

## Supplementary material

### **Biopolymers production by halotolerant bacteria isolated from Caatinga Biome**

Maria Paula Parada-Pinilla<sup>1</sup>, Maria Alejandra Ferreira<sup>1</sup>, Juan Camilo Roncallo<sup>1</sup>, Suikinai Nobre Santos<sup>2</sup>, Itamar Soares de Melo<sup>2</sup>, Alexia Nathália Brígido Assef<sup>3</sup>, Diego Veras Wilke<sup>3</sup>, Luiziana Ferreira da Silva<sup>1</sup>, Leandro Maza Garrido<sup>1</sup>, Wellington Luiz de Araújo<sup>4</sup> and Gabriel Padilla<sup>1</sup>

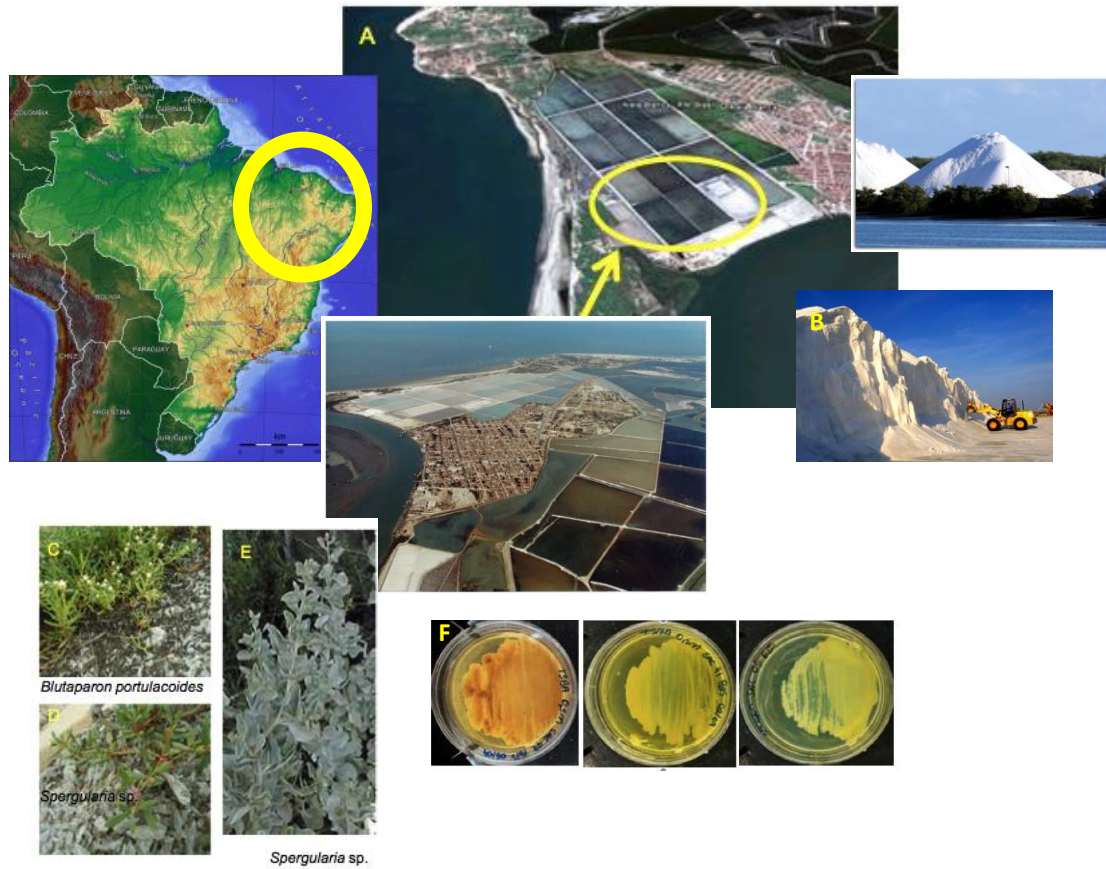
<sup>1</sup> Bioproducts Laboratory, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

<sup>2</sup> Environmental Microbiology Laboratory, Brazilian Agricultural Research Corporation (Embrapa), São Paulo, Brazil

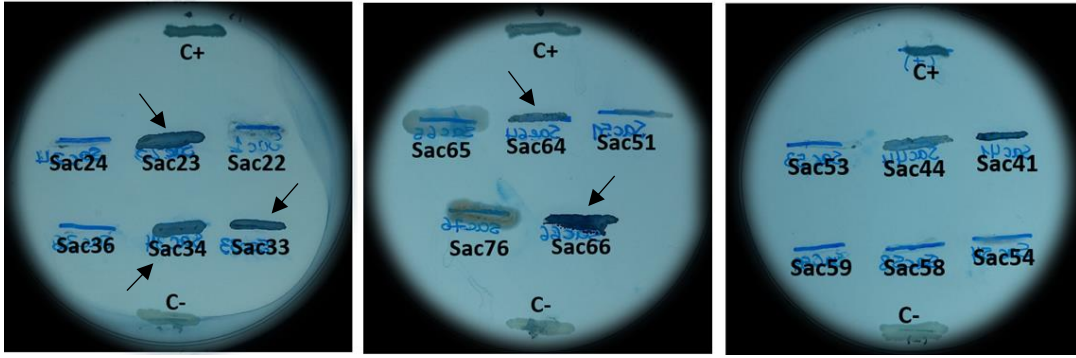
<sup>3</sup> Laboratory of Bioprospecting and Marine Biotechnology, Federal University of Ceará, Fortaleza, Brazil

<sup>4</sup> Molecular Biology and Microbial Ecology Laboratory, Institute of Biomedical Sciences, University of São Paulo, São Paulo, Brazil

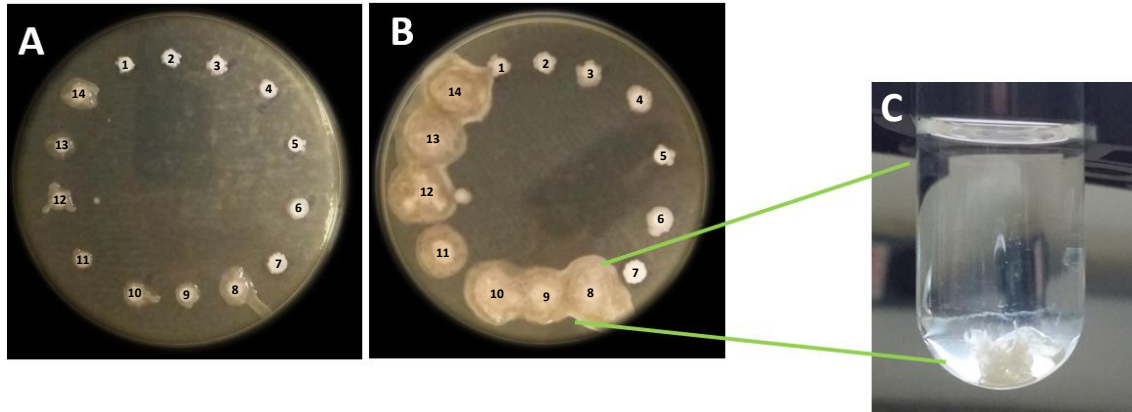
Corresponding author: Gabriel Padilla, [gpadilla@icb.usp.br](mailto:gpadilla@icb.usp.br)



**Figure S1.** Caatinga biome, Mossoró district (A), Rio Grande do Norte state, near Areia Branca saltworks (B), coordinates 4 57'22''S- 37 08'13''W. (C) Plant of native species *Blutaparon portulacoides*, popularly known as Succulent. (D-E) *Spergularia* sp., known as Pirristio. (F) Halotolerant isolates. Images courtesy of the Environmental Microbiology Laboratory (Embrapa)



**Figure 1 S2.** Screening of polyhydroxyalkanoates-producing bacteria by Sudan Black B staining method. Mineral salts solid media supplemented with 1% glucose. Incubation time 72 hours. Positive control (C+) *Pseudomonas* sp. LFM 046, negative control (C-) *Pseudomonas* sp. LFM 461. The arrows indicate selected isolates as potential PHA producers.



**Figure S3.** Screening of exopolysaccharides-producing bacteria. Translucent colonies, potentially producing EPS (A and B). Confirmation of EPS production by mixture of colonies in ethanol (C).



Bacillus filamentosus: BEH\_04720

Help

Entry	BEH_04720 CDS T03983
Definition	(GenBank) class III poly(R)-hydroxyalkanoic acid synthase subunit PhaC KO K03821 polyhydroxyalkanoate synthase subunit PhaC [EC:2.3.1.-]
Organism	beo Bacillus filamentosus
Pathway	beo00650 Butanoate metabolism beo01100 Metabolic pathways
Brite	KEGG Orthology (KO) [BR:beo00001] 09100 Metabolism 09101 Carbohydrate metabolism 00650 Butanoate metabolism BEH_04720 Enzymes [BR:beo01000] 2. Transferases 2.3 Acyltransferases 2.3.1 Transferring groups other than aminoacyl groups 2.3.1.- BEH_04720 <a href="#">BRITE hierarchy</a>
SSDB	<a href="#">Ortholog</a> <a href="#">Paralog</a> <a href="#">Gene cluster</a> <a href="#">GFIT</a>
Motif	Pfam: Abhydrolase_1 PhaC_N PHB_depo_C Hydrolase_4 DUF3141 DLH Abhydrolase_5 DUF2048 <a href="#">Motif</a>
Other DBs	NCBI-ProteinID: AK094970 UniProt: A0A0H4KN34
LinkDB	<a href="#">All DBs</a>
Position	949233..950294 <a href="#">Genome map</a>
AA seq	353 aa <a href="#">AA seq</a> <a href="#">DB search</a> MEKLLATMPKEYKHSARRFKRAVEILTTEPEPEVGLTPKETIWKKNKAKLYRVVPKQETT HRTPILMVYALINKPYILDLTGKNSLVEYLLDRGFDVYLLDWGTPGLEDDQMKLDDYILD YIPRAVKKVLRTSGASDLSILGYCMGGTMTSIFASLHDDLPIKNLIFMTSPFDFSETGLY GVFLDERYFNLDKVVDTLGNIPDMIDFGNKMLKPIITNFYGPYVTLMDRSENQRFVESWK LMQKIVVGGGIPFPGEAYRQWIRDFYQQNLIKGELTVRGRKVDLSKIKASVLNIAADRDH IAMPQQVESLMNVISSKDKEFKLLNTGHVSVVFGPKAVKETYPICIGDWLVKRS
NT seq	1062 nt <a href="#">NT seq</a> +upstream 0 nt +downstream 0 nt atggaaaaattgttagctacgatgccgaaggagtataaacattctgctagacgctttaa cgggctgtggaattttaacaacagaaccgagcctgaagtagggttaactcctaaggag accatttggaaaaagaataaagcaaaactttatagatatgtccaaaaacaagaaactact catcgtacacctatcttaattggtatagctcttatacaataaacgtatattcttgattg acaaaggggaacagctctttagaataatcttcttgatcgcggattgacgtgatctatta gattggggcactcctggcctgaagaccaagatatgaagcctgatgattatattcttagac tacattccaagagcagtgaaaaaagtgttgctacgtcaggagctagtgatttgcctac ttaggatattgtatgggtggaacgatgacgtctatatttgcctctctacatgatgattta ccaattaagaaccttattttatgactagtccttttgatttctcagaacaggactttat ggagtattcttagatgaacgttactttaacttgataaagttagtgatacacttggcaat attcctcctgatattgatttgggaataagatgcttaaacgattacgaacttctat ggtccatacgtaacgcttatggatcggtcagaaaaatcagcgttttggtgaaagctggaaa ctgatgcagaagtggtaggagatggattccattcccagggtgaagcttatcgtcagtg attcgcgattttatcaacaaaaaagctaatacaaggggaactcactgtaagaggtcga aaagttagacttctaaaattaagcaagcgttttaaatattgcccagatcgagatcat attgcaatgccgcagcaagtagaatcattaatgaacgttatttccagcaaggacaagaa tttaagcttctaaatacagggcatgtttcagttgtatttggacaaaaagctgtaaaggaa acatatccttgacattggagactggttagtgaaacgttcgtaa

Figure S4. Identification of phaC gene subunit in the genome of the Bacillus filamentosus hbe603 strain using the KEEG database.

## BLASTP Search Result

Database: genes

Protein sequence database entries related to beo:BEH\_04720 - 500 hits

Show alignment

Entry	K number	bits	E-val
<input checked="" type="checkbox"/> beo:BEH_04720 class III poly(R)-hydroxyalkanoic acid synthas...	K03821	727	0.0
<input checked="" type="checkbox"/> bfx:BC359_14865 class III poly(R)-hydroxyalkanoic acid synth...	K03821	629	0.0
<input checked="" type="checkbox"/> bmh:BMWSH_4007 phaC; PHA synthase PhaC	K03821	624	0.0
<input checked="" type="checkbox"/> bmd:BMD_1216 phaC; polyhydroxyalkanoic acid synthase, PhaC s...	K03821	624	0.0
<input checked="" type="checkbox"/> bmq:BMQ_1231 phaC; polyhydroxyalkanoic acid synthase, PhaC s...	K03821	624	0.0
<input checked="" type="checkbox"/> bmeg:BG04_3531 phaC; poly(R)-hydroxyalkanoic acid synthase, ...	K03821	622	0.0
<input checked="" type="checkbox"/> anx:ACH33_13635 poly(R)-hydroxyalkanoic acid synthase	K03821	562	0.0
<input checked="" type="checkbox"/> bmyo:BG05_4581 phaC; poly(R)-hydroxyalkanoic acid synthase, ...	K03821	558	0.0
<input checked="" type="checkbox"/> asoc:CB4_03453 phbC; Poly-beta-hydroxybutyrate polymerase	K03821	558	0.0
<input checked="" type="checkbox"/> bww:bwei_3691 phaC; poly(R)-hydroxyalkanoic acid synthase, c...	K03821	557	0.0
<input type="checkbox"/> bwe:BcerKBAB4_1232 poly(R)-hydroxyalkanoic acid synthase, cl...	K03821	557	0.0
<input type="checkbox"/> bwd:CT694_07075 class III poly(R)-hydroxyalkanoic acid synth...	K03821	556	0.0
<input type="checkbox"/> bcy:Bcer98_1044 poly(R)-hydroxyalkanoic acid synthase, class...	K03821	555	0.0
<input type="checkbox"/> bcb:BCB4264_A1369 phaC; poly(R)-hydroxyalkanoic acid synthas...	K03821	555	0.0
<input type="checkbox"/> bmyc:DJ92_3964 phaC; poly(R)-hydroxyalkanoic acid synthase, ...	K03821	555	0.0
<input type="checkbox"/> btn:BTF1_04290 poly(R)-hydroxyalkanoic acid synthase subunit...	K03821	555	0.0
<input type="checkbox"/> bti:BTG_14155 poly(R)-hydroxyalkanoic acid synthase subunit ...	K03821	555	0.0
<input type="checkbox"/> bcg:BCG9842_B3975 phaC; poly(R)-hydroxyalkanoic acid synthas...	K03821	555	0.0
<input type="checkbox"/> btw:BF38_2537 phaC; poly(R)-hydroxyalkanoic acid synthase, c...	K03821	554	0.0
<input type="checkbox"/> btf:YBT020_07235 poly(R)-hydroxyalkanoic acid synthase, clas...	K03821	554	0.0
<input type="checkbox"/> bt1:BALH_1180 poly(R)-hydroxyalkanoic acid synthase, class I...	K03821	554	0.0
<input type="checkbox"/> btk:BT9727_1208 phaC; poly(R)-hydroxyalkanoic acid synthase ...	K03821	554	0.0

**Figure S5** Protein blast of the PhaC subunit found in *Bacillus filamentosus* hbe603 strain using the GenomeNet Bioinformatics Tools (Kyoto University Bioinformatics Center)

**Table S1**

Composition of the culture media used in the study for the production of biopolymers.

Culture Media	Composition	g/L
MSM	Na <sub>2</sub> HPO <sub>4</sub>	3.5
	KH <sub>2</sub> PO <sub>4</sub>	1.5
	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1.0
	MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.2
	CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.01
	Fe (III)NH <sub>4</sub> -citrate	0.06
	1 ml trace elements solution containing (g/l):	
	H <sub>3</sub> BO <sub>3</sub>	0.3
	CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.2
	ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.1
	MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.03
	NaMoO <sub>4</sub> ·2H <sub>2</sub> O	0.03
	NiCl <sub>2</sub> ·6H <sub>2</sub> O	0.02
	CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.01
EPS-M	Saccharose	100
	Yeast extract	20
	K <sub>2</sub> HPO <sub>4</sub>	15
	MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.2
	MnSO <sub>4</sub>	0.015
	FeSO <sub>4</sub>	0.015
	CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.03
	NaCl	0.015
HA-M	Saccharose	100
	Yeast extract	20
	K <sub>2</sub> HPO <sub>4</sub>	15
	MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.2
	MnSO <sub>4</sub>	0.015
	FeSO <sub>4</sub>	0.015
	CaCl <sub>2</sub> ·2H <sub>2</sub> O	0.03
	NaCl	0.015

**Table S2**

16S rRNA sequence analysis of the isolates from rhizosphere of a saline region, Areia Branca saltworks of the caatinga biome.

Strain	Number of nucleotides (bp)	Closed type strain (% similarity)	Accession number
Sac1*	1405	<i>Bacillus paralicheniformis</i> KJ-16 <sup>T</sup> (99,9)	MT479139
Sac7*	1408	<i>Bacillus paralicheniformis</i> KJ-16 <sup>T</sup> (99,8)	MT479140
Sac9*	1411	<i>Bacillus paralicheniformis</i> KJ-16 <sup>T</sup> (99,9)	MT479141
Sac33**	1294	<i>Bacillus filamentosus</i> SGD-14 <sup>T</sup> (100)	MT479148
Sac34**	1331	<i>Bacillus filamentosus</i> SGD-14 <sup>T</sup> (100)	MT479149
Sac36**	1228	<i>Exiguobacterium aestuarii</i> TF-16 <sup>T</sup> (99,5)	MT479150
Sac41**	1198	<i>Bacillus filamentosus</i> SGD-14 <sup>T</sup> (99,4)	MT479153
Sac44**	1350	<i>Bacillus paralicheniformis</i> KJ-16 <sup>T</sup> (99,9)	MT479154
Sac54*	1259	<i>Curtobacterium oceanosedimentum</i> ATCC 31317 <sup>T</sup> (98,7)	MT479157
Sac58**	1209	<i>Curtobacterium citreum</i> DSM 20528 <sup>T</sup> (99,6)	MT479158
Sac64**	1385	<i>Bacillus paralicheniformis</i> KJ-16 <sup>T</sup> (99,8)	MT479160
Sac65**	1185	<i>Pseudomonas alcaliphila</i> JCM 10630 <sup>T</sup> (97,4)	MT479161
Sac66**	1342	<i>Bacillus filamentosus</i> SGD-14 <sup>T</sup> (99,8)	MT479162
Sac76**	1124	<i>Bacillus filamentosus</i> SGD-14 <sup>T</sup> (100)	MT479163
Sac10*	1141	<i>Bacillus salacetis</i> SKP7-4 <sup>T</sup> (99,3)	MT479142
Sac16*	1187	<i>Bacillus paralicheniformis</i> KJ-16 <sup>T</sup> (99,9)	MT479143
Sac18*	1111	<i>Bacillus paralicheniformis</i> KJ-16 <sup>T</sup> (99,5)	MT479144
Sac22**	1203	<i>Bacillus paralicheniformis</i> KJ-16 <sup>T</sup> (99,5)	MT479145
Sac23**	1242	<i>Bacillus filamentosus</i> SGD-14 <sup>T</sup> (100)	MT479146
Sac24**	1203	<i>Bacillus albus</i> N35-10-2 <sup>T</sup> (100)	MT479147
Sac37**	1177	<i>Curtobacterium citreum</i> DSM 20528 <sup>T</sup> (99,2)	MT479151
Sac38**	1199	<i>Staphylococcus warneri</i> ATCC 27836 <sup>T</sup> (98,9)	MT479152
Sac45**	1198	<i>Bacillus haynesii</i> NRRL B-41327 <sup>T</sup> (97,4)	MT479155
Sac51*	1124	<i>Bacillus paralicheniformis</i> KJ-16 <sup>T</sup> (99,7)	MT479156
Sac59**	1203	<i>Staphylococcus warneri</i> ATCC 27836 <sup>T</sup> (100)	MT479159

\* Strains isolated from the rhizosphere of *Spergularia* sp plant

\*\* Strains isolated from the rhizosphere of *Blutaparon portulacoides* plant



**Table S3**

NaCl tolerance profile of bacterial strains isolated from Caatinga Biome.

Genus	Strains	Range for growth NaCl (M)
<i>Bacillus</i>	Sac1, Sac7, Sac9, Sac10, Sac16, Sac18, Sac22, Sac23, Sac24, Sac33, Sac34, Sac41, Sac44, Sac45, Sac51, Sac64, Sac66, Sac76	0.1-2.0
<i>Curtobacterium</i>	Sac37, Sac54, Sac58	0.1-2.0
<i>Staphylococcus</i>	Sac38, Sac59	0.1-2.0
<i>Pseudomonas</i>	Sac65	0.1-2.0
<i>Exiguobacterium</i>	Sac36	0.1-1.0

**Table S4**

P(3HB) production from different carbon sources and different concentrations of NaCl (M) by two *Bacillus filamentosus* strains. Average and standard deviation of triplicate cultivation after 72 h.

Isolates	Carbon Source	NaCl (M)	CDW (g/l)	P(3HB) (% CDW)	P(3HB) (g/l)
Sac33	Glucose	0	2.7±4.4	30.5±2.2	0.9±1.4
		0.6	2.4±3.6	17.6±0.9	0.4±0.4
		1.0	3.0±2.5	15.6±1.7	0.5±0.9
		2.0	1.1±0.0	1.5±2.6	0.0±0.0
	Xylose	0	1.6±5.2	36.6±2.5	0.6±1.4
		0.6	1.6±1.3	16.9±0.6	0.3±0.2
		1.0	1.4±1.6	14.1±3.6	0.2±0.7
		2.0	1.2±0.3	0.1±0.1	0.0±0.0
	Glycerol	0	1.8±1.8	21.7±4.6	0.4±0.4
		0.6	2.3±2.9	18.5±2.8	0.4±0.2
		1.0	2.1±4.4	17.0±0.4	0.4±0.6
		2.0	1.6±2.3	0.0±0.0	0.0±0.0
Sac34	Glucose	0	3.1±0.4	27.7±2.7	0.8±0.9
		0.6	3.6±2.1	21.7±1.2	0.8±0.3
		1.0	4.2±1.2	8.7±1.2	0.4±0.6
		2.0	2.1±1.6	0.0±0.0	0.0±0.0
	Xylose	0	2.0±1.8	27.0±2.0	0.5±0.4
		0.6	2.4±2.0	21.1±1.9	0.5±0.2
		1.0	2.1±0.1	14.2±1.4	0.3±0.2
		2.0	0.7±2.4	0.0±0.0	0.0±0.0
	Glycerol	0	1.9±0.8	20.3±2.1	0.4±0.4
		0.6	2.1±2.9	14.8±1.4	0.3±0.8
		1.0	1.6±4.2	10.9±2.7	0.2±0.0
		2.0	1.6±2.5	0.0±0.0	0.0±0.0

**Table S5**

Quantification of Hyaluronic Acid (HA) using Alcian blue staining reaction. Average and standard deviation of triplicate cultivation after 7 days.

Code	Strain	Optical Density <sub>(540nm)</sub>	HA (mg/L)
ATCC 39920*	<i>Streptococcus equi</i> subsp. <i>Zooepidemicus</i>	0,171 ± 0,0	640,6
Sac1	<i>Bacillus paralicheniformis</i>	0,214 ± 0,0	561,6
Sac7	<i>Bacillus paralicheniformis</i>	0,300 ± 0,0	420,0
Sac9	<i>Bacillus paralicheniformis</i>	0,171 ± 0,0	640,6
Sac10	<i>Bacillus salacetis</i>	0,187 ± 0,0	610,6
Sac16	<i>Bacillus paralicheniformis</i>	0,783 ± 0,0	34,2
Sac18	<i>Bacillus paralicheniformis</i>	0,295 ± 0,0	426,4
Sac22	<i>Bacillus paralicheniformis</i>	0,301 ± 0,0	418,5
Sac23	<i>Bacillus filamentosus</i>	0,163 ± 0,0	655,6
Sac24	<i>Bacillus albus</i>	0,266 ± 0,0	472,6
Sac33	<i>Bacillus filamentosus</i>	0,159 ± 0,0	663,6
Sac34	<i>Bacillus filamentosus</i>	0,177 ± 0,0	628,9
Sac36	<i>Exiguobacterium aestuarii</i>	0,190 ± 0,0	603,5
Sac37	<i>Curtobacterium</i> sp.	0,155 ± 0,0	671,4
Sac38	<i>Staphylococcus</i> sp.	0,191 ± 0,0	601,6
Sac41	<i>Bacillus filamentosus</i>	0,161 ± 0,0	658,2
Sac44	<i>Bacillus paralicheniformis</i>	0,233 ± 0,0	527,0
Sac45	<i>Bacillus haynesii</i>	0,799 ± 0,0	33,3
Sac51	<i>Bacillus paralicheniformis</i>	0,805 ± 0,1	33,1
Sac54	<i>Curtobacterium</i> sp.	0,131 ± 0,0	719,0
Sac58	<i>Curtobacterium citreum</i>	0,163 ± 0,0	655,9
Sac59	<i>Staphylococcus warneri</i>	0,152 ± 0,0	676,3
Sac64	<i>Bacillus paralicheniformis</i>	0,173 ± 0,0	636,8
Sac65	<i>Pseudomonas</i> sp.	0,206 ± 0,0	575,9
Sac66	<i>Bacillus filamentosus</i>	0,173 ± 0,0	636,8
Sac76	<i>Bacillus filamentosus</i>	0,311 ± 0,0	402,8

\* Positive control

**Table S6**

Hyaluronic acid (HA) standard curve. Average and standard deviation of triplicate experiments.

HA (mg/L)	Optical Density <sub>(540nm)</sub>
0	0,804 ± 0,0
50	0,758 ± 0,1
100	0,681 ± 0,0
200	0,464 ± 0,0
300	0,348 ± 0,0
500	0,273 ± 0,0

