Exopolysaccharides from *Bacillus licheniformis*: Production, partial characterization and emulsifying activity

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Microbial exopolysaccharides (EPSs) are soluble or insoluble polymers secreted by microorganisms. The use of such molecules is widespread due to their unique or superior physical properties relative to traditional plant polysaccharides. In this category are xantan, pullulan, fructan, dextran, etc. Fructans are a diverse group of polysaccharides that contain one or more β-linked fructose units. In the most prominent structural types, inulin and levan, polysaccharide chain originates from the fructose part of a sucrose molecule, proceeding *via* β-(2,1) and β-(2,6) linkages, respectively. *Bacillus*, *Aerobacter*, *Streptococcus*, *Pseudomonas*, *Corynebacterium* are just some of EPS producing genera.

The aim of the present study was to compare growth and EPS production of Bacillus licheniformis in two different media, and to partially characterize isolated polysaccharides.

Strain of *Bacillus licheniformis* NS032 used in this experiment was isolated from petroleum sludge sample taken from Oil Refinery, Novi Sad.[1] The identification of isolated strains was achieved by API tests (Tables 1 and 2, Figure 1), fatty acid methyl ester (FAME) composition (Table 3) and by sequence analysis of 16S rRNA genes (Figure 2). The strain grows on the majority of the selected hydrocarbons as the sole source of carbon, and show tolerance to the heavy metals: Ni^{2+} (5 mmol l^{-1}), Cu^{2+} (2.5 mmol l^{-1}), Cr^{2+} (2.5 mmol l^{-1}) and Cd^{2+} (1.25 mmol l^{-1}), which indicates a broad capacity for the degradation and ability to survive.

For EPS production, fermentations were conducted in nutrient (NB) and sucrose broths (SB).[2] EPSs were isolated from media by centrifugation on 10000 rpm and precipitation with three volumes of ethanol. Monosaccharide composition was determined by paper chromatography, after total hydrolysis using 2 M trifluoroacetic acid. Emulsifying activity was determined as E_{24}.[3]

Table 1: Growth of *Bacillus licheniformis* NS032 on different carbon sources (API 50CH)

Tube	Test	Growth	Tube	Test	Growth	Tube	Test	Growth	Tube	Test	Growth	Tube	Test	Growth
0	0		10	GAL	yes	20	MDM	no	30	MEL	yes	40	TUR	yes
1	GLY	yes	11	GLU	yes	21	MDG	yes	31	SAC	yes	41	LYX	no
2	ERY	no	12	FRU	yes	22	NAG	yes	32	TRE	yes	42	TAG	yes
3	DARA	no	13	MNE	yes	23	AMY	yes	33	INU	yes	43	DFUC	no
4	LARA	yes	14	SBE	no	24	ARB	yes	34	MLZ	no	44	LFUC	no
5	RIB	yes	15	RHA	yes	25	ESC	yes	35	RAF	yes	45	DARL	no
6	DXYL	yes	16	DUL	no	26	SAL	yes	36	AMD	yes	46	LARL	no
7	LXYL	no	17	INO	yes	27	CEL	yes	37	GLYG	yes	47	GNT	no
8	ADO	no	18	MAN	yes	28	MAL	yes	38	XLT	no	48	2KG	no
9	MDX	no	19	SOR	yes	29	LAC	no	39	GEN	yes	49	5KG	no

Figure 1: Photograph of API 50CH test

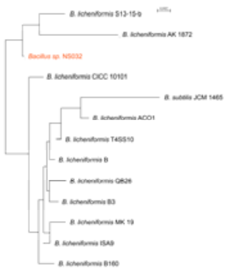

Figure 2: Phylogenetic tree obtained by the neighbor - joining method

Table 2: API 20E/50CHB test

Tube	Test	Reaction
50	ONPG	yes
51	ADH	yes
52	LDC	no
53	ODC	no
54	CIT	no
55	H2S	no
56	URE	no
57	TDA	no
58	IND	no
59	VP	yes
60	GEL	yes
61	NIT	yes

Table 3: Cellular fatty acid composition of isolated strain, % of total detected

Fatty acid[a]	NS032	Fatty acid[a]	NS032	Fatty acid[a]	NS032
*i*12:0	ND[b]	14:0	0.29	*i*17:0	15.32
12:0	ND	*i*15:0	29.37	*a*17:0	ND
12 2OH	ND	*a*15:0	33.63	17:0	ND
12 3OH	ND	15:0	ND	Σ18:1[c]	ND
*i*13:0	ND	*i*16:0	3.18	18:0	0.58
*a*13:0	ND	Σ16:1[c]	ND	18:0 10 methyl	ND
13:0	ND	16:0	9.49	*cy*19:0	ND
*i*14:0	ND	*cy*17:0	ND		

[a] Fatty acids are designated in terms of the total number of carbon atoms: number of double bonds. The prefixes *a* and *i* indicate *anteiso* and *iso* branching, *cy* refers to cyclopropane fatty acids, OH indicates the presence of hydroxyl group.
[b] Not determined, values < 0.20 % are ommited.
[c] Total sum of monounsaturated acids.

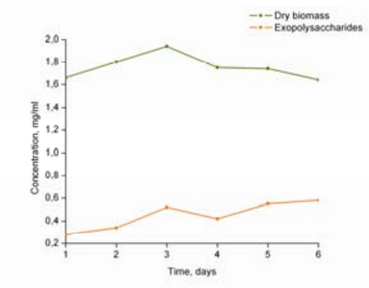

Figure 3: Growth and EPS production on nutrient medium

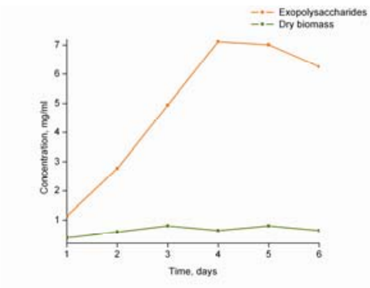

Figure 4: Growth and EPS production on sucrose medium

Table 4: Emulsifying activity

Sample	Upper layer height, mm	Inner layer height, mm	Lower layer height, mm	E_{24}, %
Water	11	-	12	-
1% Biosolve	9	6	13	27.27
1% EPS-NB	7	5	15	22.73
1% EPS-SB	9	3	11	15.00

Obtained data (Figures 3, 4) showed that Bacillus licheniformis strain grew much better on nutrient medium than on sucrose medium. Growth maximum was attained at the third day of fermentation. In sucrose broth, EPS production reached 7 g/L, and this level was two fold higher than that obtained in nutrient broth. Crude polysaccharide preparations were built from fructose and glucose (EPS obtained from sucrose medium) or galactose, glucose and fructose monosaccharide units (EPS from nutrient medium) and exhibited high emulsifying activity compared with chemical surfactant (Table 4). EPS from sucrose medium were purified by repeated dissolution and precipitation with iso-propanol and, based on the results of acid hydrolysis, it was shown that investigated polysaccharide was fructan.

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