2.1 Preparation of Primary culture

The isolation of LAB from various probiotic supplements was achieved by using microbial culturing techniques. A selective media, such as MRS (de Man, Rogosa, and Sharpe) agar, was used to favor the growth of LAB. Pure cultures of LAB were obtained from probiotic products. Identification of the isolated strains, as LAB strains was carried out through biochemical tests, molecular techniques (such as 16S rRNA gene sequencing), and phenotype profiling. The results revealed a broad spectrum of LAB species, with common isolates including *Lactococcus, Pediococcus, Leuconostoc, Lactobacillus, Streptococcus, and Enterococcus.* The LAB are considered as effective probiotics by, their survive in acidic environments of the stomach and also withstand the bile salts in the small intestine. The LAB strains isolated in this study generally displayed good tolerance to acidic conditions, bile salt tolerance and antimicrobial activity. From the 11 isolates, we selected 3 LAB for further studies that is screening of EPS production.

Analysis of EPS production properties of 3 LAB strains

The level of EPS production of 3 selected LAB strains cultivated in MRS broth modified by adding 10% (w/v) sucrose [7]. From the primary culture transfer the 10% of the inoculum to the EPS production media and allow it for 48hrs incubation at 37^0C with constant rotatory in a bacteriological rotatory incubator [Fig 1]. LAB cell debris were separated from culture media via centrifugation at 8,000 rpm for 25 min [8]. A 5 ml aliquot of the supernatant was mixed with 25 ml of 95% chilled ethanol (− 20 °C), and incubated at 4 °C for 16 h. The precipitated EPS was centrifuged at 8,000 rpm for 20 min, and the supernatant was discarded. The crude EPS precipitate was collected and dried at room temperature or in an oven below 45^0C. The extracted EPS was subjected for the estimation by using the standard procedure of Anthrone method.

2.3 Selection of Exopolysaccharide-Producing LAB Strains

2.3.1. By Colony Morphology:

Visual examination of colony morphology is the simplest and most widely used methods for evaluating exopolysaccharide (EPS) production [9]. In this approach, lactic acid bacteria (LAB) strains are monitored for EPS production based on their colony morphology when grown on MRS agar medium, with various carbon sources such as sucrose with appropriate incubation conditions [10]. EPS production is typically indicated by the formation of mucoid, slimy, or ropy colonies [11,12].

2.4. Quantification of EPS

2.4.1. Colorimetric Methods

Anthrone-sulfuric acid method is one of the methods used for the quantification of the carbohydrate content in EPS.

2.4.2. Anthrone-Sulfuric Acid Method

Anthrone reagent, which is made up of combination of 0.1% Anthrone (w/v) and concentrated Sulphuric acid (80% v/v), is used to estimate the total amount of carbohydrates in an EPS sample [15]. In this method, the EPS sample (0.5 mL) is mixed with Anthrone reagent (2.5 mL), and incubated for 15 min in boiling water (90 °C) bath [16]. The mixture is chilled on ice for 15 min dark conditions and the absorbance is taken at 620 nm.

2.5. Microorganism selection and mass production of EPS:

From the above procedure out of 3 LAB isolates we have selected CS-2, for the mass production of Exopolysaccharides. Based on quantification results.

2.6. Methods for Characterization of EPS

To determine the chemical and structural characteristics, like functional groups, monosaccharide composition, kinds of sugar subunits, ring conformation, morphology, molecular weight, degree of branching, and types of glycosidic linkages, the characterization of EPS is necessary, several methodologies are used to analyze and recognize the molecular weight, structure, and composition of EPS [17]. The different analytical techniques, such as chromatography, XRD, FTIR, NMR, Zeta potential (ZP) are used for physical and chemical characters of EPS.

2.6.1. Characterization Techniques

2.6.1. Energy Dispersive X-ray Spectroscopy (EDX):

EDX is a technique used for characterizing the elemental composition and structure of polysaccharides. It gives valuable information about the presence of different elements, their concentrations, and their spatial distribution within the sample. EDX helps identify these elements and assess the purity of the EPS, mineral content, presence of bound ions or metal contaminants. The released X-rays are used to determine the weight and atomic percentages of the elements [18].

2.6.2. Fourier transform-infrared spectroscopy (FTIR)

The functional groups in the purified EPS CS-2 were analyzed by using an attenuated total reflectance (ATR-FTIR) spectrophotometer with the spectrum range of 4000–400 cm^{-1}. The FT-IR spectroscopy, determines the

distribution of the functional groups in the pure EPS [19]. In this method, the freeze-dried EPS sample is mixed with dry potassium bromide (KBr) in the ratio of 1:100, crushed finely, and compressed into a pellet with a thickness and diameter of 1 and 10 mm, respectively [20].

2.6.3. Nuclear magnetic resonance (NMR)

Nuclear magnetic resonance (NMR) spectroscopy is used to determine the molecular structures of chemicals, within the EPS, such as the carbon arrangements and proton profile, along with configuration of glycosidic bonds [9]. The chemical structure of hydrogen and carbon environments in the molecule of EPSs is examined using 1D NMR spectroscopy [21]. From the 1D NMR spectroscopy, the structural elucidation of the exopolysaccharide, isolated from *Lactococcus lactis* (CS-2), was carried out, specifically ^1H and ^13C NMR, with DMSO-d_6 as the solvent.

2.6.4. Scanning electron microscopy (SEM)

The scanning electron microscopy is used to determine the surface morphology of EPS-CS-2. SEM is an analytical technique used for visualizing the microstructure of biopolymers and microbial extracellular products [22, 23]. Here the Hitachi S-3400 SEM is used for the analysis. Imaging was performed using the secondary electron (SE) signal. With the 6.6 mm working distance, an accelerating voltage of 10.0 kV was applied, with a magnification of 4000×. The specimen bias was set at 0 V, and the micron marker length corresponded to 10 μm. The different stage movement parameters included an X-position of 61.053 mm, Y-position of 21.248 mm, and Z-position of 7.0 mm, with both rotational (R) and tilt (T) angles at 0°. The system operated under high vacuum conditions.

2.6.5. X-ray diffraction (XRD)

X-ray diffraction (XRD) is technique commonly used to determine the crystalline configuration of the EPS. The source of X-ray is operating at 40 kV and 30 mA is applied. The system consisting of a D/teX Ultra detector and employed continuous scan mode to enhance data acquisition efficiency [24].

2.6.6. GC-MS:

The GC-MS analysis of sample was conducted at IADFAC Laboratories Pvt. Ltd., Bangalore. The sample, identified as EPS (with a Sample ID: 2120), which was analyzed at Level 1. The procedure here is followed was based on the protocol specified in the internal laboratory documentation (File path: *D:\GCMS-QP2010+\Ullas\IADFAC SCAN.Qgm*). This protocol follows with standard procedures commonly employed for GC-MS-based characterization in specialized laboratories.

2.7. Molecular docking

Molecular docking analysis was performed to evaluate the binding potential of selected bioactive compounds against target proteins (BRAF, EGFR2, HDAC8, PPARA, VEGFR2 and RET). The ligand structure was obtained from PubChem and prepared using standard energy minimization methods. Docking simulation were carried out using Auto Dock vina within defined active site regions. The binding affinities were analyzed based on docking score and the ligand protein interactions were visualized using molecular visualization tools such as PyRx, Open Babel [38].

2.8. Statistical analysis

All experiments were conducted in triplicate, and the data are presented as mean ± standard deviation and data were analysed using one-way ANOVA, followed by Turkey's test to determine significant differences between mean values ($P < 0.05$). All the graphs in the article were prepared using Microsoft offices excel.

3. RESULTS

3.1. Isolation and Primary Screening of EPS Producing Bacteria

A total of several bacterial isolates was obtained from fermented dairy samples, including curd, buttermilk, and fermented milk. Among these, only a few isolates exhibited mucoid colony morphology on MRS agar supplemented with 10% sucrose, indicating exopolysaccharide (EPS) production **[Fig 1,2].** The most promising isolate, designated as *Lactococcus lactis* (CS-2), showed smooth, creamy-white, and highly viscous colonies **[Fig 3].** The isolate was further purified through repeated washing and dialysis to obtain a stable EPS-producing culture.

3.2 Quantification of EPS

The EPS sample turns into a blue-green color due to the presence of carbohydrates. The total quantity of EPS isolated from *Lactococcus lactis* is yield of 0.391 g/L**.**

3.3. Physicochemical Characterization of EPS

The extracted EPS was characterized using FTIR, SEM–EDX, XRD, NMR, and GC–MS analyses. FTIR confirmed the presence of characteristic functional groups and indicated the purity of the EPS. SEM–EDX analysis provided insights into the surface morphology and elemental composition, while XRD analysis revealed its semi-crystalline nature. NMR and GC–MS analyses further elucidated the structural and compositional features of the EPS.

3.4. Elemental Composition by EDX Analysis

Energy Dispersive X-ray (EDX) spectroscopy revealed that the EPS primarily consisted of carbon, oxygen, sodium, phosphorus, and chlorine **[Fig. 5].** Oxygen was the predominant element (59.16 ± 1.30 wt.%), reflecting the abundance of hydroxyl and carboxyl functional groups typical of polysaccharides. Carbon accounted for 22.09 ± 3.39 wt.%, confirming its role as the structural backbone of sugar monomers. Sodium was present at 15.71 ± 0.66 wt.%, likely originating from residual salts or as bound cations interacting with the negatively charged EPS matrix. Minor amounts of phosphorus (2.07 ± 0.31 wt.%) and chlorine (0.97 ± 0.13 wt.%) were also detected. These findings support the carbohydrate nature of the EPS and suggest possible influences from the culture medium or purification process.

3.5. FTIR Spectral Analysis

FTIR analysis of the EPS revealed several characteristic absorption peaks corresponding to key functional groups **[Fig.4].** Broad peaks at 2543 and 2501 cm^{-1} were attributed to hydrogen-bonded O–H stretching vibrations, indicating the presence of carboxylic acid groups, possibly uronic acid residues. Peaks in the range of 2225–2115 cm^{-1} suggested triple bond stretching, which may correspond to nitrile or alkyne groups. The absorption band at 1458 cm^{-1} was associated with C–H bending vibrations, while the peak at 1409 cm^{-1} indicated phenolic O–H bending or CH_2 wagging. A band at 1314 cm^{-1} suggested C–N stretching or O–H deformation. Notably, a strong peak at 1014 cm^{-1} confirmed C–O–C stretching vibrations, characteristic of glycosidic linkages. Overall, the FTIR profile confirmed the presence of hydroxyl, carboxyl, and ether groups, supporting the polysaccharide nature of the EPS.

3.6. Structural Elucidation by NMR Analysis

The structural features of the EPS were further investigated using ^{1}H and ^{13}C NMR spectroscopy. The ^{1}H NMR spectrum (0–11 ppm) displayed prominent signals around 2.5–3.5 ppm, corresponding to ring protons and anomeric hydrogens, indicating a hexose-based polysaccharide backbone. The ^{13}C NMR spectrum (0–200 ppm) showed characteristic signals between 60–110 ppm, representing carbohydrate ring carbons. Signals around 39–40 ppm suggested the presence of methyl or methylene groups, possibly due to structural modifications such as acetylation.

The absence of resonances above 110 ppm indicated a lack of aromatic or uronic acid components. Based on these observations, the EPS is likely a branched heteropolysaccharide composed of hexose units such as glucose or galactose, with α- or β-glycosidic linkages and possible branching at 1→3 or 1→4 positions. The variability in chemical shifts suggests structural heterogeneity in sugar composition and linkage patterns.

3.7. Surface Morphology by SEM Analysis

Scanning Electron Microscopy (SEM) analysis at 4000× magnification revealed a smooth and continuous surface morphology of the EPS **[Fig.6]**. The observed texture is typical of dried polysaccharide films. Irregular bright particles distributed across the surface were likely residual salts or protein aggregates from the purification process. These particles ranged from approximately 200 nm to 2 µm in size and appeared randomly distributed.

3.8. Crystallinity Analysis by XRD

X-ray diffraction (XRD) analysis revealed a sharp diffraction peak at $2\theta \approx 28.46°$, with a high intensity and a narrow full width at half maximum (FWHM) of 0.1261° **[Fig. 8].** The crystallite size, calculated using the Scherrer equation, was approximately 67.9 nm. Although EPS is typically amorphous, the presence of a sharp peak indicates partial crystallinity and ordered microstructures. The observed peak splitting suggests structural heterogeneity or mixed-phase characteristics. Overall, the EPS exhibited a predominantly amorphous structure with semi-crystalline features, which may influence its functional properties.

3.9. GC–MS Analysis of EPS Composition

GC–MS analysis identified a total of 32 compounds in the EPS sample **[Fig7],** indicating a complex metabolic profile. Glycyl-L-proline was the most abundant compound (28.21%), suggesting protein degradation or nitrogen metabolism. Other major compounds included glycerin (8.55%) and 1,3-propanediol derivatives, reflecting carbohydrate metabolism.

Compounds such as 4H-pyran-4-one derivatives likely originated from EPS degradation or medium components. Overall, the GC–MS profile highlights the co-production of small organic molecules during EPS biosynthesis and reflects the metabolic versatility of the organism. These findings provide valuable insights into the biochemical composition and potential biotechnological applications of the EPS.

3.7. Molecular docking

Molecular docking analysis revealed differential binding affinities of the screened bioactive compound constituents against multiple therapeutic targets, namely BRAF, EGFR2, HDAC8, PPARA, VEGFR2, and RET, with binding energies ranging from weak to strong interactions. Among all evaluated compounds, Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(phenylmethyl) exhibited the most favorable binding profile across all targets, with docking scores of −7.8 kcal/mol (PPARA), −7.3 kcal/mol (BRAF and EGFR2), −7.1 kcal/mol (VEGFR2), −6.6 kcal/mol (HDAC8), and −6.4 kcal/mol (RET), indicating strong and consistent multi-target binding potential.

On the other hand, moderate binding affinities were obtained for molecules such as glycyl-L-proline, benzene acetaldehyde, 3,5-dimethyl-2-isobutylpyrazine, and pyrazine analogs, with docking energy values ranging from -5.0 to -6.0 kcal/mol. Small and less complex molecules like cyclopropene and certain diols were found to have poor binding efficiency (up to −2.2 to −4.3 kcal/mol), reflecting limited structural complementarity within the active sites of the target proteins **[Table 1].**

Overall, the docking results highlight a subset of compounds with pronounced multi-target binding potential, particularly against VEGFR2, PPARA, EGFR2, and BRAF, suggesting their possible role in the simultaneous modulation of oncogenic signaling cascades, angiogenesis, and metabolic reprogramming pathways.

3.7.1. Post-dock Analysis

From the interaction analysis, binding of ligand to all target proteins was mainly influenced by both hydrophobic and hydrogen bonds. In BRAF, several Pi-alkyl interactions such as those made with amino acids ALA483, ALA481, LEU514, and VAL471, together with a hydrogen bond at 2.30 Å with residue CYS532, suggest that there is stable binding of ligand within the binding pocket. Hydrophobic interactions in EGFR mainly involve

Pi-alkyl interactions between the ligand and amino acids such as LEU718, VAL726, and ALA743, as well as Pi-sigma interaction with LEU844.

In the case of HDAC8, hydrophobic interactions mainly involve formation of a hydrogen bond with the amino acid ASP101, which is at 2.67 Å, as well as Pi-Pi stacking and Pi-sulfur interactions between PHE152, PHE208, and MET274, thus indicating strong and specific binding of the ligand. PPARA showed a Pi-sigma interaction with amino acid MET320, suggesting moderate stability. In RET, hydrophobic interactions involved several Pi-alkyl interactions with amino acids ALA756, ALA807, LEU881, and VAL738, together with a Pi, complemented by a Pi–sigma interaction with LEU730, contribute to hydrophobic stabilization of the complex.

However, it is worth noting that the protein VEGFR2 exhibits a versatile set of interactions, such as π-π stacking with PHE1047, π-cation interactions with ARG1051, as well as hydrogen bonds with ASN923, ARG929, and THR926 (1.81–2.94 Å), suggesting that the drug compound binds strongly and stably. From this information, we can conclude that the drug binds in a stable state through complementary hydrophobic and polar interactions at several target sites **[Figure &Table 1].**

4. DISCUSSION

The purpose of this work is, the screening and isolation of exopolysaccharide (EPS) producing lactic acid bacteria (LAB) from probiotic supplements. Resulted in the identification of a potent EPS producer, *Lactococcus lactis* CS-2. LAB, species such as *Leuconostoc*, *Pediococcus*, and *Lactococcus*, are widely recognized for their role in fermentative food processes and health benefits. Based on colony morphology, EPS precipitation, and carbohydrate quantification using the Anthrone method screening process is completed, and effectively selected the to three strains, among them CS-2 displays the highest yield of EPS. The two types morphology observed on MRS agar plates enhanced with dissimilar carbon sources; they are mucoid and ropy colony. This morphological approach, although is the one of the most effective primary screening methods for the production of EPS, as supported by prior studies [9–12]. The CS-2 strain exhibited consistent tolerance to acidic pH and bile salts, a hallmark of probiotic potential, which exhibits with previous findings where *Lactococcus* strains have shown resilience under gastrointestinal conditions. EPS production was optimized using a modified MRS broth by adding 10% sucrose, which is known to enhance the biosynthesis of polysaccharides by serving as a precursor. The yield obtained (0.391 g/L) is within the expected range for *Lactococcus*-derived EPS under similar culture conditions. This is comparable to the other studies where EPS production in *Lactococcus lactis* varied between 0.2–0.5 g/L depending on the substrate and fermentation time [14,16]. By Characterizing the EPS by using analytical tools like FTIR, EDX, SEM, NMR, GC-MS and XRD provides a comprehensive understanding of its chemical structure such as elemental composition, surface morphology, and crystalline nature. FTIR analysis revealed the presence of key functional groups such as hydroxyl, carboxylic, and glycosidic linkages, which are characteristic features of heteropolysaccharides. These findings are consistent with previous reports that emphasize the importance of infrared spectroscopy in identifying polysaccharide structures and functional groups [26,27,28]. Similar spectral patterns have been widely reported for EPS derived from lactic acid bacteria, confirming the reliability of FTIR in polysaccharide characterization [29,30].

EDX analysis further supported these observations by confirming the dominance of carbon and oxygen, along with trace elements such as sodium and phosphorus. These minor elements may influence physicochemical properties such as charge distribution and solubility, which are critical for the functional behavior of EPS in biological systems.

One-dimensional ^1H and ^13C NMR spectra provided deeper structural insights, confirming the heteropolysaccharide nature of the EPS. The spectra indicated the presence of both α- and β-anomeric configurations along with a diverse range of sugar residues. Such structural diversity is typical of LAB-derived EPS and contributes significantly to their biological functionality [31,32]. Heteropolysaccharides from lactic acid bacteria are known for their structural complexity, which directly influences their physicochemical and bioactive properties [33,34].

The multifunctional potential of EPS, including antioxidant, immunomodulatory, and rheological properties, is closely linked to this compositional heterogeneity. Previous studies have highlighted that EPS from Lactococcus lactis exhibit significant industrial and biomedical relevance due to their diverse sugar composition and structural organization [35,36].

SEM analysis revealed a relatively smooth and continuous EPS matrix interspersed with irregular crystalline or aggregated particles. These structures are likely residues of salts or proteins introduced during extraction and drying processes. Such morphological features are commonly reported in EPS studies and reflect both intrinsic polymer structure and sample preparation conditions [37].

XRD analysis demonstrated that the EPS possesses a predominantly amorphous nature with some degree of crystallinity. This semi-crystalline behavior may arise from partial ordering of polysaccharide chains, which is typical in microbial EPS and can influence mechanical strength and solubility.

GC-MS profiling identified a range of low molecular weight metabolites, including polyols, dipeptides, and pyrazine derivatives, suggesting active microbial metabolism during EPS biosynthesis. The detection of compounds such as glycerol and other dihydroxy derivatives indicates enhanced carbohydrate metabolism, supporting the EPS-producing capability of the strain.

Overall, the findings of this study confirm that Lactococcus lactis CS-2 is a potent producer of structurally complex and functionally versatile exopolysaccharides. The combination of advanced analytical techniques demonstrates that its EPS possesses significant biochemical diversity and structural complexity, making it a promising candidate for probiotic, industrial, and biomedical applications. These results are in agreement with earlier studies that highlight the importance of LAB-derived heteropolysaccharides in functional food and health-related applications [38,35].

The molecular docking study that the selected bioactive compounds exhibited varying binding affinities towards multiple therapeutic targets such as BRAF, EGFR2, HDAC8, PPARA, VEGFR2, and RET. Among these Pyrrolo [1,2-a] pyrazine derivatives showed the strongest and most consistent binding, indicating promising multi-target activity. Strong interaction with VEGF2, HDAC8, PPARA, BRAF, EGFR2 and RET. suggest their potential role in inhibiting angiogenesis and tumor progression pathways. The results also highlight the importance of binding energy values, where lower energies indicate more stable ligand–protein complexes [39,40,41].

Interaction analysis demonstrated that hydrogen bonds and hydrophobic interactions play a crucial role in stabilizing the complexes. Key amino acid residues such as CYS532, ASP101, and ASN923 were involved in hydrogen bonding, enhancing specificity and stability. Additionally, π–π stacking and π-alkyl interactions further contributed to strong binding within the active sites. VEGFR2 showed the most diverse interaction pattern, indicating it as a major target for these compounds. In contrast, smaller molecules exhibited weaker binding due to limited structural compatibility, supporting previous reports on structure–activity relationships [42].

5. CONCLUSION

The study highlights the isolation and characterization of an EPS-producing strain, *Lactococcus lactis* CS-2, from the various probiotic sources. The isolate CS-2 demonstrated strong EPS production capability, particularly when media is supplemented with 10 % sucrose at optimal growth conditions. The partially purified EPS-CS-2 was characterized by using analytically by FTIR, NMR, and SEM techniques for examining its physicochemical and biological properties. The biochemical, molecular, and FTIR analyses confirmed the polysaccharide nature of the EPS produced. These findings suggest that *L. lactis* (CS-2) has a potential application in the health and food industry. Further investigation, which may include extensive clinical trials, is required to verify the purported health benefits of EPS-CS-2. Further studies should focus on scaling up the production process, exploring the structural composition of the EPS in detail, and evaluating its functional and health-promoting properties in food systems or in vivo models.

6. AUTHOR'S CONTRIBUTION STATEMENT

Tulasabai Chavhan and Kumar B P, Anushka Devnikar: Conceptualization, Methodology, Funding acquisition and Writing. Navya H M: Review and editing of draft, Formula analysis and Validation. Prabhurajeshwar: Guide,Supervision, Investigation and Data curation.

7. FINDING

No finding

8. CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

9. COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies with human participants performed by any of the authors.

10. DATA AVAILABILITY

The data that was used in this study will be given to the corresponding author upon a fair request

11. PUBLISHER'S NOTE

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12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the writing or editing of this manuscript, and no images were generated or modified using AI.

Abbreviations

EPS-Exopolysaccharide

XRD- X-ray diffraction

SEM- Scanning electron microscope

FTIR: Fourier Transform Infrared

NMR: Nuclear magnetic resonance

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Tables and Graphs:

Figure 1: Screening of EPS production from the 24hrs old culture

Figure 2: EPS of CS-II precipitated by chilled Ethanol

Figure 3: EPS production is typically indicated by the formation of mucoid /slimy colonies

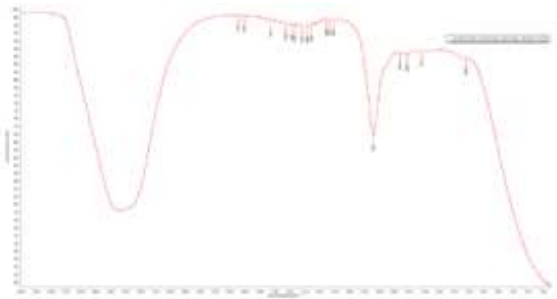


Figure 4: FTIR spectrum of EPS-CS2 at wavenumbers range of 4000–400 cm–

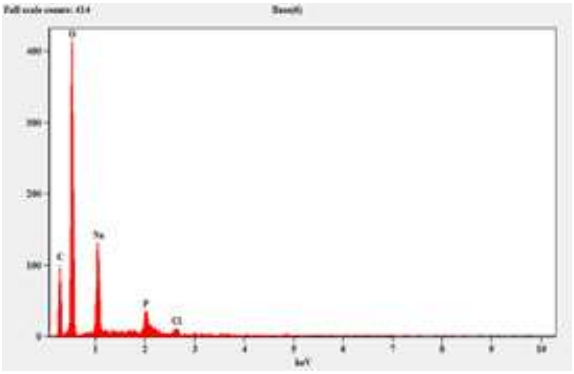


Figure 5: EDX Analysis of Exopolysaccharide from Lactococcus lactiss

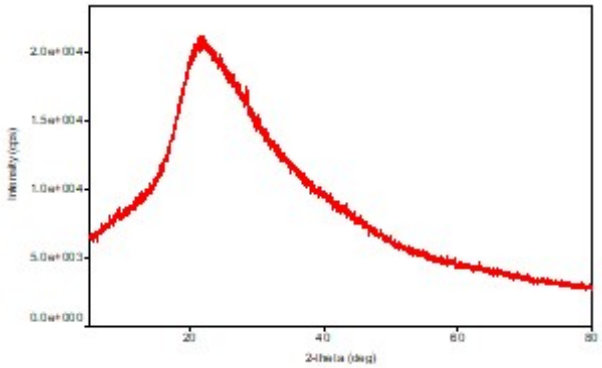
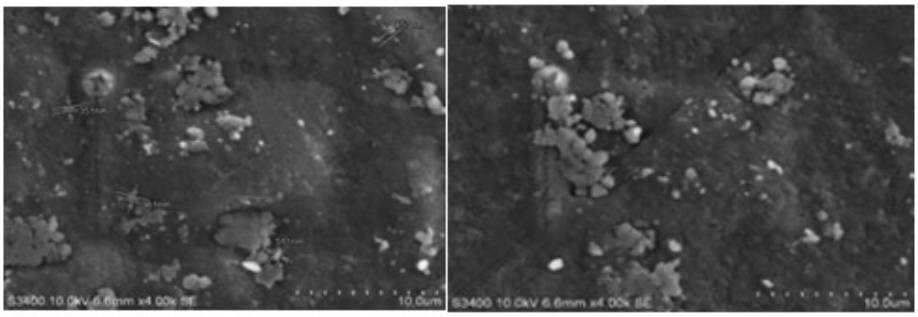


Figure 6: SEM micrograph at 4000× magnification



(A) (B)

A) Nanostructured features with particle sizes measured at approximately 351,361,457,511nm.

B) Aggregated particles (bright white clusters) on the surfaces

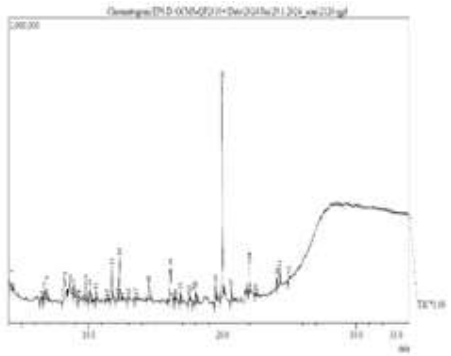


Figure7: Analysis of GCMS peak of EPS CS-II

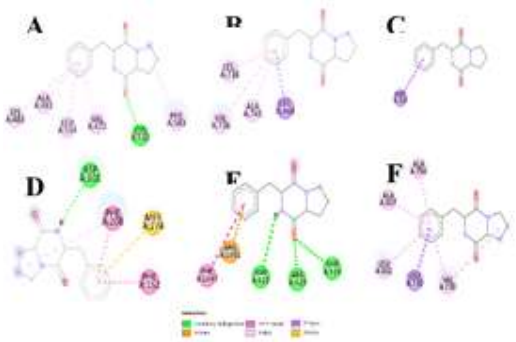


Figure 8: XRD Spectral analysis of EPS CS-II

Figure 9: Molecular docking–derived protein–ligand interaction profiles of Pyrrolo[1,2-a] pyrazine-1,4-dione, hexahydro-3-(phenylmethyl) within the active sites of key therapeutic targets: (A) BRAF, (B) EGFR, (C) PPARA, (D) HDAC8, (E) VEGFR2, and (F) RET.

Table 1: Molecular interaction profile of the selected ligand with target proteins (BRAF [6V2U], EGFR [4I22], HDAC8 [2VSX], PPARA [3FEI], RET [7DU9], and VEGFR2 [4AGC]), showing interacting amino acid residues, types of intermolecular interactions, and bond distances (Å), highlighting the structural basis of ligand–protein binding and complex stability.

Interaction information of ligands with respective proteins			
Protein	**Interacting residues**	**Types of bonds**	**Distance (Å)**
BRAF [6V2U]	ALA:483	Pi-Alkyl	5.28
	ALA:481	Pi-Alkyl	4.99
	LEUA:514	Pi-Alkyl	4.78
	VALA:471	Pi-Alkyl	4.56
	CYSA:532	Conventional hydrogen bond	2.30
	PHEA:583	Pi-Alkyl	4.78
EGFR [4I22]	LEUA:718	Pi-Alkyl	5.21
	VALA:726	Pi-Alkyl	5.33
	ALAA:743	Pi-Alkyl	5.29
	LEUA:844	Pi-Sigma	3.63
HDAC8 [2VSX]	ASPA:101	Hydrogen bond	2.67
	PHEA:208	Pi-Alkyl and Pi-Pi stacked	5.41 & 4.97
	META:274	Pi-Sulfur	5.52
	PHEA:152	Pi-Pi stacked	4.0
PPARA [3FEI]	META:320	Pi-Sigma	3.76
RET [7DU9]	ALAA:756	Pi-alkyl	4.53
	ALAA:807	Pi-alkyl	4.96
	LEUA:881	Pi-alkyl	4.52
	LEUA:730	Pi-Sigma	3.98
	VALA:738	Pi-alkyl	5.03 & 4.79
VEGFR2 [4AGC]	PHEA:1047	Pi-Pi stacked	4.59
	ARGA:1051	Pi-Cation & Pi-Alkyl	3.67 & 4.79
	ASNA:923	Hydrogen bond	2.94
	ARGA:929	Hydrogen bond	1.81 & 2.67
	THRA:926	Hydrogen bond	2.65