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Extraction and optimization of exopolysaccharide production from lactic acid bacteria and its application in biosorption of chromium from waste water

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Abstract:

Exopolysaccharide (EPS) produced by lactic acid bacteria (LAB) is important in determining the rheological properties of dairy products and also as a natural candidate for industrial use as thickener, stabilizer, emulsifier, gelling agent and water-binding agent in foods. The aim of this study was to isolate LAB showing good potential for EPS production. Various dairy products were screened to obtain six LAB strains capable of EPS production. The EPS produced by each of the strains was assayed and the one giving maximum yield was selected for further study. The highest EPS producer was identified using Bergey's manual as a Lactobacillus strain. The effect of various paramaters such as carbon source, pH, temperature and cultivation time on EPS production by the strain was studied. Maximum EPS production was obtained on incubation at 30^{0}C for 120 hours at pH 7.5 with glucose as the carbon source. The extracted EPS was analyzed by thin layer chromatography and found to be a homopolymer composed of repeating units of galactose. The EPS was

further tested for its ability to remove heavy metals from water. A 70% reduction in chromium concentration was obtained after a 20 day incubation period with the heavy metal. Thus the EPS extracted from the Lactobacillus isolate has potential in biosorption of heavy metals from waste water.

Key words: Exopolysaccharide, lactic acid bacteria, Lactobacillus, biosorption, chromium

Introduction

Lactic acid bacteria (LAB) are a group of related bacteria that produce lactic acid as a result of carbohydrate fermentation. These microbes are broadly used in the production of fermented food products, such as yogurt (*Streptococcus* spp. and *Lactobacillus* spp.), cheeses (*Lactococcus* spp.), sauerkraut (*Leuconostoc* spp.) and sausage. They are food grade organisms, possessing the generally-regarded-as safe (GRAS) status. LAB are used in the food industry because their growth lowers both the carbohydrate content of the foods that they ferment and the pH due to lactic acid production. The pH may drop to as low as 4.0, low enough to inhibit the growth of most other microorganisms including the most common human pathogens, thus allowing these foods prolonged shelf life. (Zehra et al 2008).

LAB can produce several types of polysaccharides that are classified according to their location in the cell (Degeest, et al 2001). Some LAB secrete loose polysaccharide layers on their surface known as Exopolysaccharides (EPSs). The EPS produced by LAB improves the texture and viscosity of naturally fermented milk products and prevents syneresis. The EPS of LAB also improves the rheological properties of dairy products (Zehra et al 2008). When they are added to food,

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polysaccharides show functions as thickeners, stabilizers, emulsifiers, gelling agents and water binding agents. They also contribute to preservation and enhance aroma and flavour of milk and dairy products. Moreover it has been suggested that some EPS produced by LAB confer health benefits to the consumer.

Another area of application of EPS is in biosorption of heavy toxic metals like lead, chromium, copper etc from waste water. Heavy metal contamination is a very significant environmental issue as metals are highly toxic to biota; they decrease metabolic activity and diversity, and affect the qualitative and quantitative structure of microbial communities. Microbial biosorption of heavy metals provides an economic and safe alternative compared to other physicochemical methodologies for the same. LAB EPS helps in biosorption of heavy toxic metals like lead, copper etc from waste water (Elen Aquino Perpetuo et al 2011).However one of the major shortcomings of the LAB EPS is low yield.

The objective of this study was to isolate LAB producing high amounts of EPS, optimize the production of EPS and study its application in the biosorption of the heavy metal chromium.

Materials and Methods

Collection of samples: Curd samples, buttermilk and raw milk were collected from dairies in Chembur and Nerul, Mumbai and stored at 4 deg C for analysis.

Isolation of lactic acid bacteria: A loopful of each sample was streaked on sterile de Man, Rogosa and Sharpe (MRS) agar medium which is selective for the growth of lactic acid bacteria. The plates were incubated at. After incubation at 37°C for 24 to 48 h under microaerophilic conditions, individual colonies with different morphologies were picked and grown in MRS broth. The colonies were purified by plating again. Isolates showing

Gram positive and catalase negative characteristics were then screened for Exopolysaccharides production.

Screening for EPS production: Isolates producing mucoid/ropy colonies were screened for EPS production. The isolates were grown in MRS broth, following which the cells were harvested by centrifugation for 10 min at 11000g. 2 volumes of cold ethanol was added to culture supernatant and stored overnight at 4^0C. Precipitated material was collected by centrifugation (20 min at 2500g) and resuspended in distilled water. The suspension was mixed with two volumes of cold ethanol, centrifuged at 2500g and pellets dried at 100^0C. The pellet was finally dissolved in 1 ml of distilled water and its EPS content was estimated by phenol sulphuric acid method using dextran as standard (Dubios et al., 1956).

Identification of high yield EPS producing strain: The biochemical tests for identification of Lactic acid bacteria was carried out as per as per Bergey's manual of systematic bacteriology, 2nd edition. The culture was maintained by sub culturing once a month on MRS agar slants and its morphology and gram nature were noted. Catalase test, oxidase test, nitrate reduction test, casein hydrolysis, gelatin hydrolysis, sugar fermentation test and Endospore test were preformed. Also growth characteristics at temperature 15^0C and 45^0C, ph 5 and 8 were studied in MRS broth tubes for a period of 48 hrs.

Optimization of EPS production: EPS production is affected by several parameters such as media components and cultivation conditions. Therefore to increase the yield of EPS the following media components and cultivation conditions were investigated by the one factor at a time method (Yang Wang et al., 2010): carbon source with replacement of glucose by sucrose, fructose, lactose, galactose, maltose; initial pH (4.5,5,5.9,6.6,7.5); cultivation time (24, 30,52,72,96,120 hr); and

cultivation temperature(30,37,55^{0}C). In each experiment, one factor was changed with other factors remaining constant. The effects of these factors were evaluated by quantifying the EPS by phenol sulphuric acid method (Dubios et al., 1956).

Characterization of EPS: The monosaccharide composition of polysaccharide hydrolysate of LAB was determined by thin layer chromatography. The sample and standards were subjected to TLC and the Rf values were calculated.

Application of EPS in biosorption of heavy metal: The EPS was extracted by Ethanol precipitation-Sulphuric acid method and dissolved in 20ml of distilled water. To 20ml of EPS solution, 30g/L of chromium solution was added and incubated at RT for 20 days. The amounts of chromium present initially and after incubation with extracted EPS, were estimated by Diphenyl carbazide method. For determination of chromium in water samples, 10ml of the sample was added in the test tube. To each test tube, 12 drops of 3M sulphuric acid was then added, followed by addition of 0.5ml of Diphenyl carbazide solution and allowed to stand for 5 minutes for colour development. The amount of Cr (VI) present was determined either by absorbance at 540nm or by visual comparison with standard solutions.

Results and Discussion

Isolation of EPS producing lactic acid bacteria

From the tested samples, six lactic acid bacteria were isolated. The primary characterization of the isolates indicated that they were gram positive rods and cocci, catalase negative and microaerophilic. The isolates were coded as IS1-IS6. Visual inspection of bacterial colonies for ropy or mucoid appearance on MRS agar plates showed that of the six isolates, two isolates (IS3&IS5) presented a more mucoid phenotype than the others.

These were then subjected to further screening for EPS production. EPS produced by IS3 was estimated to be 69mg/ml, while the IS5 produced 190mg/ml of EPS. The higher EPS producing isolate IS5 was identified and used for further analysis.

Identification of the highest EPS producing strain IS5:

IS-5 was identified as belonging to the genus *Lactobacillus* on the basis of its morphological and biochemical characteristics as per Bergey's manual of systematic bacteriology, second edition.

Table 1: Biochemical characterization of IS-5

Tests	Observed results	Standard Biochemical results for Lactobacillus
Gram reaction	Gram positive	Gram positive
Predominant cell shape-rod -cocci	Rods	Rods
Motility	Non motile	Uncommon
Catalase	-	-
Spore formation	Not seen	-
Cytochrome oxidase test	-	-
Gelatine hydrolysis	-	-
Casein hydrolysis	-	-
Nitrate reduction	+	Uncommon
Growth at 6.5% NaCl	+	+
Growth at – pH 5 pH 8	+ -	+ -
Growth at -15 ^{0}C 45 ^{0}C	+ +	Variable Variable
Fermentation mode	Heterofermentative	Heterofermentative

Key: + = positive, - = negative

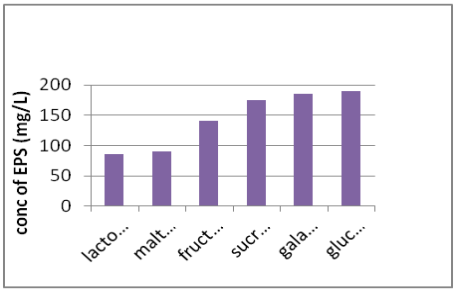
Optimization of various cultural conditions for EPS production:

Effect of different carbon sources on EPS production:

The influence of carbon sources (fructose, lactose, galactose, sucrose, glucose and maltose) on EPS production by the

Lactobacillus strain was studied. The most efficient carbon source was found to be glucose (Fig.1).

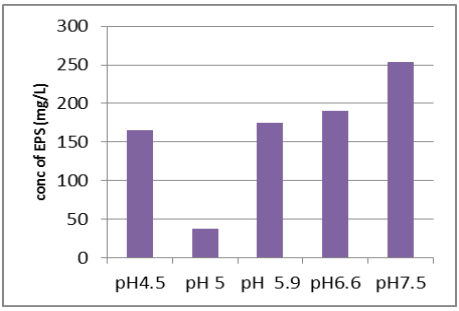
Fig. 1: Effect of carbon sources on EPS production



Effect of different pH values on EPS production:

The influence of different pH values (4.5, 5.0, 5.9, 6.6 and 7.5) on EPS production was studied. The optimum pH for EPS production by the isolate was found to be 7.5 (Fig.2).

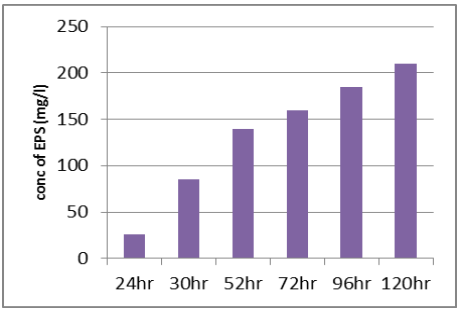
Fig.2: Effect of pH on EPS production



Effect of incubation time on EPS production.

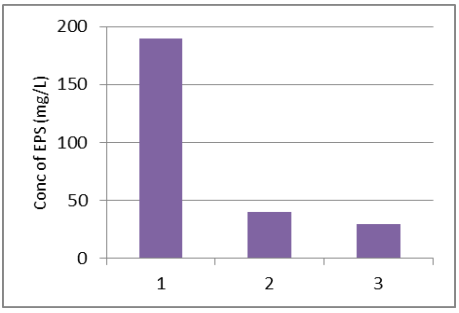
The influence of incubation time (24, 30, 52, 72, 96,120 hr) on EPS production was studied. As incubation time was increased the EPS production was found to increase (Fig.3).

Fig.3: Effect of incubation time on EPS production



Effect of incubation temperature on EPS production: The concentration of EPS produced at 30^0C was much higher than that produced at the other temperatures tested. Thus the optimum temperature for EPS production was concluded to be 30^0 C (Fig.4)

Fig.4: Effect of incubation temperature on EPS production



Key: 1=30^0 C, 2=37^0 C, 3=55^0 C

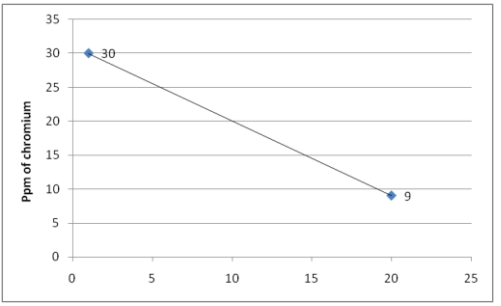
Monosaccharide analysis of EPS

The sugar composition of EPS extracted from the isolate was studied by Thin layer Chromatography. The standard sugars used were: glucose, galactose, fructose, ribose, arabinose, sucrose, and xylose. A single spot was obtained on running the sample and its Rf value was closest to that of galactose. Hence it was concluded that the EPS produced by the isolate was a homopolysaccharide composed of galactose units.

Application of EPS in biosorption of heavy metal chromium:

The biosorption ability of EPS extracted from the isolate was studied by incubating it with 30mg/l of chromium solution for 20 days and determining the concentration of left over chromium (after separation of the EPS) by the Diphenyl carbazide method. The EPS was observed to be quite efficient in its biosorption ability as it brought about a 70% reduction in the chromium concentration (Fig.5).

Fig. 5: Biosorption of chromium by LAB EPS in 20 days



Conclusion:

Lactic acid bacteria produce exopolysaccharides which have important applications in food industries as natural preservatives, emulsifiers, and agents for bioremediation and waste water treatment.

In this study, six EPS producing lactic acid bacteria were isolated from various dairy products. The isolate showing maximum EPS production was subjected to further study. It was identified as a Lactobacillus species after morphological and biochemical characterization based on Bergey's Maunal of Systematic Bacteriology. The effect of various paramaters such as carbon source, pH, temperature and cultivation time on EPS production was studied. Maximum EPS production was

obtained on incubation at 30^0C for 120 hours at pH 7.5 with glucose as the carbon source. Thin layer chromatographic analysis revealed that the EPS was a homopolymer consisting of repeating units of galactose. The EPS was found to bring about 70% reduction in chromium level after 20 days incubation with the heavy metal. This indicates that it has good potential for use in biosorption of chromium from waste water.

Future prospects:

The extracted EPS can be tested for biosorption of other heavy metals such as lead, copper, zinc, mercury and arsenic from waste water. It can also be tested for application as a viscosifier, stabilizer, emulsifier and gelling agent to modify the rheological properties of food products. The probiotic effects of the EPS such as cholesterol lowering, immunomodulating and anti-tumor abilities can be studied. Its application as a blood volume expander and flow improver can also be explored. Attempts can be made to increase the yield of EPS by genetic modification and metabolic engineering.

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