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Original Article

Bioprospecting of indigenous resources for the exploration of exopolysaccharide producing lactic acid bacteria

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ABSTRACT

Exploration of biodiversity lead towards the discovery of novel exopolysaccharide (EPS) producing microbes that have multiple applications. The safety compatibility status of lactic acid bacteria (LAB) makes it an attractive candidate for the production of EPS in industries. Therefore, new bacterial isolates are continuously being identified from different habitats. Current research was conducted to explore indigenous biodiversity for the production of dextransucrase, which is involved in the synthesis of dextran. Dextran is an EPS which is used in different industries. In this study, thirty-nine LAB were isolated from different food samples. The isolates were identified as genus *Leuconostoc*, *Weissella* and *Streptococcus* based on genotypic and phenotypic characteristics. Screening revealed that only eight isolates can produce dextransucrase in high titres. Fermentation conditions of dextran producing LAB was optimized. The results indicated that *Weissella confusa* exhibited maximum specific activity (1.50 DSU mg^{-1}) in 8 h at 25 °C with pH 7.5. Dextran produced from *Weissella* proved to be a useful alternative to commercially used dextran produced by *Leuconostoc mesenteroides* in industries for various applications.

1. Introduction

The need to explore biodiversity for the production of novel metabolites is increasing progressively. Bioprospecting is the most frequently used term for screening of biological resources for the extraction of commercially important compounds. It generally relies on the provision of novel biodiversity [1]. Microbes are considered as powerful source for the development of new bioproducts. A diversity of chemical structure and functionality has been observed in compounds obtained from microorganisms. Lactic acid bacteria (LAB) is one of the most explored microbial community among all identified genera with the potential to produce different metabolites. LAB are ubiquitous in nature and commonly found in various fruits, vegetables, meat, dairy products, cereals and seafoods. During the past few decades, exploration of natural resources resulted in identification of several new taxa of lactic acid bacteria [2–4]. LAB comprises of diverse genera including *Streptococcus, Pediococcus, Leuconostoc, Lactococcus, Weissella, Oenococcus, Sporolactobacillus, Teragenococcus, Vagococcus, Lactobacillus, Aerococcus, Carnobacterium* and *Enterococcus* [5]. These genera have proven record in production of important commercial metabolites [6,7].

LAB have potential applications in food, pharmaceuticals and chemicals. They are also considered as microbial cell factories as they can produce different types of extracellular polysaccharides [8,9]. All exopolysaccharides have broad commercial applications due to the versatility in their structural and functional properties. A wide range of exopolysaccharides are produced from lactic acid bacteria including dextran, mutan, alternan, reuteran, inulin and levan [10]. Among them, dextran has various commercial applications since its identification in 1861 [11]. Several species of *Leuconostoc* have been reported to produce exopolysaccharides [12–14]. However, recent studies focused on its isolation from other LAB such as *Weissella* and *Pediococcus* [15,16]. The exploration of new isolates is important because the characteristics of dextran varies from species to species. The applicability of this exopolysaccharide highly depends on the molecular weight, type of linkage and degree of branching [17]. Dextran produced form *Leuconostoc mesenteroides, Leuconostoc citreum and Weissella confusa* have α-1 → 2 (4.1%), α-1 → 2 (3.1%) and α-1 → 3 (11%), α-1 → 3 linked

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branching (2.3%), respectively. Dextran from *Leuconostoc citreum* is preferred as a prebiotic glucooligosaccharide as compare to other lactic acid bacteria whereas, dextran from *Weissella* is more linear than commercially used *Leuconostoc mesenteroides* B512F [18]. Linear dextran is highly soluble and have high viscosity while, branching decreases the solubility of the polymer [19].

Considering the fact that biopolymers have applicability in diverse field depending upon their structural characteristics. The current study is aimed to explore biodiversity of lactic acid bacteria from indigenous sources for the production of dextransucrase and dextran. After identification of a potential isolate, the fermentation conditions were optimized to enhance the secretion of the enzyme.

2. Materials and methods

2.1. Chemicals and reagents

All the chemicals and reagents used in the current study are of analytical grade and purchased from different companies. MRS broth, yeast extract, bacteriological peptone, vancomycin disk were purchased from Oxoid (Basingstoke, Hampshire, England); sucrose, dipotassium hydrogen phosphate, calcium chloride, sodium chloride were products of Serva (Heidelberg, Germany); arginine and magnesium sulphate were purchased from Scharlau (Barcelona, Spain). The reagents were prepared in deionized water unless otherwise stated.

2.2. Sample collection and bacterial isolation

For the isolation of bacterial strains, twenty-five indigenous samples including decayed fruits (*Mangifera indica*, *Malus domestica*, *Vitis vinifera*, *Prunus domestica* and *Prunus persica*), vegetables (*Brassica oleraceae var. capitate*, *Lycopersicion esculentum* and *Brassica oleraceae var botyris*) and a dairy product (raw milk) were collected from different areas of Karachi, Pakistan. After collection, samples were chopped and transferred into MRS (de Man, Rogosa and Sharpe Agar) broth medium and incubated at 25 °C for up to 24 h under agitation (100 rpm). Serial dilutions were carried out up to 10^{-6} diluents. From each dilution, 100 μl was spread on sucrose (20 g L^{-1}) agar plate and incubated at 25 °C up to 48 h. Isolated colonies with glutinous texture were selected and identified. The pure colonies were preserved on tomato juice agar slant and stored at 4 °C for further analysis [20].

2.3. Bacterial growth measurements

Bacterial growth was measured spectrophotometrically by transferring the culture in MRS broth medium and incubated for 24 h at 25 °C. After incubation, the samples were centrifuged at 4 0,000 × *g* for 10 min at 4 °C. The bacterial cell pellet was suspended in normal saline (1.5 ml) and optical density (OD) was measured at 600 nm using normal saline as a blank. Afterwards, for the calculation of the number of bacterial cells present in the sample, the respective OD of each sample was compared with that of McFarland turbidity index [21].

2.4. Qualitative screening for dextransucrase and dextran production

All the selected isolates were screened for biopolymer synthesis. Dextransucrase is an inducive enzyme and specifically require sucrose in the medium for the production of dextran. Therefore, for the screening of the dextransucrase and dextran production all the isolates were cultivated in MRS broth containing sucrose (10 g L^{-1}) and incubated at 25 °C for 24 h. After incubation, strains were selected on the basis of polymer production. The fermented broth become turned viscous due to dextran production.

2.5. Identification of bacterial isolates by taxonomic characterization

Pure bacterial isolates were identified through different morphological analysis and by performing various biochemical test.

2.5.1. Taxonomic characterization

For morphological identification of bacterial strains, Bergey's manual of determinative bacteriology was used [22]. Colonial characteristics and cell morphology were observed for identification. The bacterial isolates were subjected to different biochemical tests for the biochemical characterization including sugar fermentation and arginine hydrolysis tests. For sugar fermentation, bacterial cultures were inoculated into the medium containing 10 g L^{-1} of different sugars (Arabinose, Sucrose, Maltose, Dextrose, Lactose, Mannitol, Trehalose, Xylose, Fructose) for up to 48 h at 25 °C. Colour change and gas production was observed for fermentation. To test for arginine hydrolysis, samples were analysed as described by Tindall et al. [23]. Arginine dihydrolase activity was determined by inoculating the strain in nutrient broth medium containing arginine (5 g L^{-1}) and bromocresol purple as a pH indicator. The inoculated strains were incubated at 35 °C for 48 h.

2.5.2. Molecular characterization

DNA sequence analysis was performed for further identification of bacteria. Genomic DNA was extracted from overnight bacterial culture using DNA extraction kit (Promega, USA). For amplification, polymerase chain reaction (PCR) of 16S rDNA was performed using universal primer set as 16S forward 5′-GAGTTTGATCCTGGCTCAG-3′ and 16S reverse 3′-AGAAAGGAGGTGATCCAGCC-5′. The program parameters are as follows: initial denaturation at 95 °C for 5 min, 95 °C for 1 min for 35 cycles, 55 °C for 45 s, 72 °C for 1 min followed by final elongation of 72 °C for 10 min and a hold at 25 °C. The amplified DNA was analysed using agarose gel electrophoresis (1%) and purified through PCR purification kit (Promega, USA). The purified products were then sequenced and sequence similarity was searched through BLAST (GenBank, http://www.ncbi.nlm.nih.gov/blast/). Sequences were aligned and phylogenetic tree was constructed by neighbour joining method using Mega software (version 7.0.1).

2.6. Quantitative screening for dextransucrase and dextran production

The pure bacterial isolates were screened for maximum dextransucrase activity. The inoculum was transferred into sucrose (20 g L^{-1}) containing broth (10 ml) and incubated at 25 °C for 24 h. The seed culture was transferred into flask (90 ml) and incubated further for 24 h. After fermentation, bacterial cells were harvested for 10 min at 40,000 × *g*. The cell free filtrate (CFF) was used for further determination of enzyme activity and total protein. Sucrose containing medium constitutes of (g L^{-1}): 5.0, yeast extract; 5.0, peptone; 15.0, dipotassium hydrogen phosphate; 0.5, calcium chloride; 0.1, magnesium sulphate; 0.1, manganese chloride and 0.1, sodium chloride. The initial pH of the fermentation broth was adjusted at pH 7.5 before sterilization.

Dextransucrase activity was determined by measuring the amount of reducing sugar liberated from sucrose using glucose as standard through method as described by Kobayashi and Matsuda [24]. For enzyme activity, CFF (50 μl) was incubated with 1.0 ml of sucrose (125 mg ml^{-1}) at 35 °C for 15 min. After incubation, the reaction was stopped by incorporating 1 N solution of NaOH (50 μl). One unit of enzyme activity is measured as Dextransucrase Units (DSU) and is defined as:

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