Optimization of growth conditions for the isolation of dextran producing *Leuconostoc* spp. from indigenous food sources

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Abstract: *Leuconostoc* are known to produce dextran, which have great commercial importance in chemical, medical and food industry. The present study is an attempt to select the best medium for the isolation of indigenous dextran producing *Leuconostoc,* measuring their enzyme activities for dextransucrase, production of dextran and identification of dextran producing *Leuconostoc* CMG706, CMG707, CMG710 and CMG713. Since, dextran producing *Leuconostoc* produce slimy colonies, twenty-four slime producing bacterial strains were isolated from different food sources, fruits and vegetables. Three different isolation medium were evaluated for the isolation of *Leuconostoc* only and the best one was found to be one containing sucrose and sodium azide. Further, all slime producing bacterial strains were screened for enzyme activity of dextransucrase, which is responsible for dextran production. Four bacterial strains CMG706, CMG707, CMG710 and CMG713 giving high enzyme activities were selected for dextran production and identified.

Keywords: *Leuconostoc*, dextran, dextransucrase, isolation, lactic acid bacteria.

INTRODUCTION

The genus Leuconostoc belongs to the 'sensu stricto' group of lactic acid bacteria (LAB). About 12% of the LAB isolated from plant materials are *Leuconostoc* species. Some are isolated from the surface of wide range of vegetables and fruits, and others from refrigerated meats and dairy products*. Leuconostoc* group have special commercial importance due to their ability to produce aromatic compounds, valuable polysaccharides and malolactic fermentation. Like other groups of LAB, *Leuconostocs* also need complex media due to their multiple demands for amino acids, peptide, carbohydrates, vitamins and metallic ions.

A number of *Leuconostoc* differential and selective media are known for the isolation of *Leuconostoc* such as: M.R.S. (De Man *et al*., 1960), LUSM (Benkerroum *et al*., 1993), McCleskeys medium (McCleskey *et al*., 1947) and Mayeux medium (Mayeux and Colmer, 1961). However, no selective and single medium is yet reported for the isolation of dextran producing *Leuconostoc* spp. from various food sources, fruits and vegetables. M.R.S. medium is selective for isolation of Lactic acid bacteria (DeMan *et al*., 1960), which can also be used for the isolation of *Leuconostoc* as Lactic acid bacteria. Another character of *Leuconostoc* is that they resist vancomycin upto 500 µg/ml and this has led to the use of vancomycin as selective medium (LUSM medium) for the isolation of *Leuconostoc* (Benkerroum *et al*., 1993). McCleskey medium (McCleskey *et al*., 1947) is suitable for the isolation of all slime producing bacteria including *Leuconostoc.* Another medium i.e. Mayeux agar (Mayeux *and Colmer,* 1961) has also been reported for the isolation of *Leuconostoc.*

Dextrans have important medical applications in the production of fine chemicals such as plasma substitutes and Sephadex. Also used for texture improvement in food industry, e.g. in milk based drinks, yogurts and ice creams (Neubauer *et al.*, 2003*)*. Dextran is reported to be produced from sucrose by strains of *Leuconostoc* sp (Hucker and Pederson, 1930). However, dextran can also be produced by *Streptococcus* and *Acetobacter* in sucrose-containing medium. The growing cells secrete an enzyme called dextransucrase into the medium, where it converts sucrose into dextran (Behravan *et al*., 2003).

Dextran producing *L. mesenteroides* CMG713 (Accession no: DQ208970) has been-studied previously for maximum yield of dextran (Sarwat *et al*., 2008) characterized for several phenotypic and biochemical parameters as reported by Garvie, 1986 and Holt, 1994. Effects of various parameters such as PH, temperature, NaCl and sucrose concentration on growth and slime/dextran production were also investigated (Ahmed and Sarwat, 2012).

Present study describes selection of best medium for the isolation of indigenous dextran producing *Leuconostoc,* measuring their enzyme activities for dextransucrase, production of dextran ~~and~~ Identification of dextran producing *Leuconostoc* CMG706, CMG707, CMG710 and CMG713 was performed by modified QTS-24 (DESTO) indigenous product similar to API.

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MATERIALS AND METHODS

For the isolation of dextran producing *Leuconostoc species*, different isolation media i.e. M.R.S medium, 15% sucrose containing medium and 5% sucrose and sodium azide containing medium were used.

Media used for isolation

Medium 1: M.R.S (de Man, Rogosa and Sharpe) broth prepared M.R.S medium from Bioline was used.

Medium 2: 5% Sucrose containing broth

Sucrose 5% (Merck),Tryptone 1% (Difco), Yeast Extract 0.1% (Merck), K_2HPO_4 0.25% (Merck) was prepared and sterilized by autoclaving at 121˚C for 20 minutes. pH of the medium was adjusted at 7.0 before sterilization.

Medium 3:5% Sucrose and sodium azide containing broth

Sucrose 5% (Merck), Tryptone 1% (Difco), Yeast Extract 0.1% (Merck), K_2HPO_4 0.25% (Merck) was prepared and sterilized by autoclaving at 121˚C for 20 minutes. pH of the medium was adjusted at 7.0 After autoclaving 0.005% sodium azide was added to the medium.

Medium 4: For isolation of slimy shiny colonies

Sucrose 2% (Merck), nutrient broth 1.3% (Oxide) and nutrient agar 1.6% (Oxide). PH of the medium was adjusted at 7.0. After autoclaving 0.005% sodium azide was added to the medium.

Isolation of dextran producing Leuconostoc species

For the isolation of dextran-producing *Leuconostoc* strains, a large number of food sources such as, cream, biryani (traditional rice dish) and a piece of tomato from cooked tomato dish was used. Fruits and vegetables were obtained from the local markets of Karachi, Pakistan (table 1 and 2). Initially a small piece of each sample was inoculated in medium 1, 2 and 3 respectively (table 1) and incubated overnight at 25˚C. After overnight incubation, viscous broths and non viscous broths were spread by serial dilution 10^{-6} to 10^{-10} on medium4 agar plates containing 2% sucrose and incubated overnight at 25˚C. Slimy shiny colonies were then picked and single colony was restreaked on each agar plates for isolation of dextran producing *Leuconostoc* strains (fig. 1). Only one colony from each sample was picked and preserved.

Purification of isolated colonies

All isolated slimy shiny colonies were purified by restreaking a single colony on 2% sucrose agar plates and incubated overnight at 25˚C. Purity was confirmed by observing colonial morphology i.e. (slimy shiny colonies) on medium4 agar plates (fig. 1) and cellular morphology i.e. (diplococci in chains) by Gram's staining.

Preservation of bacteria

The purified bacteria were preserved in 15% glycerol at -70˚C.Whereas, routine cultures were propagated in M.R.S. and medium 4 without sodium azide at 4˚C.

Enzyme production

Slime producing colonies were then confirmed for the production of enzyme (dextransucrase) responsible for dextran production by inoculating 18hr grown culture in fresh enzyme production medium and incubated at 25˚C for 18 hr (Ul Qader, 2001).

Enzyme activity

The enzyme activity was determined by centrifuging 18hr old culture medium at 10,000 rpm for 20 minutes and obtaining the cell-free supernatant. Standard enzyme assay method of Kobayashi and Matsuda, 1974 was used. The enzyme activity is represented in Dextransucrase Unit (DSU/ml) (Lopez and Monsan, 1980).

Dextran production

For dextran production enzyme medium containing 10% sucrose containing medium was used. Medium (90 ml) was inoculated by a 10.0 ml, 24 hr grown culture and further incubated at 30˚C. After 20 hrs of incubation, sample was centrifuged and precipitated with ethanol for the isolation of dextran from cell free fluid (Sarwat *et al*., 2008).

Precipitation of dextran

Isolation of dextran was achieved by precipitating cell-free supernatant with chilled 98% ethanol. The precipitated dextran was then filtered and centrifuged at 10,000 rpm for 20 minutes and dried under vacuum on $CaCl_2$ at room temperature i.e. 30˚C.

Identification of bacteria

CMG706, CMG707, CMG710 and CMG713 were identified according to Bergey's Manual of Systematic Bacteriology (Garvie, 1986) and Bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994) with the help of modified QTS-24 (DESTO) indigenous product similar to API, developed specially for this study only.

RESULTS

Isolation of dextran producing Leuconostoc

Twenty-four, dextran producing *Leuconostoc species* were successfully isolated from various food sources, fruits and vegetables **(t**able 1**)**. Out of 24 samples inoculated in medium1 i.e. M.R.S broth and incubated at 25˚C for 24 hrs, only 6 samples were found to be positive for slimy shiny colonies when streaked on medium 4 agar plates (fig. 1). The second isolation medium 2 was sucrose 5% containing medium which was incubated at 25˚C for 24 hrs, but only 12 slimy shiny colonies were isolated out of 24 samples inoculated. Third isolation medium3 was 5%

sucrose medium containing sodium azide incubated at 25˚C for 24 hrs. Slimy shiny colonies were isolated from all the 24 samples inoculated.

Table 1: Isolation of dextran producing bacteria using different media at 25˚C

S. No.	Source	M.R.S	5% sucrose	5% sucrose+ sodium azide
Fruits				
1	Falsa or phalsa	-ve	+ve	+ve
2	Sapodilla	+ve	+ve	+ve
3	Peach	+ve	-ve	+ve
4	Apricot	-ve	-ve	+ve
5	Jujube	-ve	-ve	+ve
6	Grapes	-ve	+ve	+ve
7	Mango	-ve	-ve	+ve
Vegetables				
8	Cabbage	+ve	+ve	+ve
9	Onion	-ve	-ve	+ve
10	Dish-cloth gourd	+ve	-ve	+ve
11	Cabbage	-ve	-ve	+ve
12	Cauliflower	-ve	-ve	+ve
13	Okra	-ve	+ve	+ve
14	Cucumber	-ve	-ve	+ve
15	Purple cabbage	-ve	-ve	+ve
16	Apple	-ve	+ve	+ve
17	Bitter-gourd	-ve	+ve	+ve
18	Carrot	+ve	+ve	+ve
19	Ginger	-ve	+ve	+ve
20	Sugar beet	-ve	+ve	+ve
21	Musk-melon	-ve	+ve	+ve
Food items				
22	Tomato(cooked)	+ve	+ve	+ve
23	Cream	-ve	-ve	+ve
24	Rice (biryani)	-ve	-ve	+ve

Key: +ve = Positive for dextran producing bacteria, -ve = Negative for dextran producing bacteria

Enzyme production

Enzyme activities ranging from 08 (DSU ml/hr) to 33 (DSU ml/hr) were obtained when isolated colonies were screened for enzyme dextransucrase (table 2).

Dextran production

Dextran yield ranging from 3.4 gm/100ml to 4.5 gms/100 ml was obtained when CMG706, CMG707, CMG710 and CMG713 were selected for dextran production (table 2). Dextran obtained was white amorphous powder as shown in (fig. 2).

Identification of bacteria

CMG706, CMG707, CMG710 and CMG713 were selected for identification (fig. 1). Identification was done with the help of modified QTS-24 (DESTO). Phenotypic and biochemical characteristics were studied and compared with the characters of *Leuconostoc* as reported by Garvie, 1986 and Holt, 1994. CMG strains were identified as *L. mesenteroides* subsp *mesenteroides.*

Fig. 2: Dextran from *L. mesenteroides* CMG713

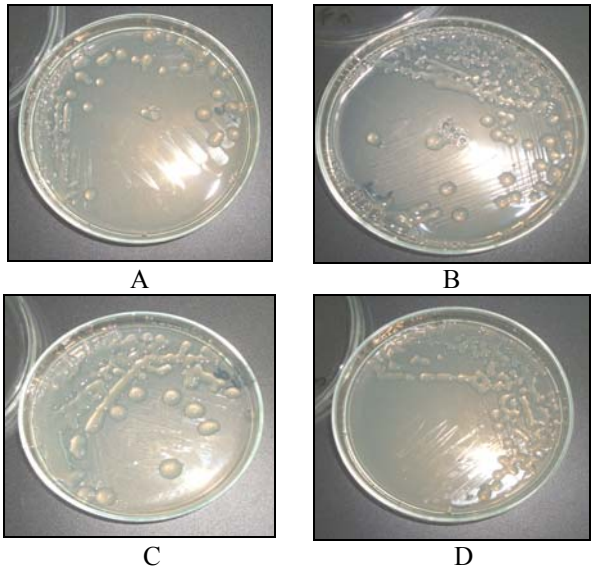

Fig. 1: Slimy shiny colonies of dextran producing *L. mesenteroides* A. CMG 706 B. CMG 707 C. CMG 708 D. CMG 713

DISCUSSION

Results have shown that dextran producers are commonly present in nature and can be isolated from wide variety of fruits, vegetables and from other food sources (tables 1

Table 2: Different sources and enzyme activities of dextran producing *Leuconostoc* species

CMG Strain Code	Source	Botanical names	Enzyme activity DSU/ml/hr	Dextran (gms)
701	Cream		10	3.5
702	Rice(Biryani)	H*Oryza sativa*H or *HOryza glaberrima*H	26	3.9
703	Cabbage	*Brassica oleracea capitata*	22	3.8
704	Peach	*Prunus persica*	11	3.5
705	Onion	*Allium cepa*	23	3.8
706	Apricot	*Prunus armeniaca*	33	4.5
707	Dish-cloth gourd or Luffa	*Luffa aegyptiaca (Luffa cylindrical)*	31	4.2
708	Cabbage	*Brassica oleracea capitata*	14	3.6
709	Cauliflower	*Brassica oleracea botrytis*	26	3.8
710	Okra, lady's fingers or gumbo	*Abelmoschus esculentus*	32	4.5
711	Jujube	*Ziziphus zizyphus*	20	3.8
712	Cucumber	*Cucumis sativus*	28	3.9
713	Grapes	*Vitis vinifera*	30	4.0
714	Mango	H*Mangifera indica*H common mango	10	3.5
715	Purple Cabbage	*Brassica oleracea capitata*	08	3.4
716	Apple	*Malus domestica*	21	3.8
717	bitter melon or bitter gourd	*Momordica charantia,*	12	3.5
718	Carrot	*Daucus carota*	11	3.5
719	Ginger	*Zingiber officinale*	11	3.5
720	Sugar beet	*Beta vulgaris*	22	3.8
721	Muskmelon	*Cucumis melo*	06	3.4
722	Falsa	*Grewia asiatica*	22	3.8

and 2). Negative results observed during the initial isolation from medium 1 i.e. M.R.S. broths, shows that the media was selective for Lactic acid bacteria but not for dextran producing bacteria; rather, dextran producing bacteria were found to be inhibited by other bacteria and fungi, which is a problem when using sugar with low pH of the medium. Though Medium 2 was found to be selective medium for slime producing bacteria but non-selective for *Leuconostoc species* until and unless *Leuconostoc* could survive in mix flora. Sucrose concentration of 5% was rather found to be an enriched medium for the growth of undesirable bacteria and fungi. slimy shiny colonies were isolated from all the 24 samples inoculated (table 1), the reason could be that sodium azide inhibited the growth of fungi and undesired bacteria which normally contaminate *Leuconostoc* sp. Sodium azide was used for the selective isolation of *L. mesenteroides* because sodium azide has been reported to be inhibitory for most lactic acid bacteria, including Lactobacilli, but not *for Leuconostoc* or *Lactococcus diacetylactis* (Benkerroum *et al.,* 1993). It was also observed that sucrose is essential for the isolation of dextran producing *Leuconostoc* sp. as it converts sucrose to dextran and makes isolation broths viscous. Further it was observed that plates without sodium azide became contaminated with bacteria or fungi but plates containing sodium azide inhibited the growth of contaminants, promoting growth of dextran producing *Leuconostocs* only. Fig.1 shows slimy shiny growth of dextran producing *Leuconostoc species.* Further, it could be concluded that adding sucrose and sodium azide to any enrich media composition will prove to be selective for the isolation of dextran producing *Leuconostocs.* During the handling of the cultures it was also observed that production of slime varies from liquid to highly viscous slimy growth in broths and on agar plates. It was found that dextran production is directly proportional to enzyme activity. Highest enzyme activities and dextran yield was obtained from *L. mesenteroides* CMG706 isolated from apricot, CMG707 isolated from Luffa, CMG710 isolated from okra and CMG713 isolated from grapes (table 2). Results show that variation exists among *Leuconostoc* isolated from different food sources with respect to enzyme and dextran production (table 2). Differences has been found among dextrans from several strains of *Leuconostoc* with respect to their glucosidic linkages, degree and type of branching, mass and physical and chemical characteristics (Cote & Robyt, 1982; Figures & Edwards, 1981; Zahnley & Smith, 1995). However, no reason is yet reported for variation among enzyme activities of *Leuconostoc* isolated from different food sources under similar isolation and growth conditions. Maybe source has nothing to do with the variation which exists naturally among *Leuconostoc*. Therefore, it can be concluded that production of dextran and dextransucrase from *Leuconostoc* sp. depends upon type of strain,

environmental conditions and growth medium. It was also observed earlier that under optimized conditions for maximum dextran production, the enzyme production was also increased (Sarwat *et al*., 2008). The characters described in Bergeys manual for *Leuconostoc mesenteroides* were compared with the CMG strains and it was found that dextran producing *Leuconostoc mesenteroides* CMG706, CMG707, CMG710 and CMG713 have similar phenotypic and biochemical characteristics as reported by Garvie,1986 and Holt, 1994. These characteristics of CMG713 have been reported earlier in detail (Ahmed and Sarwat *et al.,* 2012).

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