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Aysha CH
Faculty, Verghese Kurien
Institute of Dairy and Food
Technology, Kerala Veterinary
and Animal Sciences University,
Mannuthy, Thrissur, Kerala,
India

Aparna Sudhakaran V
Faculty, Verghese Kurien
Institute of Dairy and Food
Technology, Kerala Veterinary
and Animal Sciences University,
Mannuthy, Thrissur, Kerala,
India

AK Beena
Faculty, Verghese Kurien
Institute of Dairy and Food
Technology, Kerala Veterinary
and Animal Sciences University,
Mannuthy, Thrissur, Kerala,
India

Archana Chandran
Faculty, College of Dairy Science
and Technology, Kerala
Veterinary and Animal Sciences
University, Pookode, Wayanad,
Kerala, India

Rahila MP
Faculty, College of Dairy Science
and Technology, Kerala
Veterinary and Animal Sciences
University, Pookode, Wayanad,
Kerala, India

Beena RL
College of Dairy Science and
Technology, Kerala Veterinary
and Animal Sciences University,
Kaimanam, Trivandrum, Kerala,
India

Smitha J Lukose
Faculty, Verghese Kurien
Institute of Dairy and Food
Technology, Kerala Veterinary
and Animal Sciences University,
Mannuthy, Thrissur, Kerala,
India

Corresponding Author:
Aparna Sudhakaran V
Faculty, Verghese Kurien
Institute of Dairy and Food
Technology, Kerala Veterinary
and Animal Sciences University,
Mannuthy, Thrissur, Kerala,
India

Evaluation of dextransucrase activity in lactic acid bacteria for exopolysaccharide production potential

Aysha CH, Aparna Sudhakaran V, AK Beena, Archana Chandran, Rahila MP, Beena RL and Smitha J Lukose

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Abstract

The objective of this study was to isolate lactic acid bacteria from some vegetables and traditional fermented foods, with potential for the production of exopolysaccharides (EPSs), which are an important factor in assuring the proper consistency and texture of fermented food. Twenty strains of lactic acid bacteria were isolated and screened for exopolysaccharide producing potential. Among the twenty strains, Lactic acid bacteria isolated from potato, rice gruel, fermented dosa batter and ivy gourd were found to be positive for exopolysaccharide production when examined by Congo red assay. The four EPS positive isolates were identified as *Weissella confusa* R, *Weissella confusa* AN, *Pediococcus pentosaceus* P and *Lactocaseibacillus plantarum* IVY. All four isolates were examined for dextransucrase activity and the amount of dextran produced in De Man Rogosa Sharpe broth as well as paneer whey. *Weissella confusa* R isolated from fermented rice gruel exhibited the highest dextransucrase activity (58.66 ± 0.01 U) as well as dextran content (53.38 ± 0.03 U).

Keywords: Dextransucrase activity, exopolysaccharide, lactic acid bacteria, dextran, whey medium

Introduction

Recent trends of clean label and naturally derived additives have attracted considerable attention towards Bacteria derived Exopolysaccharide (EPS). Exopolysaccharides (EPS) produced by lactic acid bacteria are of great interest because of their GRAS (Generally Regarded As Safe) status, improved rheological properties of fermented foods, as well as functional and health related applications.

Dextran is a homopolysaccharide type of EPS that is synthesized extracellularly from sucrose by the dextransucrase enzyme. Hence, its activity can be used for the identification and characterization of high performing LAB EPS producers. In this context, dextransucrase activity was used as a preliminary selection criterion for EPS producers along with conventional plating techniques. The use of EPS produced *in situ* during the manufacture of food is a point of attraction for the food industry and it may be categorized as a new generation of biothickeners.

Materials and Methods

Isolation and identification of Lactic acid bacterial isolates

Lactic acid bacteria were isolated from fermented rice gruel, dosa batter, potato and ivyguard. Serial dilutions of samples were prepared using normal saline and were isolated in MRS agar by following standard microbiological procedures. Plates were incubated at 37 °C for 24 hours. Preliminary characterisation of the isolates was carried out based on catalase test and Gram staining. All the LAB cultures were streaked on MRS (de Man Rogosa Sharpe) agar for purification and were propagated in MRS broth. For long-term storage, the cultures were maintained in 70 percent glycerol at -18 °C. The molecular level identification by 16s rRNA sequencing of the isolates was conducted at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram.

Characterization of isolates

Sterilized MRS broth tubes were inoculated with 1% freshly activated cultures and incubated for 24 hours at four different temperatures (25 °C, 30 °C 37 °C, and 42 °C).

Growth was qualitatively measured using a spectrophotometer to measure the optical density of the tubes at 600 nm. Uninoculated broth tubes were provided as controls. The carbohydrate fermentative profile of isolates was determined using Andrade's peptone water (Himedia, Mumbai). The isolates were tested for dextrose, sucrose, lactose, fructose, mannose, raffinose, mellibiose, cellobiose, salicin, galactose and xylose. The color change from bright pink to dark pink indicated carbohydrate consumption and acid generation.

Congo red assay

Congo red assay for confirmation of EPS synthesis was done as per Freeman, Falkiner, and Keane (1989). Congo red agar was prepared using Brain Heart Infusion agar with 5% sucrose and 9% congo red dye (1%). The freshly activated isolates were streaked on Congo red agar and incubation was done at 37 °C for 24 hours.

Preparation of whey medium

Paneer whey was collected from KVASU Dairy plant, Mannuthy. It was heated in a boiling water bath for 1 hour and filtered. Whey medium was then formulated as detailed below and autoclaved for further use [2].

Component	Weight (g/ml)
Whey	1000 ml
K ₂ HPO ₄	1.0
Sucrose	50.0
Yeast extract	1.0
p H adjusted to 7.0	

Dextranucrase activity

Dextranucrase activity was estimated using 3,5 dinitrosalicylic acid (DNSA) method as suggested by Sawale and Lele (2009). The isolates were grown in MRS broth with 5% sucrose and whey medium with 5% sucrose at 37 °C for 24 hours. Cell free supernatant (CFS) was collected by centrifugation at 10000 rpm at 4 °C for 30 minutes. Dextranucrase activity was measured by estimating the total amount of reducing sugars (calculated as fructose) produced using 3,5-dinitrosalicylic acid (DNSA) method. 10% sucrose in 0.05M acetate buffer at p H 5.2 was used as substrate. 0.9ml of substrate was mixed with 0.1ml of CFS and incubated at room temperature for 15 minutes. The reaction was stopped by the addition of 1ml of DNSA reagent and was heated in a boiling water bath for 15 minutes. After cooling, 10 ml of distilled water was added and mixed well. The reducing sugars produced were estimated spectrophotometrically at 540 nm. One unit (U) of dextranucrase activity is defined as the amount of enzyme that liberates one Fructose mol of reducing sugar from sucrose in one minute at p H 5.2.

Estimation of dextran

Dextran content was estimated using the phenol sulphuric acid method as suggested by DuBois *et al.* (1956). The isolates were grown in MRS broth with 5% sucrose and whey medium at 37 °C for 24 hours. Five millilitres of 2.5N

HCl was added to 0.1ml of the CFS and kept for digestion in a boiling water bath for 3 hours. After cooling, sodium carbonate powder was added until the effervescence stopped. The sample was then made up to 25 ml using a measuring cylinder and centrifuged at 5000rpm for 10 minutes. To 10 µL of supernatant, 90 µL of distilled water was added and vortexed well after the addition of 1ml of 5% phenol solution. 5ml of 96% sulphuric acid was added and mixed well and incubated at room temperature for 30 minutes. Dextran content was spectrophotometrically measured at 490nm. Dextrose (0.1 mM to 30 mM) was used as a standard for the estimation of dextran content.

Results and Discussion

Fermented foods are rich reservoirs of lactic acid bacteria (LAB), which can produce exopolysaccharides (EPS), natural polymers that improve texture, viscosity, and stability in food matrices. Traditional Indian ferments such as rice gruel and dosa batter, as well as vegetables like potato and ivy gourd, include a variety of EPS-producing LAB strains, including *Weissella confusa* and *Pediococcus* spp., indicating adaptability to carbohydrate-rich, low-pH environments during spontaneous fermentation.

In this study, twenty lactic acid bacteria (LAB) isolates from vegetables and traditional fermented foods, potato, rice gruel, fermented dosa batter, and ivy gourd were screened for exopolysaccharide (EPS) production potential using the Congo red assay. These EPS-positive LAB were further examined visually, biochemically, and molecularly (16S rRNA sequencing), as well as growth profile and carbohydrate fermentation patterns. The four potential EPS producing strains were identified as *Weissella confusa* R, *Weissella confusa* AN, *Pediococcus pentosaceus* P and *Lactocaseibacillus plantarum* IVY. The colonies of *Weissella confusa* R and *Weissella confusa* AN were slimy and transparent, while those of *Lactocaseibacillus plantarum* IVY were white, non slimy colonies. The colonies of *Pediococcus pentosaceus* P were slightly mucoid and translucent.

Biochemical characterization of Lactic acid bacterial isolates

The ability of the isolates to utilize different carbon sources was assessed by using carbohydrate discs in Andrade's peptone water. A change in the colour of the media from light pink to dark pink was taken as positive. *Lactocaseibacillus plantarum* IVY was negative for raffinose and xylose fermentation, while *Weissella confusa* AN did not ferment lactose, raffinose, mellibiose and cellobiose. *Weissella confusa* R failed to ferment galactose, raffinose and mellibiose where as *Pediococcus pentosaceus* P was a raffinose non fermenter.

The optical density of all isolates at four different temperatures is depicted in Table 1. Maximum optical density was observed for growth at 30 °C in the case of *Weissella confusa* R, *Weissella confusa* AN and *Pediococcus pentosaceus* P, while *Lactocaseibacillus plantarum* IVY exhibited highest optical density at 37°C.

Table 1: Growth at different temperature

Growth at different temperature				
Isolates	OD at 600nm			
	250C	300C	370C	420C
<i>Lacticaseibacillus plantarum</i> IVY	2.07±.00a	2.15±.01a	2.16±.00a	1.62±.05a
<i>Weissella confusa</i> R	0.05±.01d	1.72±.06b	1.68±.01c	0.61±.02c
<i>Pediococcus pentosaceus</i> P	1.47±.01c	1.63±.01c	1.62±.01d	1.62±.01a
<i>Weissella confusa</i> AN	1.56±.01b	1.75±.01b	1.75±.00b	1.3±.02b

Congo red assay

Isolates with the ability to synthesise EPS will grow as black colonies. Among the 20 isolates, four isolates were positive for congo red assay (Figure 1). This was in agreement with the work done by Ignatova-Ivanova (2017) and coworkers where they have done Congo red agar assay with high sucrose (10%) and Congo red (5%). They reported that isolates formed black or almost-black, mucoid colonies, showing EPS/biofilm formation by lactic acid bacteria,

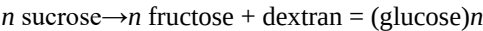
including *L. plantarum*. Similarly, in the study by Amrutha and coworkers (2023), Congo red agar assays (5% sucrose, 0.08% Congo red) was used to identify EPS-positive strains that produce black or dark-colored mucoid colonies . Among the EPS-positive isolates screened were *Pediococcus*-like strains and *Weissella confusa*-type LAB, confirming CRA positivity for EPS production across both genera.



Fig 1: Congo red assay for confirmation of exopolysaccharide production

Dextranucrase activity

The basis for dextranucrase activity is its role as a glucosyltransferase, which builds dextran by transferring glucose residues while releasing fructose using sucrose as the only substrate. Dextranucrase uses an α-(1→6) glycosidic bond to transfer the glucosyl moiety from sucrose to a developing dextran chain. This polymerizes glucose units into a branching polysaccharide (dextran) and liberates fructose as a byproduct [7].



To assess activity, fructose (a reducing sugar) or dextran synthesis from sucrose is often quantified utilizing DNS reducing sugar techniques or gel-based "in situ" detection of dextran-producing bands [8]. The Cab3 dextranucrase produced by *W. confusa* had a high specific activity of approximately 105 U/mg, distinguishing it from other LAB-derived dextranucrases [9]. *W. confusa* VTT E-90392 efficiently synthesized dextran (11-16 g/kg dry weight) from sucrose in wheat sourdough, with substantial dextranucrase activity and moderate acidification [10]. Dextranucrase activity in whey is observed to be more than in MRS medium with sucrose, as mentioned in Table 2. This observation was in conjunction with the findings of [11], where whey permeate medium resulted in increased dextran concentration and viscosity, as well as higher dextran-forming activity and biomass compared to normal media, which suggested enhanced dextranucrase activity in whey-

based medium.

Table 2: Dextranucrase activity of all isolates when grown in MRS (with sucrose) and paneer whey (with sucrose)

Dextranucrase activity(U)		
Isolates	MRS with sucrose	Whey with sucrose
<i>Lacticaseibacillus plantarum</i> IVY	10.62±.04 ^d	35.35±.01 ^b
<i>Weissella confusa</i> R	18.29±0.04 ^b	58.66±.01 ^a
<i>Pediococcus pentosaceus</i> P	14.75±.04 ^c	21.23±.14 ^d
<i>Weissella confusa</i> AN	20.44±.18 ^a	24.43±.06 ^c

Estimation of dextran

Dextran content was higher in *Weissella confusa* R in both MRS media and whey as compared to other isolates (Table 3) [12] demonstrated that maximal dextran levels occur around pH 4.0-4.5, when dextranucrase productivity is highest; at more acidic or neutral pH, dextran yield declines in tandem with decreased dextranucrase activity, demonstrating a direct relationship between enzyme activity and dextran content. Moreover, research done by Al-anbakey, Muslim, and Hamzah (2024) showed that the amount of dextran generated in a given time is strongly related to the amount of enzyme present: larger enzyme concentrations resulted in higher dextran yields until saturation, indicating a proportional relationship and enzyme activity increased with increased sucrose content.

Table 3: Dextran content/ml of all isolates when grown in MRS (with sucrose) and paneer whey (with sucrose)

Dextran content/ml		
Isolates	MRS with sucrose	Whey with sucrose
<i>Lactocaseibacillus plantarum</i> IVY	12.75±.04 ^d	40.61±.04 ^b
<i>Weissella confusa</i> R	20.66±0.04 ^a	53.38±.03 ^a
<i>Pediococcus pentosaceus</i> P	17.27±.57 ^b	40.59±.04 ^b
<i>Weissella confusa</i> AN	14.17±.06 ^c	27.89±.06 ^c

Conclusion

This study effectively isolated and screened EPS-producing LAB from indigenous sources from Kerala, with *Weissella confusa* R emerging as a promising strain for high dextranase activity and dextran production in paneer whey. These findings highlight the value of dairy byproducts such as paneer whey as sustainable, low-cost substrates that outperform synthetic MRS broth, increasing enzyme efficiency and polymer synthesis for industrial scaling. Health-promoting properties such as prebiotic benefits, cholesterol lowering, immunomodulation, and antioxidant activity increase its potential in functional foods designed for Indian consumers. Notably, *in situ* EPS manufacturing is consistent with tendencies toward clean-label developments such as enhanced paneer or yogurt made from local whey waste. These findings emphasize the potential of locally fermented foods and dairy waste for producing natural thickeners in food industry applications, given LAB's GRAS designation and health benefits. The research highlights the significance of dextranase enzymatic activity as a functional marker for EPS biosynthesis and its application in the selection of technologically valuable LAB strains. Future studies should focus on optimizing production scales, characterizing dextran structures, and evaluating applications in fermented dairy products to improve texture and functionality.

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