Supplementary data for article:

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Supplementary material

Brachybacterium sp. CH-KOV3 isolated from an oil-polluted environment – a new producer of levan

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Content

Table S-1. Physiological-biochemical characterization of isolate CH-KOV3. + positive reaction; ++ strong positive reaction; - negative reaction; ND – not determinated.

API ZYM

Enzyme assayed for	**Control**	**Alkaline phosphatase**	**Esterase (C4)**	**Esterase Lipase (C 8)**	**Lipase (C 14)**	**Leucinearyl-amidase**	**Valinearyl-amidase**	**Cysteine arylamidase**	**Trypsin**	**α-chymotrypsin**
Substrate	/	2-naphthyl phosphate	2-naphthyl butyrate	2-naphthyl caprylate	2-naphthyl myristate	L-leucyl-2-naphthyl amide	L-valyl-2-naphthyl amide	L-cystyl-2-naphthyl amide	N-benzoyl-DL-arginine-2-naphthyl amide	N-glutaryl-phenylalanine-2-naphthyl amide
Result	-	-	-	+	+	++	++	-	-	-
Enzyme assayed for	**Acid phosphatase**	**Naphtol-AS-BI-phospho-hydrolase**	**α-galactosidase**	**β-galactosidase**	**β-glucuronidase**	**α-glucosidase**	**β-glucosidase**	**N-acetyl-β-glucosamini-dase**	**α-mannosidase**	**α-fucosidase**
Substrate	2-naphthyl phosphate	Naphthol-AS-BI-phosphate	6-Br-2-naphthyl-αD-galactopyranoside	2-naphthyl-βD-galacto-pyranoside	Naphthol-AS-BI-βD-glucuronide	2-naphthyl-αD-gluco-pyranoside	6-Br-2-naphthyl-βD-gluco-pyranoside	1-naphthyl-N-acetyl-βD-glucosaminide	6-Br-2-naphthyl-αD-manno-pyranoside	2-naphthyl-αL-fuco-pyranoside
Result	-	+	+	++	-	++	+	+	-	-

API 20 NE

Enzyme assayed for	**NO_3**	**TRP**	**GLU**	**ADH**	**URE**	**ESC**	**GEL**	**PNPG**	**GLU**	**ARA**
Substrate	Potassium nitrate	L-tryptophane	D-glucose	L-arginine	Urea	Esculin ferric citrate	Gelatin	4-nitrophenyl-βD-galacto-pyranoside	D-glucose	L-arabinose
Result	+/-	-	-	-	-	+	-	+	-	-
Enzyme assayed for	**MNE**	**MAN**	**NAG**	**MAL**	**GNT**	**CAP**	**ADI**	**MLT**	**CIT**	**PAC**
Substrate	D-mannose	D-mannitol	N-acetyl-glucosamine	D-maltose	Potassium gluconate	Capric acid	Adipic acid	Malic acid	Trisodium citrate	Phenylacetic acid
Result	-	-	-	-	-	-	-	-	-	-

API CORYNE

Enzyme assayed for	NIT	PYZ	PYRA	PAL	βGUR	βGAL	αGLU	βNAG	ESC	URE
Substrate	Potassium nitrate	Pyrazinecarboxamid	Pyroglutamic acid-β-naphthylamide	2-naphthyl phosphate	Naphthol ASBI-glucuronic acid	2-naphthyl-βD-galactopyranoside	2-naphthyl-αD-glucopyranoside	1-napthtyl N-acetyl-βD-glucosaminide	Esculin ferric citrate	Urea
Result	+	+	+	+	+	+	+	+	+	-
Enzyme assayed for	GEL	O	GLU	RIB	XYL	MAN	MAL	LAC	SAC	GLYG
Substrate	Gelatin	Negative control	D-glucose	D-ribose	D-xylose	Mannitol	D-maltose	D-lactose	D-sucrose	Glycogen
Result	-	-	-	-	-	-	-	-	-	-

API 20 E

Enzyme assayed for	ONPG	ADH	LDC	ODC	CIT	H_2S	URE	TDA	IND	VP
Substrate	2-nitrophenyl-ßd-galactopyranoside	L-arginine	L-lysine	L-ornithine	trisodium citrate	sodium thiosulfate	Urea	L-tryptophane	L-tryptophane	Sodium pyruvate
Result	+	-	-	-	-	-	-	-	-	+
Enzyme assayed for	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA
Substrate	Gelatin (bovine origin)	D-glucose	D-mannitol	Inositol	D-sorbitol	L-rhamnose	D-sucrose	D-melibiose	Amygdalin	L-arabinose
Result	-	-	-	-	-	-	-	-	-	-

Table S-1. API tests results. + - positive reaction; +W - positive reaction; ++ strong positive reaction; - - negative reaction; ND – not determinated.

NO_3 (20 NE) / NIT (CORYNE) – reduction of nitrates to nitrites; reduction of nitrates to nitrogen; TRP (20 NE) / IND (20 E) – Indole production; GLU – fermentation (glucose); ADH (API 20 E and NE) – arginine dihydrolase; URE (API 20 E, NE and CORYNE) – urease; ESC (API 20 NE and CORYNE) – hydrolysis (esculin); GEL (API 20 E, NE and CORYNE) - hydrolysis (gelatin); PNPG (API 20 NE), βGAL (CORYNE), ONPG (API 20 E) – β-galactosidase; GLU, ARA, MNE, MAN, NAG, MAL, GNT, CAP, ADI, MLT, CIT, PAC, RIB, XYL, LAC, SAC, GLYG, INO, SOR, RHA, MEL, AMY, ARA – assimilation (API 20 NE), fermentation (API CORYNE), fermentation / oxidation (API 20 E) (glucose, arabinose, mannose, mannitol, N-acetyl-glucosamine, maltose, potassium gluconate, capric acid, adipic acid, malate, trisodium citrate, phenylacetic acid, ribose, xylose, lactose, saccharose, glycogen, inositol, sorbitol, rhamnose, melibiose, amygdalin, arabinose); PYZ- pyrazinamidase; PYRA – Pyrrolidonyl arylamidase; PAL - Alkaline phosphatase; βGUR - β-glucuronidase; αGLU - α-glucosidase; βNAG - N-acetyl-β-glucosaminidase; LDC - Lysine decarboxylase; ODC - Ornithine decarboxylase; CIT - citrate utilization; H_2S - H2S production; TDA - Tryptophane deaminase; VP - acetoin production (Voges Proskauer).

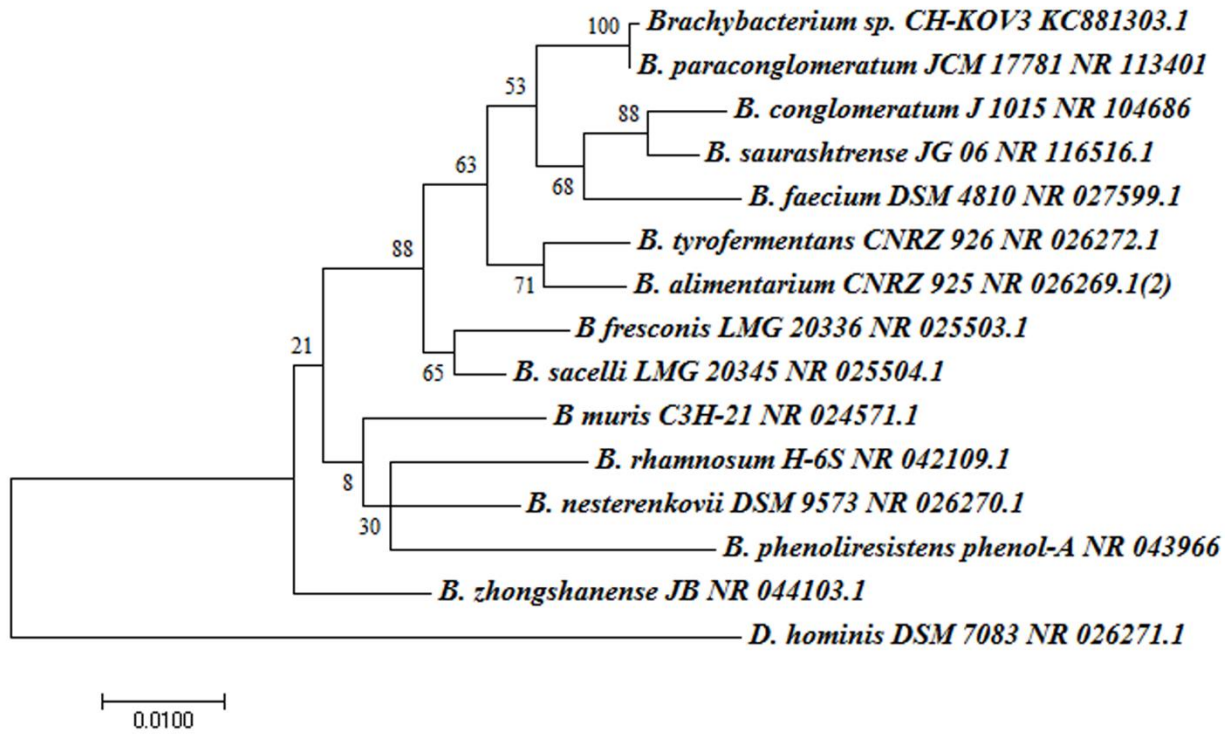

Figure S-1. Molecular Phylogenetic analysis of genus *Brachybacterium* by Maximum Likelihood method.

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model [1]. The tree with the highest log likelihood (-3134.1737) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 0.4900)). The rate variation model allowed for some sites to be evolutionarily invariable ([+*I*], 45.2915% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 15 nucleotide sequences. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment

gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 1338 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 [2].

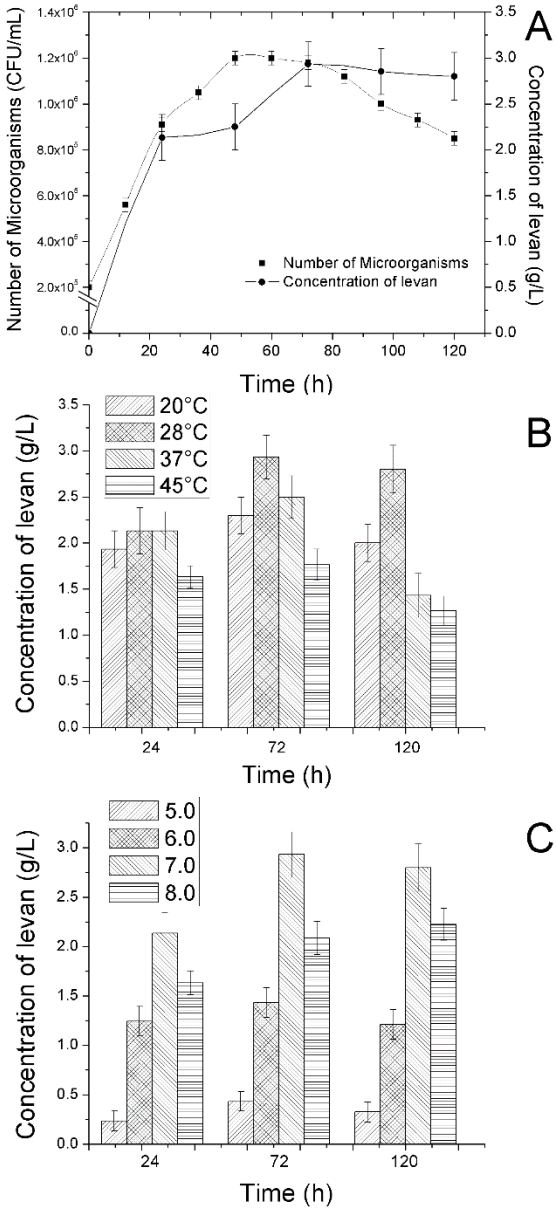

Figure S-2. (A) Growth curve of *Brachybacterium* sp. KOV-3 under optimal conditions, with 100 g/L of sucrose and concentration of levan produced; (B) Effect of temperature (20, 28, 37, and 45 ºC) on EPS production by *Brachybacterium* sp. CH-KOV3 at different time intervals (24, 72, 120 h), in BM with 100 g/L sucrose, pH 7.0, 5 days incubation and 200 rpm; (C) Effect of pH (5.0, 6.0, 7.0, and 8.0) on EPS production by *Brachybacterium* sp. CH-KOV3 at different time intervals (24, 72, 120 h), in BM with 100 g/L sucrose, 5 days at 28 ºC and 200 rpm.

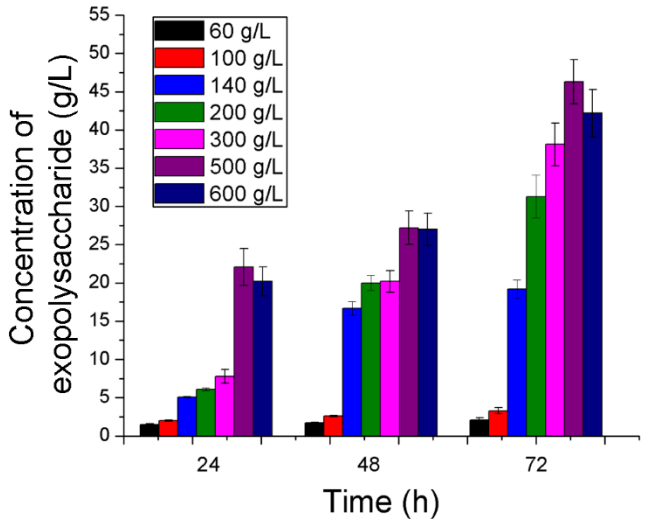

Figure S-3. Effect of sucrose concentration (60, 100, 140, 200, 300, 500, and 600 g/L) on EPS production by *Brachybacterium* sp. CH-KOV3 after 24, 48 and 72 hours. The highest yield of EPS was in media supplemented with 500 g/L of sucrose.

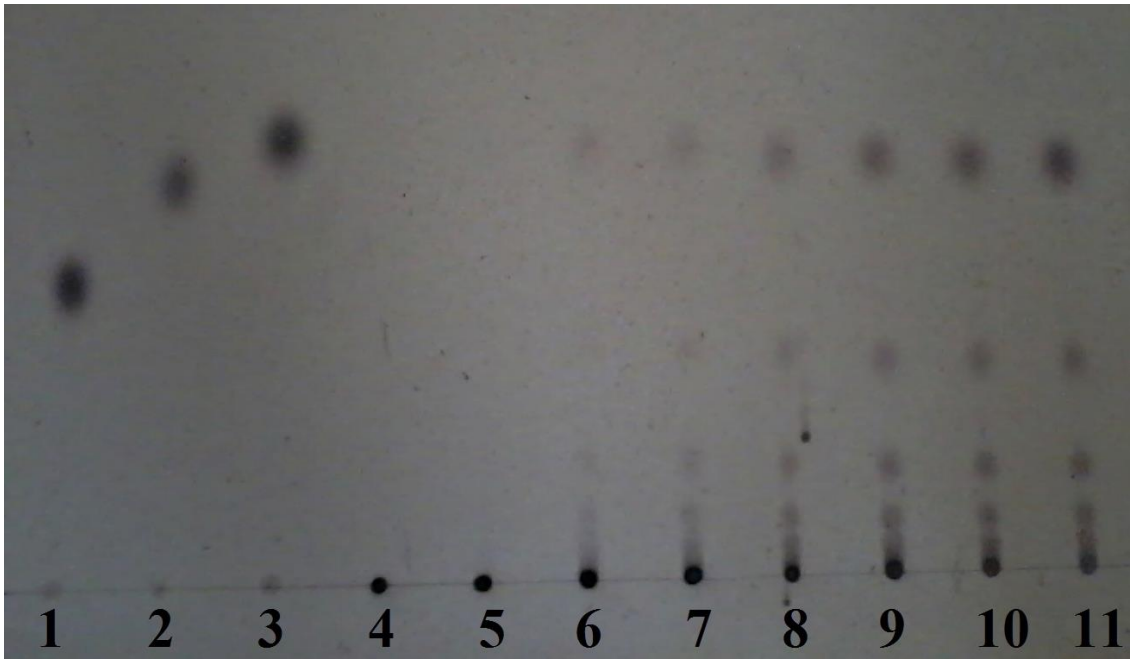

Figure S-4. Thin-layer chromatogram of partial hydrolyzate of purified EPS produced by *Brachybacterium* sp. CH-KOV3. Hydrolysis of EPS by oxalic acid performed at 80 ºC for 20 min. Products of hydrolysis were examined in the solvent system chloroform:acetic acid:water (6:7:1 V/V/V). Fructose, glucose and sucrose were used as standards. **1** – Sucrose; **2** – Glucose; **3** – Fructose; **4** – 2 min; **5** – 4 min; **6** – 6 min; **7** – 8 min; **8** – 10 min; **9** – 12 min; **10** – 14 min; **11** – 16 min.

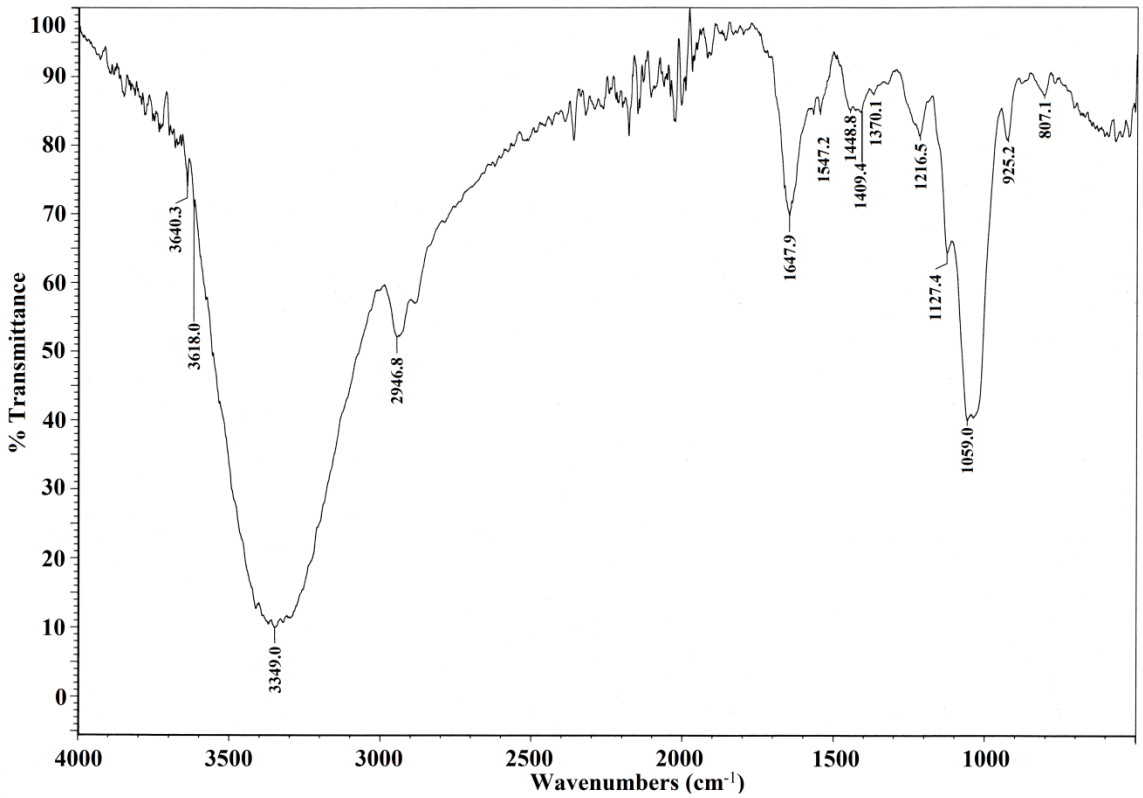

Figure S-5. The FT-IR spectrum of purified EPS.

References:

1. Tamura K, Nei M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution **10**:512-526.

2. Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution **33**:1870-1874.