Short Communication

REGULATION OF DEXTRANSUCRASE PRODUCTIVITY OF *LEUCONOSTOC MESENTEROIDES* NRRL B-512F BY THE MAINTENANCE MEDIA

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(Received October 9, 1995; Accepted November 25, 1995)

Leuconostoc mesenteroides NRRL B-512F belongs to the family Streptococcaceae and is known to synthesize dextransucrase when grown in sucrose medium. This enzyme catalyzes the formation of dextran from sucrose.

Various media for the maintenance of *L. mesenteroides* are reported (1–6,8). The organism can be maintained in sucrose-rich media (1,8) also used for isolation of *L. mesenteroides*. *Lactobacillus* MRS medium is traditionally used for maintaining all organisms belonging to family Lactobacillaceae (2). This medium is also used for long-term preservation of *L. mesenteroides* (2,6). For a short period, the culture can be stored in enzyme production medium as described by Tsuchiya et al. (10). El-Sayed et al. (3) described a different medium (tomato-tryptone medium) for culture maintenance.

Although there are many reports suggesting different media, none of them suggests that enzyme production by *L. mesenteroides* NRRL B-512F is influenced by the medium, in which it is maintained. In the present study, maintenance media described by Bhatnagar et al. (1), DeMan et al. (2), Jeanes (5), Stacey (8), Tsuchiya et al. (10) and *Lactobacillus* MRS medium (2) with modification (glucose replaced by sucrose) were used. An attempt has been made to screen a medium for culture maintenance that is capable of inducing higher dextransucrase production and ascertain the extent to which the dextransucrase production is regulated by the media used for maintaining the cultures of *L. mesenteroides* NRRL B-512F.

L. mesenteroides NRRL B-512F was procured from IMT, Chandigarh, India. Ingredients of media were from Ni-Media, India. 3,5-Dinitrosalicylic acid (DNS)

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and Folin's reagent were procured from Sigma, St. Louis, MO, U.S.A. Six different media (Table 1) were used for maintaining and weekly subculturing of *L. mesenteroides* NRRL B-512F.

Loopful of culture from stab or slant was transferred to 5 ml of enzyme production medium (EPM) as described by Tsuchiya et al. (10) for inoculum build up and incubated at 23°C. In this study, 24-h-old inoculum was used and the ratio of inoculum to medium was 1 : 20. Fermentation was carried out in triplicate sets by transferring in 100 ml of EPM in static flasks incubated at 23°C. The samples (5 ml) were withdrawn at regular intervals of 6 h, and centrifuged at 10,000 rpm for 10 min at 4°C to separate the cells. Supernatant was analyzed for enzyme activity.

Cell growth was determined by diluting broth samples 10 times with 0.9% (w/v) NaCl and by measuring the turbidity in terms of optical density (OD) at 590 nm against a sterile blank of the initial fermentation medium. OD was measured

Table 1. Composition (g/l) of different media used for maintaining *Leuconostoc mesenteroides* NRRL B-512F.

	Media ^a					
	A	B	C	D	E	F
Sucrose	140	100	20	20	—	20
Glucose	10 ^c	—	—	—	20	—
Yeast extract	—	—	5	20	5	5
Beef extract	—	0.5	—	—	10	10
Peptone	10	0.01	—	—	10	10
Tryptone	—	—	2.5	—	—	—
K ₂ HPO ₄	—	1	20	20	2	2
Sodium acetate	—	—	—	—	5	5
Triammonium citrate	—	—	—	—	2	2
Tween 80	—	—	—	—	1	1
NH ₄ NaHPO ₄	—	3	—	—	—	—
MgSO ₄ ·7H ₂ O	—	0.1	—	0.2	0.2	0.2
MnSO ₄ ·4H ₂ O	—	—	—	0.01	0.2	0.2
FeSO ₄ ·7H ₂ O	—	0.01	—	0.01	—	—
CaCl ₂ ·2H ₂ O	—	—	—	0.01	—	—
<i>p</i> -Aminobenzoic acid	—	0.05	—	—	—	—
KCl	—	0.1	—	—	—	—
NaCl	1	—	—	0.01	—	—
Sugar cane juice ^b	11	—	—	—	—	—
Agar	20	20	20	15	15	15
pH	7.0	7.6	7.4	6.9	6.4	6.4

^a A, Bhatnagar et al. (1); B, Stacey (8); C, Jeanes (5); D, Tsuchiya et al. (10); E, DeMan et al. (2); F, modified *Lactobacillus* MRS.

^b Fresh sugar cane juice was filtered and centrifuged at 4,000 rpm for 15 min to remove bagasse and other suspended impurities. The pH was adjusted to 7.0 by 10N KOH. It was then boiled for 5 min, cooled and centrifuged to remove precipitated proteins and pH was again brought to 7.0. The clear supernatant was diluted with distilled water to 15% total solids (Brix 15), checked for neutrality and sterilized.

^c Invert sugar.

using a UV-Vis spectrophotometer Gilford 260 (OH, U.S.A.). Cell dry weight was determined by filtering broth samples through pre-dried 0.2- μ m filters, drying to constant weight, comparing with blanks of sterile media and plotting the results against turbidity.

Assay of dextranucrase was carried out at pH 5.2 in 0.2 M acetate buffer. Activity was measured in terms of rate of production of reducing sugar. The reaction mixture (3 ml) contained 10% of sucrose and 0.5 ml of enzyme solution. The reaction was carried out for 1 h at 30°C. An aliquot (0.1–0.2 ml) was added to 3 ml of DNS reagent and the amount of reducing sugar was determined as described by Sumner and Sisler (9). One unit (U) of dextranucrase activity is defined as the amount of enzyme that liberates 1 μ mol of reducing sugar in 1 min, at pH 5.2 and 30°C. Specific activity is defined as units of dextranucrase per milligram of protein. Amount of protein was determined by the method as described by Lowry et al. (7) using bovine serum albumin as a standard.

L. mesenteroides NRRL B-512F maintained in different media was grown in EPM under identical culture conditions. Culture maintained in modified MRS medium (F in Table 1) gave maximum activity of 5.2 U/ml (Fig. 1). The activity obtained was higher than those reported earlier for dextranucrase, which ranged between 0.77 and 4.1 U/ml using different maintenance media (1, 3, 6, 10). In media containing high sucrose content (A and B in Table 1), the activity was only 3.8 and 4.2 U/ml, respectively. The reduced enzyme activity in the cultures maintained in

Fig. 1. Time course of extracellular dextranucrase production.

Extracellular enzyme activity profile with time (h) when *Leuconostoc mesenteroides* NRRL B-512F maintained in media (refer Table 1). F, (○); E, (△); D, (□); C, (●); B, (▲); A, (■).

sucrose-rich media, might be due to there being fewer viable cells due to either low pH or reduced biological activity of the organism caused by the coating of the cells with dextran. Culture in this state, when transferred to the EPM, led to lower cell growth and enzyme production. This made the sucrose rich media, unsuitable for long-term maintenance of *L. mesenteroides*. Medium C (Table 1) described by Jeanes (5) did not give higher enzyme production, possibly due to the lack of certain micronutrients, such as salts. The organism could be conveniently maintained in low sucrose media (D and F in Table 1) or even glucose-containing medium E (Table 1) as these cultures when transferred to EPM, produced appreciably higher enzyme activity (Fig. 1). Table 2 shows the specific activity obtained during enzyme production. Maintaining the trend modified MRS medium (F in Table 1) gave maximum specific activity of 1.2 U/mg protein in the culture supernatant. Low-sucrose media induced higher enzyme activities than the high-sucrose media.

In a separate experiment, enzyme activity was monitored, when the organism maintained in modified MRS (F in Table 1), Stacey's (8), a sucrose-rich medium (B in Table 1), and in an intermediate transfer from pure F to B medium was grown in EPM. Table 3 shows that the extracellular enzyme activity and specific activity decreased in the case when the organism was transferred from maintenance medium F to B. This supported the contention that *L. mesenteroides* NRRL B-512F retains the capacity of producing enzyme with higher activity when maintained in modified MRS medium as compared to other media especially the sucrose rich media. It was repeatedly found that transferring the culture from modified

Table 2. Specific activity of extracellular dextranucrase during production from different maintenance media.

Medium ^a	Cell concentration (g/ml)	Duration (h)	Specific activity (U/mg protein)
A	7.2	36	0.55
B	7.8	36	0.60
C	8.1	34	0.70
D	8.9	32	0.85
E	9.6	32	0.95
F	9.8	30	1.20

^a Refer Table 1.

Table 3. Effect of intermediate transfer of *Leuconostoc mesenteroides* NRRL B-512F from low-sucrose to high-sucrose medium on the activity of extracellular dextranucrase.

Medium ^a	Cell concentration (g/ml)	Duration (h)	Enzyme activity (U/ml)	Specific activity (U/mg protein)
B	7.8	36	3.9	0.60
F	9.8	30	5.8	1.20
F→B	9.0	32	4.5	0.85

^a Refer Table 1.

MRS medium to other media produced enzyme with lower activity and that reverse transfers to modified MRS medium resulted in an increase in enzyme activity. On comparing MRS and modified MRS media for prolonged maintenance of the culture, it was found that modified MRS medium gave higher dextran sucrose production than the MRS medium.

Modified *Lactobacillus* MRS proved to be better maintenance medium for *L. mesenteroides* NRRL B-512F which gave increased enzyme production when transferred to EPM. The studies have established that it is imperative to identify ideal maintenance condition and growth requirements of an organism for maximum exploitation of its attributes. The dextran sucrose activity of *L. mesenteroides* NRRL B-512F was, to a large extent, governed by the medium in which the culture was maintained. This fact is important in the process optimization for large-scale production of dextran sucrose and the product dextran.

We are thankful to Dr. Leela Iyengar and Dr. K. A. Prabhu for helpful discussions.

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