

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.

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

**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 °C) 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 °C 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 °C 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.



## API CORYNE

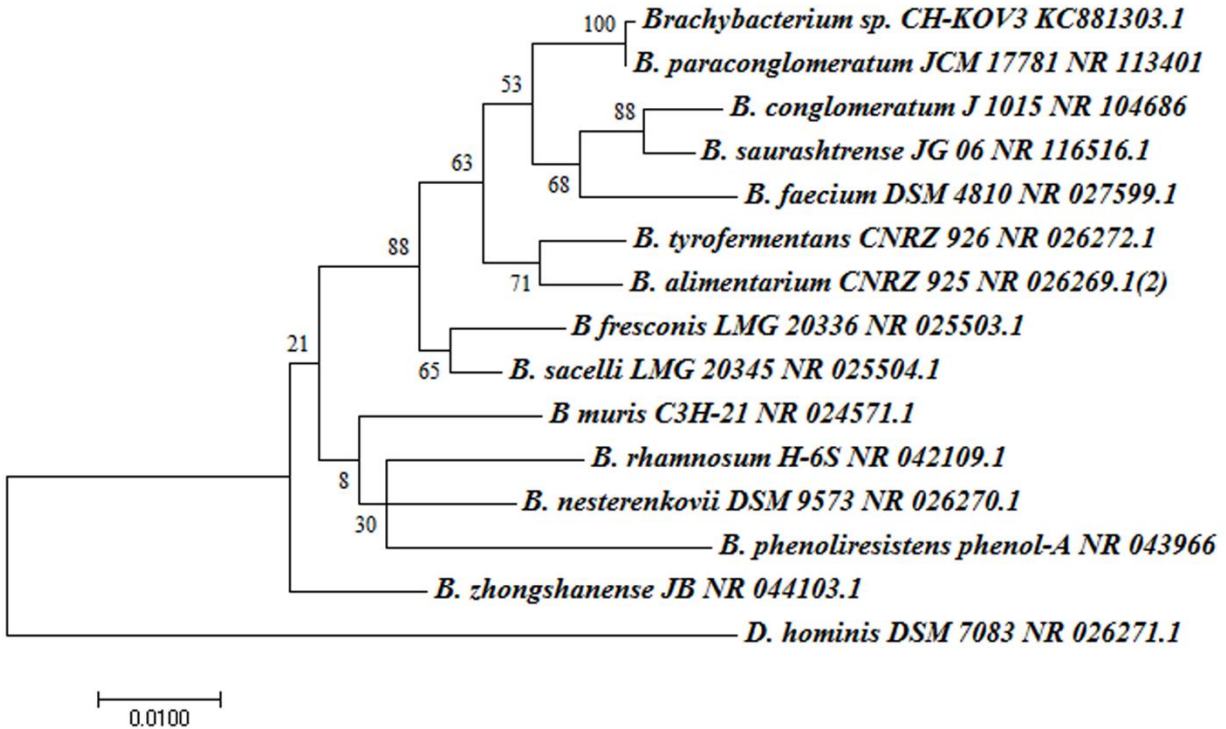
<b>Enzyme assayed for</b>	<b>NIT</b>	<b>PYZ</b>	<b>PYRA</b>	<b>PAL</b>	<b>βGUR</b>	<b>βGAL</b>	<b>αGLU</b>	<b>βNAG</b>	<b>ESC</b>	<b>URE</b>
<b>Substrate</b>	Potassium nitrate	Pyrazinecarboxamid	Pyroglutamic acid-β-naphthylamide	2-naphthyl phosphate	Naphthol ASBI-glucuronic acid	2-naphthyl-βD-galactopyranoside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

## API 20 E

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

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

$\text{NO}_3$  (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),  $\beta$ GAL (CORYNE), ONPG (API 20 E) –  $\beta$ -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;  $\beta$ GUR -  $\beta$ -glucuronidase;  $\alpha$ GLU -  $\alpha$ -glucosidase;  $\beta$ NAG - N-acetyl- $\beta$ -glucosaminidase; LDC - Lysine decarboxylase; ODC - Ornithine decarboxylase; CIT - citrate utilization;  $\text{H}_2\text{S}$  -  $\text{H}_2\text{S}$  production; TDA - Tryptophane deaminase; VP - acetoin production (Voges Proskauer).

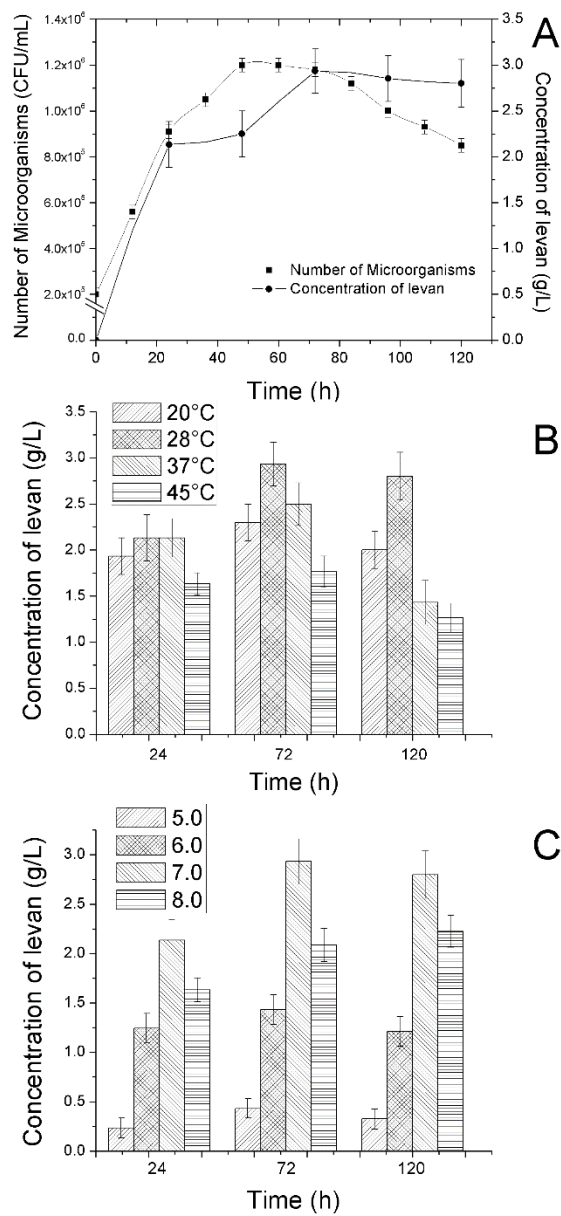


**Figure S-1.** Molecular Phylogenetic analysis of genus *Brachybacterium* by Maximum Likelihood method.

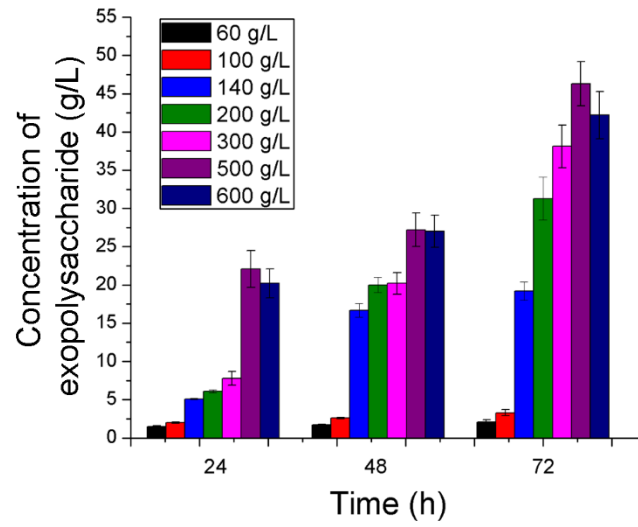
The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model [1]. The tree with the highest log likelihood (-3134.1737) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the 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].

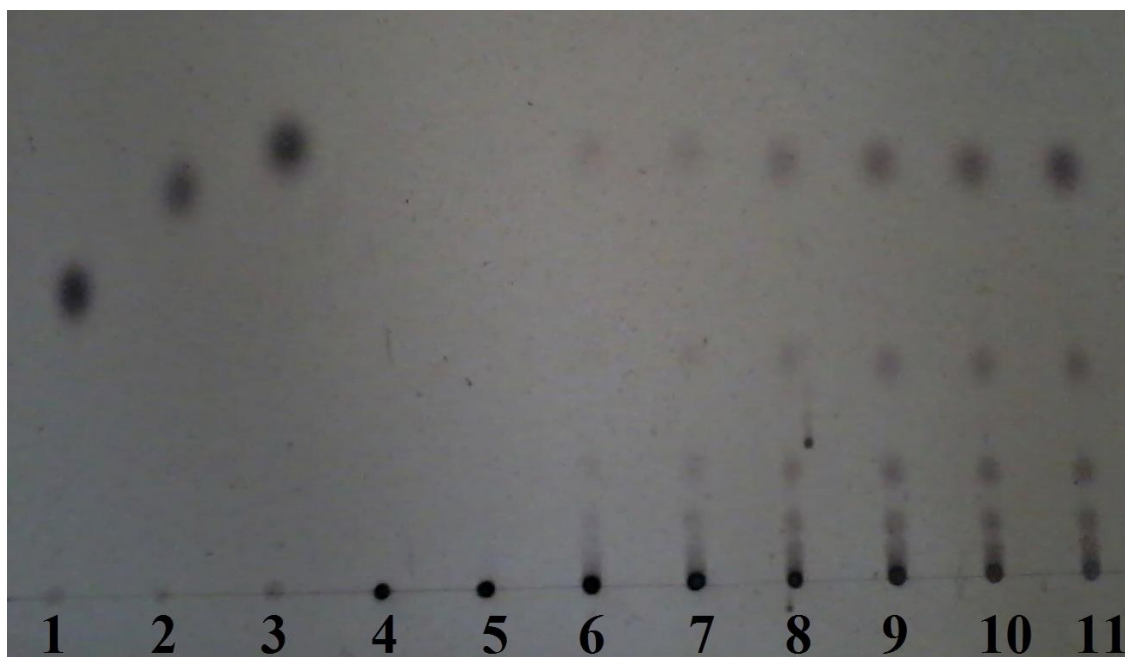




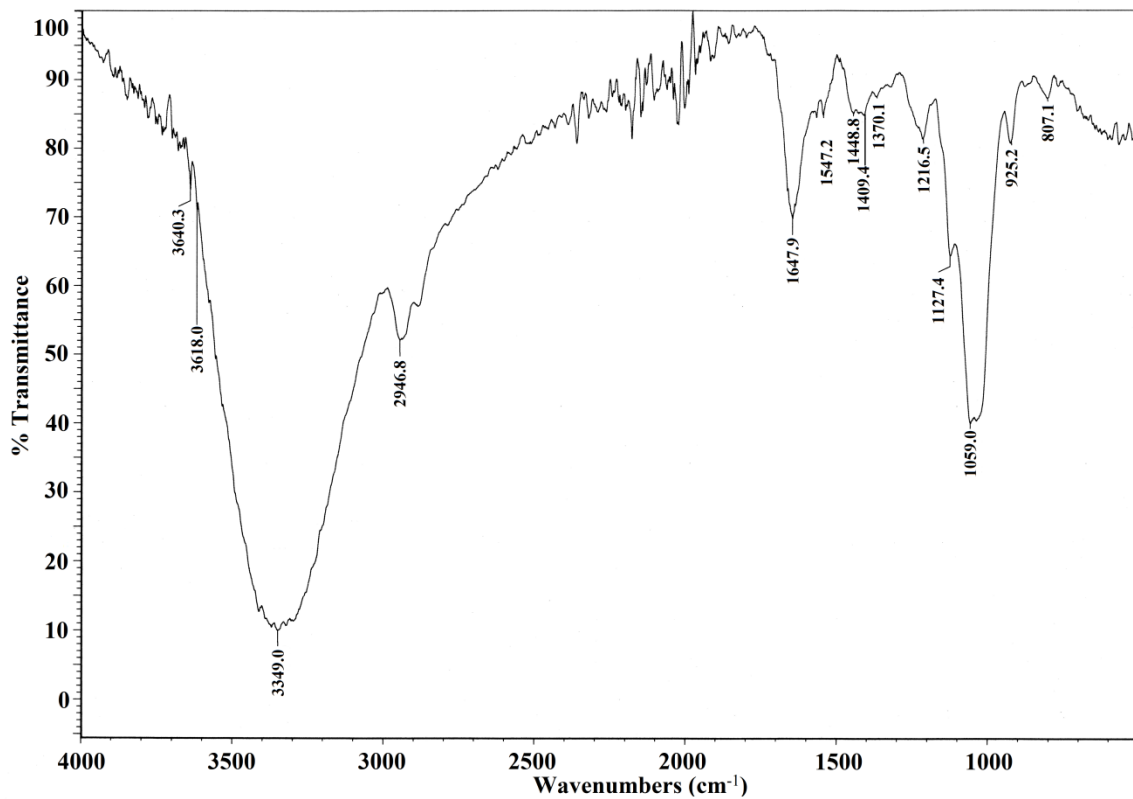
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References:

- 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.