

GENERAL CHARACTERISTICS OF TWO XYLANOLYTIC BACTERIAL STRAINS ISOLATED FROM BULGARIAN HOT SPRINGS

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Summary

The newly isolated Bacillus stearothermophilus like strains (SP and BC) possessing xylanase activity, were characterised for their morphological cultural and biochemical properties. Both strains were isolated from Bulgarian hot springs and showed thermostable alkali-tolerant extracellular xylanase production. During submerged batch cultivation they produced xylanase for relatively short period (12 - 14 h) and maximal activity (0.3 - 0.4 U/ml) was achieved after exhaustion of the carbon source in the nutrient medium. This indicated that exoxylanase biosynthesis was not growth associated and was subjected to catabolite repression.

Introduction

Application of xylanases for pre-treatment of paper pulp to remove xylan decreasing consumption of chlorine chemicals requires a thermostable xylanase, active in alkaline pH. Thermo-stable xylanases are isolated from different thermophilic bacteria but most of them are not alkali-resistant [1, 6, 12]. On the other hand bacterial xylanases, active at alkaline pH, are not stable at high temperatures [7, 9, 10, 15]. In our previous work we have isolated two alkali-tolerant thermophilic strains (SP and BC)

with xylanolytic activity by continuous cultivation from samples collected near Bulgarian hot springs. It is established that the xylanases have been thermostable at 70°C for 30 min and resistant to pH 5.5 - 8.0 (strain SP) and pH 6.0 - 7.5 (strain BC) [2]. The present investigation is undertaken with a view to characterise and identify the strains, as well as the xylanase production in shaken flask cultivation.

Materials and Methods

Methods described by Gerhard et al., Gordon et al., and Sneath [3, 4, 13] were

used for morphological, cultural and biochemical characteristics of the strains.

For xylanase production, the strains were cultivated in medium containing (g/l) birch wood xylan (Fluka) - 2.0; yeast extract (Difco) - 1.0; bacto peptone - 2.0; pH 8.0 - 8.5. The cultivation was carried out in 500 ml flasks containing 200 ml medium, at 60°C, using a platform shaker (New Brunswick) at 240 rpm.

Xylanase activity was assayed by mixing 0.05 ml culture supernatant with 0.05 ml of 1.0% birch wood xylan in phosphate buffer (pH 7.0), and was incubated for 5 min at 70°C. The reducing sugars were determined by dinitrosalicylic acid method, using

D-xylose as a standard. The supernatant, mixed with the substrate solution without incubation was used as a control. Samples were measured at 540 nm. One unit (U) of xylanase activity was defined as the amount of enzyme that produced 1 µmol xylose for 1 min, at pH 7 and temperature 70°C.

The cell concentration was expressed as the optical density (OD) at 660 nm. The cell dry weight of the cultures was estimated from OD using calibration accounting that one unit of OD was approximately 1.0 mg dry cell wt/ml for strain SP, and 0.850 mg dry cell wt/ml for strain BC.

Results and Discussion

The results for the morphological, cultural and biochemical properties of the isolated strains SP and BC are summarised in Table 1 and Table 2. They showed that the strains were closely related to each other. Comparing the characteristics of both of the strains with the description of different thermophilic *Bacillus* species [14], as well as the description of heterogeneous species *Bacillus stearothermophilus* [4, 13], the isolated strains were assigned to belong to the last one. The strains SP and BC showed some differences from the species *B. stearothermophilus* concerning growth at higher pH value, negative reaction for indol, utilisation of raffinose, arabinose and manitol, lack of gelatine hydrolysis.

In Fig. 1. the growth and xylanase production of strain SP and BC are shown. The xylanase activity of the culture supernatants appeared after the 4th hour and increased to the 12 - 14th hour of cultivation (0.3 - 0.4 U/ml), when the cultures were in the stationary phase of growth and the concentration of the reducing sugars was

very low (0.01-0.02 mg/ml). The decrease of the cell concentration of strain SP after the 10th hour was due to the cell lysis. The results indicated that the xylanase production by both of the strains was not growth associated and was carbon-source-repressed.

Among the numerous xylanase producers from the *Bacillus* species two strains of *B. stearothermophilus* were described: *B. stearothermophilus* 4125 [5, 6] and *B. stearothermophilus* T-6 [8]. The comparison between the conditions for the production and properties of the xylanases of strain SP and strain BC [2] and these for the above mentioned strains is shown in Table 3. Considering this comparison, the relatively high xylanase activity achieved for a short period of cultivation (12 - 14 h), the enzyme thermostability and pH stability, we can conclude that strains SP and BC are potentially perspective *B. stearothermophilus* producers of thermostable xylanases and they are of interest for the future studies for improvement on the cultivation conditions and respectively on the enzyme yield.

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Table 1. Morphological, cultural and biochemical properties of strains SP and BC.

Property		Strain SP	Strain BC
Morphology of the colonies on nutrient agar, 20 h at 60°C	size	1.0 – 1.5 mm	3.0 – 4.0 mm
	form	circular	circular
	margin	undulate	undulate
	elevation	convex	raised
	colour	pale	cream
Morphology of the cells in nutrient broth, 20 h at 60°C	size	Gram positive rods (3.1 - 5.4)x(0.2 - 0.9) µm	Gram positive rods (2.6 - 4.5)x(0.5 - 0.8) µm
	motility	+	+
	spores	ellipsoidal terminal	ellipsoidal terminal
Temperature of growth (°C)	minimum	40	35
	optimum	60	60
	maximum	65	65
pH ranges of growth		6.0 – 9.0	6.5 – 9.5
Growth in NaCl (%)	2	+	+
	3	+	+
	4	+	–
	5	–	–
Nutrient broth		turbid	turbid with sediment
Anaerobic growth		–	–
Azide sensitivity (0.02%)		–	–
Formation of	H ₂ S	–	–
	indol	+	+
Hydrolysis of	gelatine	–	–
	casein	+	+
	starch	+	+
Nitrate reduction		–	+
Use of citrate		–	–
Production of	urease	–	–
	catalase	–	–
	cellulase	–	–
Voges-Proskauer reaction (V-P)		–	–
pH in V-P broth		5.7	5.9
Action on lacmus milk		intensive proteolysis intensive acidification	proteolysis acidification

Table 2. Utilisation of different carbohydrates^a.

Carbohydrate	Strain SP			Strain BC		
	Growth	Acid	Gas	Growth	Acid	Gas
Arabinose	+	+	-	+	+	-
Rhamnose	-	-	-	-	-	-
Xylose	+	+	-	+	+	-
Glucose	+	+	-	+	+	-
Fructose	+	+	-	+	+	-
Galactose	+	+	-	+	+	-
Ribose	-	-	-	+	-	-
Lactose	+	+	-	-	-	-
Sucrose	+	+	-	+	+	-
Maltose	+	+	-	+	+	-
Cellubiose	+	+	-	+	+	-
Raffinose	-	-	-	-	-	-
Starch	+	+	-	+	+	-
Inulin	-	-	-	-	-	-
Dextran	+	+	-	+	+	-
Glycogen	+	+	-	+	+	-
Adonitol	-	-	-	-	-	-
Mannitol	+	+	-	+	+	-
Sorbitol	-	-	-	+	+	-
Dulcitol	-	-	-	-	-	-
Inositol	-	-	-	-	-	-
Salicin	+	+	-	+	-	-
Control, without "C"	-	-	-	-	-	-

^a The medium used consisted (g/l):

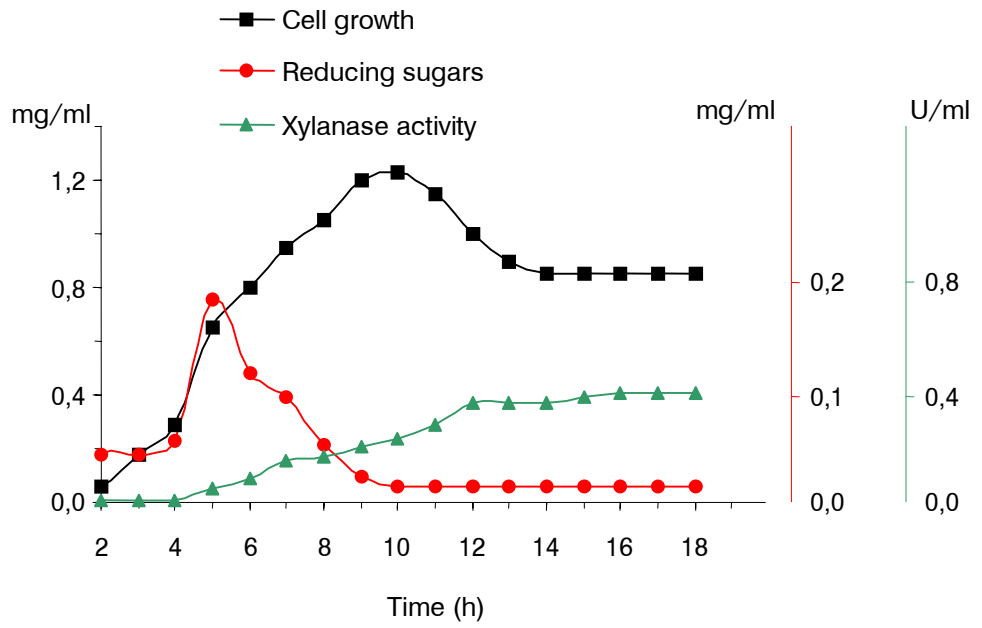
(NH₄)₂HPO₄ - 1,

KCl - 0.2,

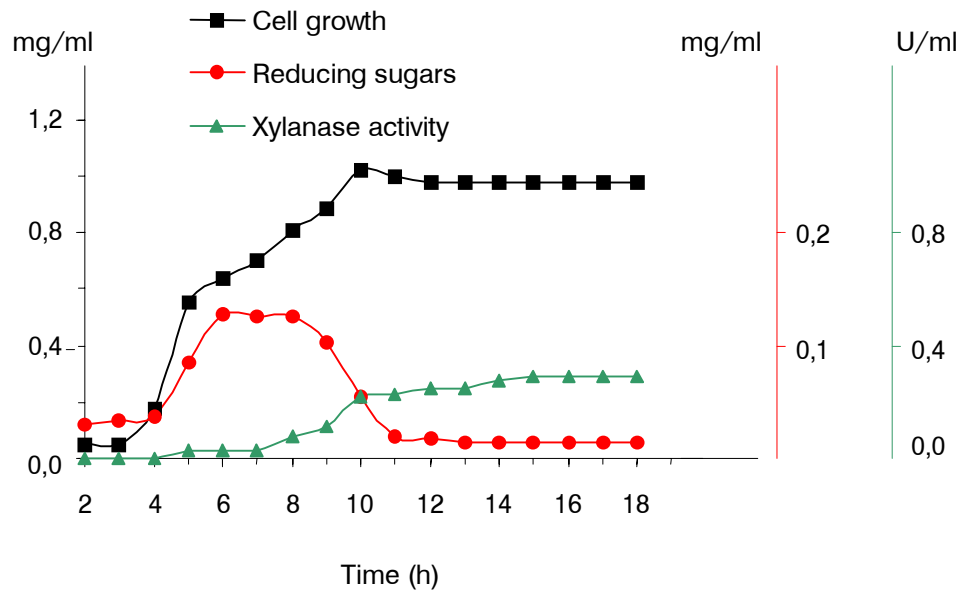
MgSO₄·7H₂O - 0.2,

thiamine - 0.001, pH - 8.4.

Cultivation at 60°C for several days.



A



B

Fig.1 Cell growth, xylanase activity and reducing sugars.

A. *Bacillus sp. SP*

B. *Bacillus sp. BC*

Table 3. Xylanase production by *B. stearothermophilus* strains.

Xylanase production	<i>B. stearothermophilus</i> 4125	<i>B. stearothermophilus</i> T6	Strain SP	Strain BC
Culture conditions				
Media with	larch wood xylan	xylose	birch wood xylan	birch wood xylan
pH	7.0	n.d.	8.0	8.0
T (°C)	65	60	60	60
Xylanase properties				
Maximum activity (U/ml)	0.1	2.0	0.5	0.5
T opt. (°C)	78	75	70	70-75
pH opt.	6.5-7.0	6.5-7.0	7.0	7.0
Thermostability	100%, 112 h, 68°C	100%, 10 h, 65°C	100%, 200 h, 30°C ³ , 100%, 40 min, 70°C	100%, 48 h, 60°C ³ , 100%, 40 min, 70°C
pH stability	n.d.	n.d.	100%, 30 min, 60°C, pH 5.5-9.6	100%, 30 min, 60°C, pH 6.0-7.5
MW (kDa)	n.d. ¹	43 ²	92 ¹	92 ¹
Ref.	5, 6	8	2	2

¹ - crude enzyme, ² - purified enzyme, ³ - unpublished data, n.d. - no data.

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