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Levan production using mutant strains of *Zymomonas mobilis* in different culture conditions

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Abstract

Several levan hyperproducing mutants of *Zymomonas mobilis* strains were selected by mutagenesis with *N*-methyl-*N*-nitro-nitrosoguanidine and caffeine. Highest levan production (41 g l^{-1}) was obtained with a mutant strain HL 29 in a culture medium containing 200 g sucrose l^{-1} and 0.5 g $(NH_4)_2SO_4$ l^{-1} stored at 7 °C for 29 days. This is the first report describing the levan synthesis by *Z. mobilis* at 7 °C.

Introduction

Zymomonas mobilis is an anaerobic, Gram-negative bacterium that produces almost theoretical yields of ethanol from glucose and is thus a promising alternative to yeast for the industrial production of ethanol (Swings & De Ley 1977, Rogers *et al.* 1982). It uses only glucose, fructose and sucrose as carbon sources. It is however capable of producing other metabolites in high concentrations (Johns *et al.* 1991) and also could be used for heterologous protein production (Conway *et al.* 1987).

We have isolated several strains of *Z. mobilis* subsp. *mobilis* able to produce levan at high concentrations. Moreover, some of these strains were flocculent under batch and continuous culture conditions (Rodríguez & Callieri 1986).

Z. mobilis produces levan from sucrose but not from glucose or fructose (Ribbons *et al.* 1962). Levan is a 2,6-linked fructose polymer with a molecular weight of about 10^7 Da, corresponding to approximately 60 000 fructose units (Swings & De Ley 1977). Levan production bears a considerable economical interest, and the major applications correspond to the pharmaceutical and food industries (Doelle *et al.* 1993). Calazans *et al.* (1997) reported anti tumor activities of levan produced by different strains of *Z. mobilis*.

In this work we describe the isolation of a levan hyperproducer *Z. mobilis* mutant as well as the main parameters that affect the production of levan in batch cultures.

Materials and methods

Microorganisms

Zymomonas mobilis subsp. *mobilis* ATCC 10988; FloB3 and 10J14 from the PROIMI collection; and mutants obtained from them. They were grown on complex medium (in g l^{-1}): sucrose, either 200 or 300; yeast extract, 10; $(NH_4)_2SO_4$, 1; $KHPO_4$, 1 and $MgSO_4 \cdot 7H_2O$, 1.
Peptone Complex medium (PCM): as complex medium plus peptone, 5.
Minimal medium (MM 1), in g l^{-1}: sucrose, either 200 or 300; $(NH_4)_2SO_4$,1; $MgSO_4 \cdot 7\ H_2O$, 1; $KHPO_4$, 1; biotin and pantothenic acid, 0.01.
Minimal medium (MM 0.5): as MM with 0.5 g $(NH_4)_2SO_4$ l^{-1}.
Minimal medium (MM): yeast extract, 0, 0.25 or 0.5

and without $(NH_4)_2SO_4$.
The media were solidified by the addition of 2% (w/v) of agar.

Inoculum

Twenty ml of complex medium with glucose, 50 g l^{-1}, as carbon source was inoculated from a stock culture and incubated without agitation 24 h at 30 °C. Inoculum volume employed was the 3.3% (v/v) of total volume.

Mutagenesis

Mutagenic treatment was described by Estévez *et al.* (1997). *N*-Methyl-*N*-nitro-nitrosoguanidine (NTG) used for mutagenic treatment reduced the population viability by 99.2%. Caffeine was used in the concentration range which had no effect on the viability of the cells. Levan hyperproducing mutants were obtained by treatment of the parental strain with NTG (0.8 mg ml^{-1}), and caffeine (5 mg ml^{-1}) added to the liquid complex medium during the early growth phase of a culture at 30 °C. After incubation at 30 °C for 2 h, the cells were washed twice with saline/phosphate buffer (100 mM, pH 7.2), and plated directly onto complex medium solid with 1 mg caffeine ml^{-1}. After 48 h of incubation at 30 °C, colonies that showed higher levan production than the parental strain were selected. Levan production of those mutants was estimated from the refringent mucilage present around the colonies.

Analytical determinations

Levan of high molecular weight was obtained according to a modification of the method described by Viikari & Gisler (1986). Levan was measured according to the Roe & Papadopoulos method (1954) for fructose determination, and expressed as levan (g l^{-1}). For biomass determination, the turbidity was measured at 540 nm and the dry weight was calculated using a calibration curve.

Results and discussion

Mutant selection

From 150 stable mutants, three levan hyperproducing strains were selected and analysed: namely HL 9, HL 11 and HL 29.

Table 1. Levan production using different *Z. mobilis* strains in sucrose based media (200 g l^{-1}) after 8 days of incubation at 30 °C. Media MM: minimal medium, MM 0.5: minimal medium with 0.5 g $(NH_4)_2SO_4$ l^{-1}, MM 1: minimal medium with 1 g $(NH_4)_2SO_4$ l^{-1}, CM: complex medium, PCM: peptone complex medium.

Strains	Levan production (g l^{-1})				
	MM	MM 0.5	MM 1	CM	PCM
ATCC 10988	4.78	3.12	6.12	4.10	1.06
PROIMI Flo B3	2.30	0.72	3.15	1.66	1.29
PROIMI 10J14	2.83	1.61	5.89	2.17	1.10
Mutant HL 11	6.70	5.20	5.95	6.10	3.07
Mutant HL 9	6.35	7.33	5.47	4.93	3.85

Table 2. Levan production using different *Z. mobilis* strains in sucrose-based media (300 g l^{-1}) after 8 days of incubation at 30 °C. All media idem legend Table 1.

Strains	Levan production (g l^{-1})				
	MM	MM 0.5	MM 1	CM	PCM
ATCC 10988	1.09	2.50	4.50	6.07	0.90
PROIMI Flo B3	1.99	0.80	1.60	1.51	1.12
PROIMI 10J14	5.52	2.23	4.83	3.31	1.11
Mutant HL 11	7.14	5.54	6.75	8.10	4.73
Mutant HL 9	6.41	7.69	6.08	8.37	6.28
Mutant HL 29	7.61	6.27	9.60	6.03	3.21

Influence of culture medium

Mutants and parental strains were grown in complex and minimal media with different sucrose and $(NH_4)_2SO_4$ concentrations. The effect of different $(NH_4)_2SO_4$ concentrations were tested because this salt had shown a negative influence on the biosynthesis of other biopolymers (Sutherland 1982) such as the escleroglucan production by *Sclerotium rolfsii* (Fariña *et al.* 1998). *Z. mobilis* strains ATCC 10988, PROIMI Flo B3 and PROIMI 10J14 were better producers in MM 1 [1 g $(NH_4)_2SO_4$ l^{-1}], while mutants HL 9 and HL 11 presented higher production in MM without the addition of the ammonium salt. Mutant HL 29 showed the best results in MM 0.5 [0.5 g $(NH_4)_2SO_4$ l^{-1}] and 200 g sucrose l^{-1} (Table 1). In culture media with 300 g sucrose l^{-1}, the optimum medium was different for each strain (Table 2).

In media containing 300 g sucrose l^{-1}, had not shown an increase in levan biosynthesis, compared with the media containing 200 g l^{-1}. The strain ATCC