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Abstract:	Two proteolytic bacterial strains, BSker2T and BSker3T, were enriched from sediments of hypersaline alkaline lakes in Kulunda Steppe (Altai, Russia) with chicken feathers as substrate, followed by pure culture isolation on hypersaline alkaline media with casein. The cells are nonmotile filamentous flexible rods. The isolates are obligate aerobic heterotrophs utilising proteins and peptides as growth substrates. Both are obligate alkaliphiles, but differed in their pH optimum: 9.5-9.8 for Bsker2T and 8.5-9 for BSker3T. The salt range for growth of both isolates is between 2 and 4.5 M total Na+ with an optimum at 2.5-3 M. No organic osmolytes were detected in cells of BSker2T, but it accumulated high intracellular concentrations of K+. The polar lipid fatty acids were dominated by unsaturated C16 and C18 species. The 16S rRNA gene phylogeny indicated that both strains belong to the recently proposed phylum Rhodothermaeota. BSker2T forms a novel genus-level branch, while BSker3T represents a novel species-level member in the genus Longimonas. On the basis of distinct phenotypic and genotypic properties, strain BSker2T (JCM 31342T=UNIQEM U1009T) is proposed to be classified as a new genus and species Natronotalea proteinilytica and strain BSker3T (JCM 31343T=UNIQEM U10110T) as a new species Longimonas haloalkaliphila.

Natronotalea proteinilytica gen. nov., sp. nov, and *Longimonas haloalkaliphila* sp. nov., extremely haloalkaliphilic members of the phylum *Rhodothermaeota* from hypersaline alkaline lakes

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Running title: *Natronotalea proteinilytica* gen. nov., sp. nov., and *Longimonas haloalkaliphila* sp. nov.

The 16S-rRNA gene sequence of strain BSker2[T] and BSker3[T] are deposited in the GenBank under the numbers KU720569 and KU72070.

Two proteolytic bacterial strains, BSker2^T and BSker3^T, were enriched from sediments of hypersaline alkaline lakes in Kulunda Steppe (Altai, Russia) with chicken feathers as substrate, followed by pure culture isolation on hypersaline alkaline media with casein. The cells are nonmotile filamentous flexible rods. The isolates are obligate aerobic heterotrophs utilising proteins and peptides as growth substrates. Both are obligate alkaliphiles, but differed in their pH optimum: 9.5-9.8 for Bsker2^T and 8.5-9 for BSker3^T. The salt range for growth of both isolates is between 2 and 4.5 M total Na^+ with an optimum at 2.5-3 M. No organic osmolytes were detected in cells of BSker2^T, but it accumulated high intracellular concentrations of K^+. The polar lipid fatty acids were dominated by unsaturated C_{16} and C_{18} species. The 16S rRNA gene phylogeny indicated that both strains belong to the recently proposed phylum *Rhodothermaeota.* BSker2^T forms a novel genus-level branch, while BSker3^T represents a novel species-level member in the genus *Longimonas.* On the basis of distinct phenotypic and genotypic properties, strain BSker2^T (JCM 31342^T=UNIQEM U1009^T) is proposed to be classified as a new genus and species *Natronotalea proteinilytica* and strain BSker3^T (JCM 31343^T=UNIQEM U10110^T) as a new species *Longimonas haloalkaliphila*.

Hypersaline lakes characterized by highly alkaline salt-saturated brines with pH from 9 to 11 can harbor diverse and dense haloalkaliphilic prokaryotic communities [1-4], which have recently been subjected to intensive fundamental and application-oriented studies [5-7]. One of the least studied aspects in this area concerns the identity of aerobic prokaryotes capable of utilizating insoluble proteinaceous substrates for growth at extremely high salt and pH conditions. Our recent focused research in this direction allowed to identify a first aerobic extremely salt-tolerant and obligately alkaliphilic gammaproteobacterium from hypersaline soda brines in south-eastern Siberia. For this organism specialized in utilization of proteins as growth substrates, we suggested the new genus and species *Natronospira proteinivora* [8]. Here we describe properties of a second group of extremely haloalkalitolerant protein-utilizing bacteria enriched from sediments of hypersaline alkaline lakes that represents a new genus and two species in the phylum *Rhodothermaeota* (former a deep lineage within the phylum *Bacteroidetes*) [9].

Surface sediments from two types of hypersaline alkaline lakes in Kulunda Steppe (Altai, Russia) were used as the inoculum for enrichment cultures: (1) from typical soda lakes with extremely high alkalinity Tanatar-1 and Tanatar-2 (July 2016, salinity=300-400 g l^{-1}, pH=9.7-10.2, total carbonate alkalinity=3.4-3.5 M) and (2) from Stamp Lake with low alkalinity (July 2015, salinity=325 g l^{-1}, pH=9.1, total carbonate alkalinity=0.15 M).

The protein-utilizing bacteria were enriched under aerobic conditions using defatted chicken feathers with β-keratin as a growth substrate. The mineral base medium containing 4 M total Na^+ (2 M Na^+ as sodium carbonates + 2 M NaCl) at pH 9.8 was used for the Tanatar sample, while the Stamp lake sediments were inoculated into 4 M NaCl-based medium adjusted to pH 9 with 1 M Na_2CO_3. Both media also included 1 g l^{-1} K_2HPO_4 and 5 g/l KCl. After

sterilization, the media were supplemented with 1 ml l^{-1} of trace metal solution and vitamin mix [10] and 1 mM $MgCl_2$. Defatted chicken feathers were added as substrate at approximately 2 g l^{-1}. Before inoculation, the sediments were resuspended 1:10 in the basic medium and the suspension was allowed to stand for 20 min., resulting in precipitation of the course fractions. 1 ml from the top fraction containing mostly colloidal sediments was then used to inoculate 40 ml cultures in 200 ml closed serum bottles placed on a rotary shaker at 37°C and at 200 rpm. The development of the enrichment culture was monitored by the extent of feather degradation and by microscopy. After 20-30 days, the cultures were serially diluted in the same medium but with casein as substrate and the maximal positive dilutions were plated onto a solid medium prepared by 1:1 mixing of the liquid medium and a 4% solution of extensively washed agar at 50°C. To compensate for the lower salinity, sterile solid NaCl was added directly to the mixture before pouring the plates. After 1-3 weeks of incubation in closed plastic bags at 37°C the dominant colony types were transferred to the respective liquid media with casein and purified by repeated plating. This, eventually, resulted in isolation of two bacterial strains: BSker2^T from the Tanatar lakes and BSker3^T from the Stamp Lake. The purity was checked microscopically (Zeiss Axioplan Imaging 2 microscope, Göttingen, Germany) and by 16S rRNA gene sequencing.

On casein agar the colonies of both strains were flat and spreading, orange-red in colour and formed a clear zone of casein hydrolysis (**Suppl. data, fig.S3**). The pigment extracted from the cells with aceton/MeOH had an absorbance maximum at 480 nm and two shoulders at around 450 and 510 nm (**Suppl. data, fig.S1).** Exponentially growing cells of both isolates were long flexible nonmotile rods. In the stationary phase the Bsker3^T cells elongated up to 100 μm and formed coiled aggregates **(Fig. 1)**. The cells were apparently covered with a thick EPS matrix since even high-speed centrifugation did not allow to obtain a compact cell pellet. The KOH test proved a Gram-negative type of cell wall.

The membrane polar lipids were extracted from the freeze-dried cells and their composition was analyzed by TLC at the DSMZ Identification Service according to [11-12]. The fatty acid methyl esters were analyzed by GC-MS according to [13-14]. Respiratory lipoquinones were extracted from the lyophilized cells by cold acetone, separated by TLC [15] and subsequently eluted and further analyzed by tandem mass spectrometry (LCG Advantage Max) in combination with HPLC-MS.

The polar lipid analysis of cell membranes of strain BSker2^T showed the presence of two glycolipids and four unidentified phospholipid species (**Supplementary fig. S2**). The respiratory quinone analysis identified a single menaquinon species MK-7 in cells of BSker2^T. In their PLFA profiles, the novel isolates were similar to the two extremely halophilic closest relatives from *Rhodothermaeota*, *Longimonas halophila* and *Salinisaeta longa*. But there was variability in the abundance of other C_{15}-C_{17} components, both between the two BSker strains and between them and the nearest relatives **(Supplementary table S1**).

Organic compatible solutes were analysed in BSker2^T cells grown at 4 M total Na^+, either at pH 8.6 (NaCl base) or pH 10 (sodium carbonate base), using HPLC and ^{1}H-NMR after extraction according to a modified Bligh and Dyer method [16-17]. The polar fraction was analyzed on a Nucleosil 100-3 aminopropyl phase HPLC column (Macherey & Nagel, Düren, Germany) using acetonitrile/water (80:20, v/v) as mobile phase at a flow rate of 1 mL/min [18]. Compounds were monitored using a combination of refractive index and UV detector. Amino-reactive compounds were analyzed by gradient HPLC with pre-column FMOC-ADAM derivatization as described previously [19]. No known organic osmolytes were detectable, neither on the aminopropyl phase column (for neutral and zwitterionic solutes) nor with FMOC derivatization (for amino reactive solutes). The latter revealed that glutamate was the dominant amino acid at a concentration of 0.45 and 0.33 mmol (g protein)$^{-1}$ for the chloride and the soda sample, respectively. Both values are within the expected range.

For *E.coli* cells a regular glutamate value of 0.15 mmol (g protein)$^{-1}$ and a transient accumulation to 0.68 mmol (g protein)$^{-1}$ upon salt stress has been reported [20]. For NMR analysis, the dry cells were extracted with 1 mL chloroform/methanol/water (10:5:4, by vol.) followed by phase separation according to [18]. The polar fraction was evaporated overnight and the dry residue was dissolved in 1 mL D_2O as lock signal. The sample was further supplemented with the internal standard benzene-1,2,4,5-tetracarboxylate sodium salt to give a final concentration of 10 mM. ^{1}H NMR spectra were recorded on a Buker Avance 300 DPX spectrometer. The soda sample (lower protein content and lower osmolarity) revealed no distinct resonances apart from the internal standard. The chloride sample displayed a number of peaks, none of which could be related to any known compatible solutes. In relation to the internal standard, the strongest signals between 1 to 4 ppm represented presumptive concentrations of unknown compounds of no more than 0.25 mmol (g protein)$^{-1}$, while for the model halophilic organism *Halomonas elongata* grown at 3.42 M NaCl an ectoine content of approx. 7 mmol (g protein)$^{-1}$ was recorded [21]. This value is almost 30x higher than what we observed here, suggesting that the "salt-out" osmotic strategy is not used by the novel extreme haloalkaliphile.

To analyze the intracellular potassium, the freeze-dried cells of BSker2^T were extracted according to a modified Bligh and Dyer protocol [16-17]. The water-soluble fraction was subjected to cation analysis by isocratic HPLC with conductivity detection (conductoMonitor III, Thermo Scientific, Waltham MA, USA) on a Metrosept Cation C4-100/4.0 column (Methrom, Herisau, Switzerland) using an eluent of 1.7 mM nitric acid and 0.7 mM dipicolinic acid at a flow rate of 0.9 ml min^{-1}. Potassium content was corrected for the proportion originating from intercellular medium and related to protein content determined by the bicinchoninic assay (Uptima, Montlucon, France). The estimated specific potassium content was 305 and 115 mg (mg cell protein)$^{-1}$ in the cells grown in NaCl base

and in soda base, respectively. The first value is close to what is usually found in haloarchaea [22] , while the much lower content in the soda-grown cells might be explained by two times less osmotic pressure of this weak electrolyte in comparison with the strongly electrolytic NaCl [2]. In conjunction with extreme halophily, this is an indication that strain BSker2^T employs the "salt-in" osmoprotection mechanism, which is also found in an extremely halophilic member of the *Rhodothermaceae* - *Salinibacter ruber* [23].

The 16S rRNA gene sequence-based phylogenetic analysis was performed in Mega 6 package [24] using Maximum Likelyhood algorithm. The results demonstrated that BSker2^T forms a novel genus lineage within the family *Rhodothermaceae*, phylum *Rhodothermaeota*, with a maximum pairwise sequence similarity of 92 % to its validly characterized halophilic members *Longimonas halophila* [25], *Salisaeta longa* [26] and *Longibacter salinarum* [27]. On the other hand, BSker3^T apparently represents a novel species in the genus *Longimonas* with 97% sequence similarity to the extremely halophilic *L. halophila* (**Fig. 2**). The phenotypic comparison of the BSker isolates with the two closest relatives is given in **Table 1**. Interestingly, despite a significant phylogenetic distance, the unusual cell morphology and some other important characteristics (such as substrate profile, extreme salt tolerance, the type of lipoquinones) were common among the soda lake isolates and the three halophilic genera mentioned above. The G + C content in the genomic DNA was analyzed by the DSMZ Identification Service using the HPLC method [28]. The determined values for BSker2^T and BSker3^T were 55.9 and 58.2 mol%, respectively.

The BSker strains are obligately aerobic organoheterotrophs which grow best with various proteins and peptides, including the following: casein, gelatin, filter-sterilized bovine serum albumin and haemoglobin; various peptones and yeast extract. Heat-sterilized alpha-keratine (fine powdered fraction), soya protein, lactalbumin and bovine collagen were only utilized by strain BSker2^T. The protease activity, qualitatively tested in strain BSker2^T by the

agar-diffusion approach, was cell-associated (**Supplementary Fig. S3**). In addition, BSker2^T was able to utilize amylose (**Supplementary Fig. S4**) in the presence of low (100 mg l^{-1}) background concentration of casein hydrolysate. Polymeric substrates tested but not utilized included amylopectin, birch wood xylan, amorphous forms of cellulose and chitin and emulsified olive oil. Among the monomeric substrates tested were sugar hexoses and pentoses, sugar alcohols and C_2-C_6 organic acids. Both strains grew (again only in the presence of a minimum of 100 mg l^{-1} of casein hydrolysate) with glycerol and maltose. In addition, BSker2^T also utilized cellobiose. Anaerobic fermentative growth with maltose and peptone was not observed.

With respect to its salt demand, both BSker2^T and BSker3^T can be qualified as extreme halophiles with their total Na^+ range for growth between 2 and 4.5 M (optimum around 3 M) (**Fig. 3a**). In contrast to the extremely halophilic relatives, the BSker strains were not dependent on high Mg concentrations. On the other hand, the strains also differed from most of soda lake bacterial isolates by obligate growth dependence on the presence of high Cl^- concentrations (minimum 0.5 M). The latter might be related to its usage as a counter anion for intracellular potassium accumulation.

At optimal salinity, the pH range for growth with casein was substantially different for the two strains . The soda lake isolate BSker2^T had a profile typical for obligate natronophiles with the pH range from 8.2 to 10.2 (optimum around 9.5), while BSker 3^T was only moderately alkaliphilic with an optimum at pH 8.5-9 (**Fig. 3b**).

In conclusion, , the two aerobic bacterial isolates from hypersaline alkaline lakes represent the first examples of extremely halophilic and alkaliphilic bacteria specialized in utilization of proteinaceous compounds and with an apparent usage of the "salt-in" osmoprotection strategy. With this combination of properties, they are clearly different from their nearest

phylogenetic relatives and are proposed to be classified as a novel genus and species *Natronotalea proteinilytica* (strain Bsker2^T) and *Longimonas haloalkaliphila* sp. nov. (Bsker3^T).

DESCRIPTION OF *NATRONOTALEA* GEN. NOV.

Natronotalea (Na.tro.no.ta'le.a Gr. n. *natron*, arbitrarily derived from the Arabic n. *natrun* or *natron*, soda; L. fem. n. *talea*, a staff, stick - a long rod; N.L. fem. n. *Natronotalea* a soda-loving long rod)

Extremely haloalkaliphilic protein-utilizing aerobic member of the family *Rhodothermaceae*, phylum *Rhodothermaeota*, found in hypersaline alkaline lakes. The type species is *Natronotalea proteinilytica.*

DESCRIPTION OF *NATRONOTALEA PROTEINILYTICA* SP. NOV.

Natronotalea proteinilytica (pro.te.i.ni.ly'ti.ca N.L. neut. n. *proteinum*, protein; N.L. fem. adj. *lytica* (from Gr. fem. adj. lytikê), dissolving; N.L. fem. adj. *proteinilytica* dissolving proteins)

Cells have the Gram-negative type of cell wall, long flexible rods, 0.5 x 5-15 μm, nonmotile, forming EPS. The colonies are flat, spreading up to 5 mm, orange-red. The cell pigment has an absorbance maximum at 480 nm. The polar lipids include 4 unidentified phospho- and two glyco- lipids. The respiratory quinones are represented by MK-7. The polar lipid fatty acids are dominated by unsaturated 16:1ω7c and 18:1ω7c. It is a strictly aerobic organoheterotroph utilizing various proteins and peptides for growth. It can also grow, but less actively, with amylose, maltose, cellobiose and glycerol. It is obligately alkaliphilic, with a pH range for growth from 8.2 to 10.2 (optimum at 9.5-9.8). It is a chloride-dependent extreme halophile which requires a Na^+ range for growth from 2 to 4.5 M (optimum at 2.5-3 M). The upper temperature limit for growth (at optimal pH and salinity) is 48°C. The G + C content of the genomic DNA in the type strain is 55.9 mol% (HPLC). The type strain BSker2^T (JCM 31342^T=UNIQEM U1009^T**)** was isolated from sediments of hypersaline soda lakes in Kulunda Steppe (Altai, Russia). The 16S rRNA gene sequence accession number of the type strain in GenBank is KU720569.

DESCRIPTION OF *LONGIMONAS HALOALKALIPHILA* SP. NOV.

Longimonas haloalkaliphila (Gr. n. *hals halos*, salt; N.L. n. *alkali*, soda ash (from Arabic *al-qalyi*, the ashes of saltwort); N.L. adj. *philus* (from Gr. adj. *philos -ê -on*), friend, loving; N.L. fem. adj. *haloalkaliphila*, salt and alkali-loving)

Cells have the Gram-negative type of cell wall, nonmotile, long, flexible rods, 0.5-0.6 x 8-30 μm in exponential growth phase, and up to 100 μm long in aggregates in aged cultures. The colonies are flat, spreading up to 8 mm, orange-red. The cell pigment has an absorbance maximum at 480 nm. The polar lipid fatty acids are dominated by unsaturated 16:1ω7c and 18:1ω7c. It is a strictly aerobic organoheterotroph utilizing a limited number of proteinaceous and peptide substrates for growth. Less active growth was observed with maltose and glycerol. It is obligately but only moderately alkaliphilic, with a pH range for growth from 7.8 to 9.3 (optimum at 8.5-8.8). It is a chloride-dependent extreme halophile which requires a Na^+ range for growth from 2 to 4.5 M (optimum at 2.5-3 M). The upper temperature limit for growth (at optimal pH and salinity) is 50°C. The G + C content of the genomic DNA in the type strain is 58.2 mol% (HPLC). The type strain BSker3^T (JCM 31343^T=UNIQEM U1010^T**)** was isolated from sediments of a hypersaline alkaline lake in Kulunda Steppe (Altai, Russia). The 16S rRNA gene sequence accession number of the type strain in GenBank is KU720570.

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Conflict of interest:
The authors declare that there is no conflict of interests.

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335 **Table 1**. Comparative properties of the BSker strains and their closest halophilic relatives from the
336 phylum *Rhodothermaeota*: *Longimonas halophila* [25], *Salisaeta longa* [26] and *Longibacter*
337 *salinarum* [27]. nd - no data

Property	**BSker2^T**	**BSker3^T**	*Longimonas halophila*	*Salisaeta longa*	*Longibacter salinarum*
Cell morphology	Long flexible rod (0.5 x 5-15 μm)	Long flexible rod (0.5-0.6 x 8-30 μm; >100 μm in old cultures)	Long rod (0.4-0.6 x 5-9 μm)	Long flexible rod (0.8 x 15-30 μm)	Long rod (0.3-0.4 x 6-12 μm)
Pigmentation	Red-orange	Red-orange	Red	Red	Red
Relation to oxygen	Obligate aerobe	Obligate aerobe	Facultative anaerobe (fermentation)	Obligate aerobe	
Growth substrates	Proteins, peptides, starch, maltose, cellobiose, glycerol	Proteins, peptides, maltose, glycerol	Glucose, sucrose, maltose, fructose, ribose	Glycerol, glucose, maltose	Glycerol, sucrose, mannitol, strach*
Salinity range (opt.), M Na^+	2-4.5 (2.5-3.0)	2-4.8 (2.5-3.0)	0.7-4.3 (1.0-1.4)	1.6-4.1 (2.5)	0.3-3.3 (1.3-2.0)
High Mg demand	no	no	yes	yes	no
pH range (opt.)	8.2-10.2 (9.5-9.8)	7.6-9.3 (8.5-8.8)	6.5-8.5 (7.5-8.0)	6.5-8.5	6.5-8.5 (7.5-8.0)
Max. growth T (°C)	46	48	50	50	50
Dominant PLFA (in order of dominance)	16:1ω7c, 18:1ω7c i17:0, i16:0; 16:0	16:1ω7c, 18:1ω7c ai17:0, i16:0, i17:0, i17:1ω9c	16:1ω7c, i16:0, 18:1ω7c; i15:0, 16:0, ai17:0	16:1ω7c, 16:0; i15:0; i16:0, ai17:0	i17:1ω9c, 16:1ω8c, i15:0, i17:0; ai17:0
Predominant lipoquinone	MK-7	nd	MK-7	nd	MK-7
G + C, mol%	55.9	58.2	61.5	62.9	58.1
Habitat	Hypersaline soda lakes (s-w Siberia)	Hypersaline alkaline lake (s-w Siberia)	Solar saltern (China)	Dead Sea (Israel)	Solar saltern (China)

338 *since this organism did not utilize maltose, its capability to grow with starch is questionable
339

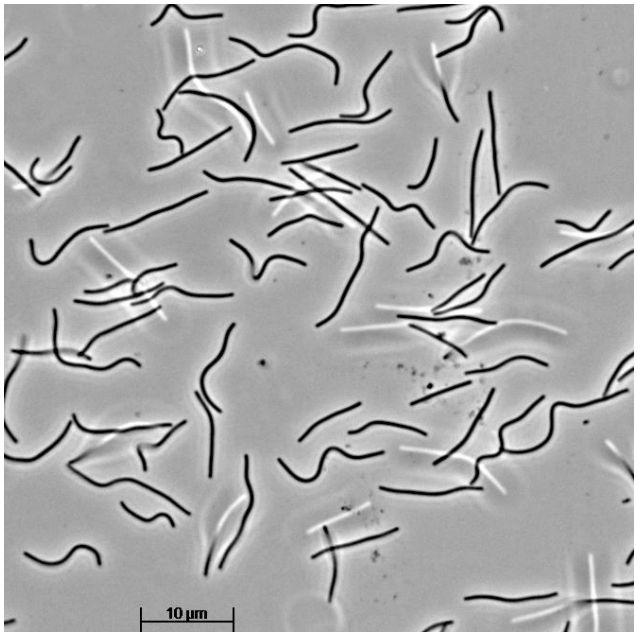
Legends to the figures

Fig. 1 Cell morphology of strain BSker2^T (**a**) and BSker3^T (**b-c**) grown with casein at 4 M total Na^+ and 37ºC, phase contrast microphotograps. (a and b), cells from exponential and stationary growth phase, respectively; (**c**), complex aggregation of extremely elongated cells of BSker3^T in late stationary growth phase.

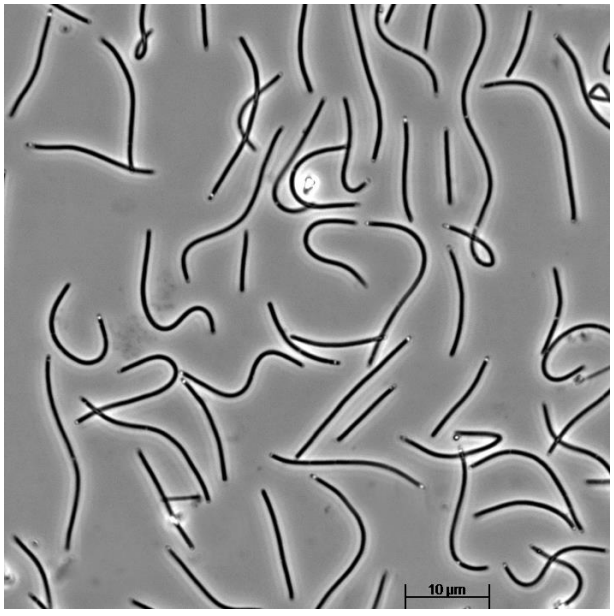
Fig. 2. Maximum Likelihood 16S rRNA gene sequence-based phylogenetic tree showing position of strains BSker2^T and BSker2^T (in bold) within the phylum *Rhodothermaeota*. Branch lengths (see scale) correspond to the number of substitutions per site with corrections, associated with the model (GTR, G + *I*, 4 categories). All positions with less than 95% site coverage were eliminated. Totally 1305 positions were used in the alignment of 24 sequences. Numbers at nodes indicate bootstrap values of 1000 repetitions. Strains BSker2^T and BSker3^T are in bold. A representative of *Bacteroidetes* phylum, *Marivirga tractuosa* DSM4126 (Genbank accession CP002349.1) was used as an outgroup.

Fig. 3. Influence of pH at 3 M total Na^+ (**a**) and Na^+ at pH 9 (BSker3^T) - 9.5 (BSker2^T) (**b**) on growth with casein at 37ºC. Incubation time: 42-55 h BSker2^T and 96 h for BSker3^T.

(a)



(b)



(c)

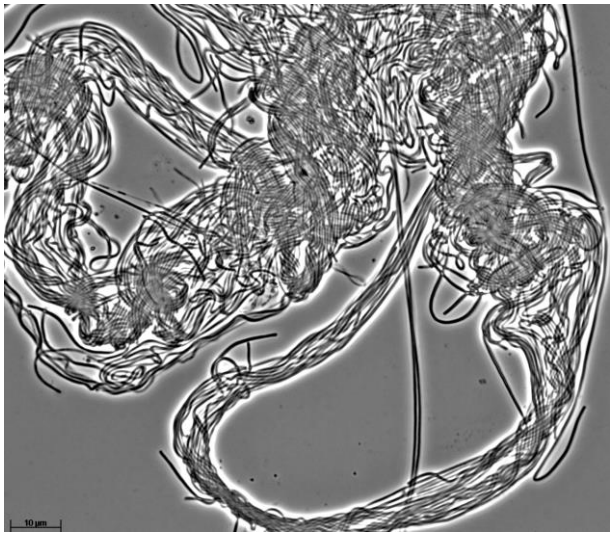


Fig.1

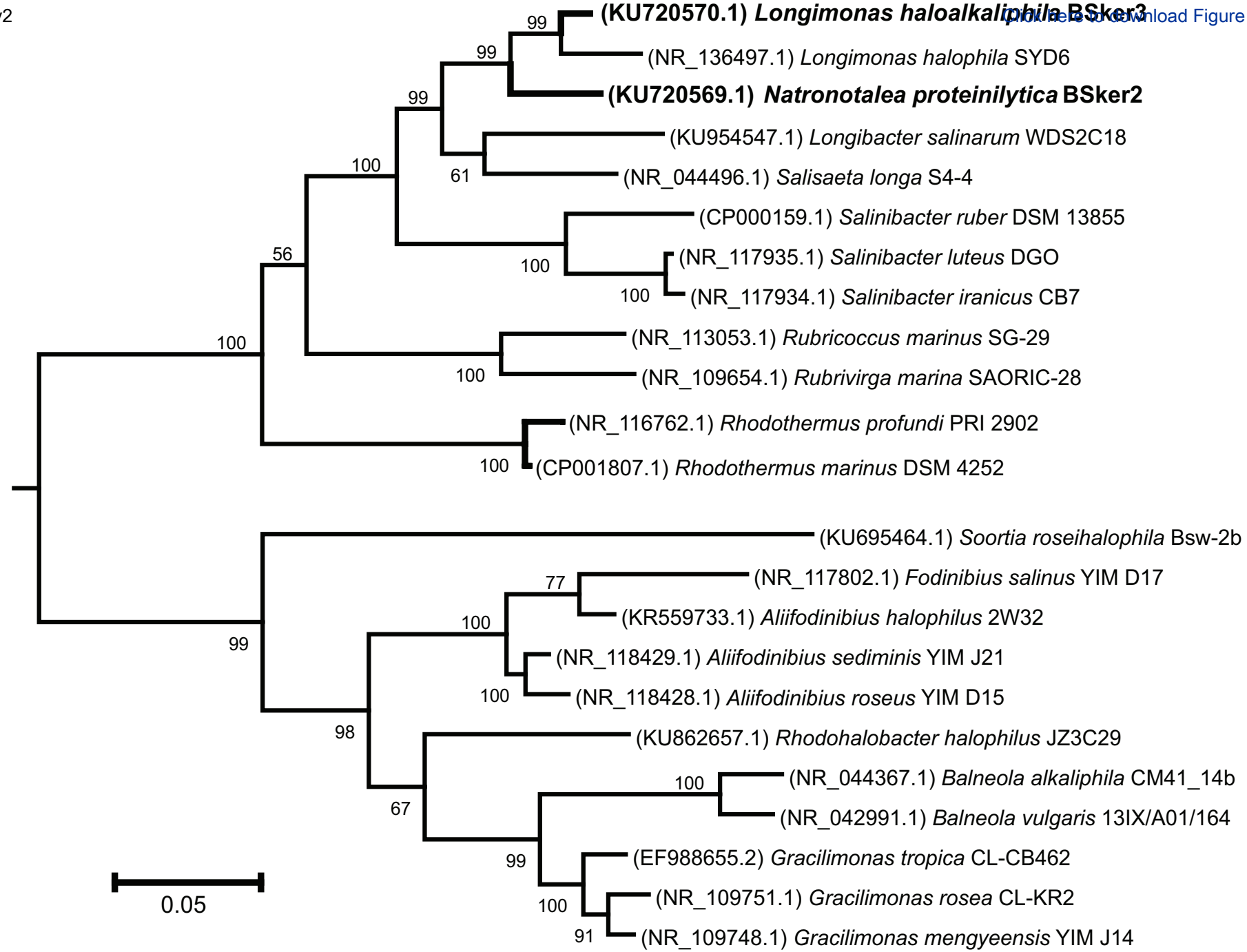


Fig.2

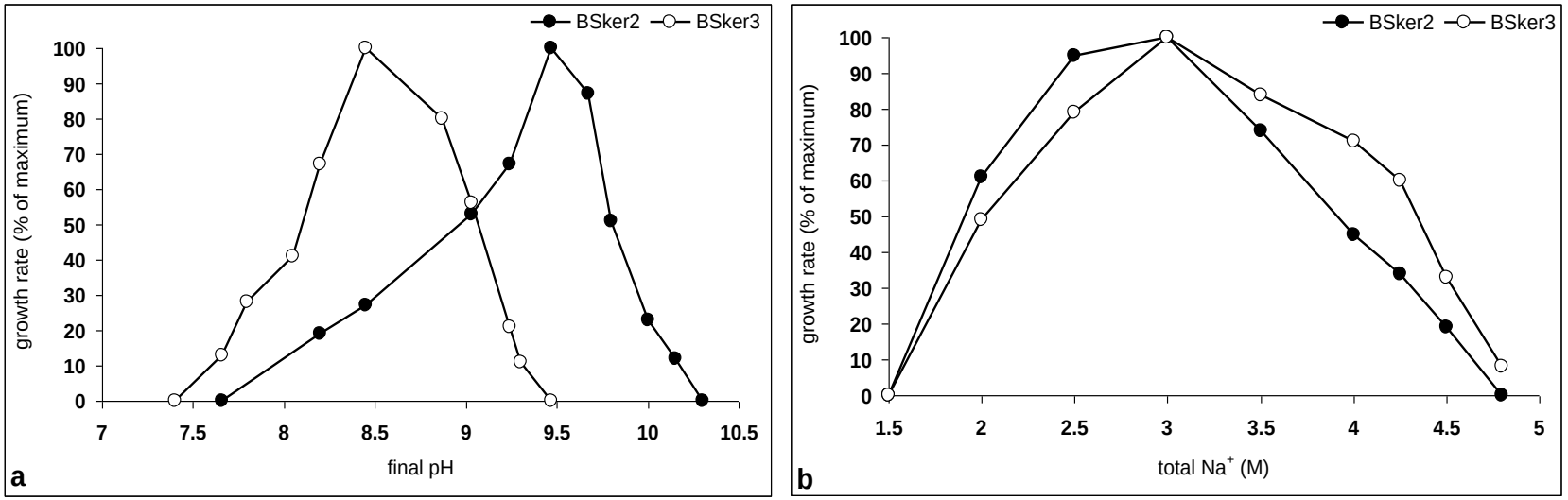


Fig.3

Supplementary data to:

Journal: International Journal of Systematic and Evolutionary Microbiology

Natronotalea proteinilytica gen. nov., sp. nov, and *Natronotalea halophila* sp. nov., extremely salt-tolerant alkaliphilic members of the phylum *Rhodothermaeota* from hypersaline soda lakes

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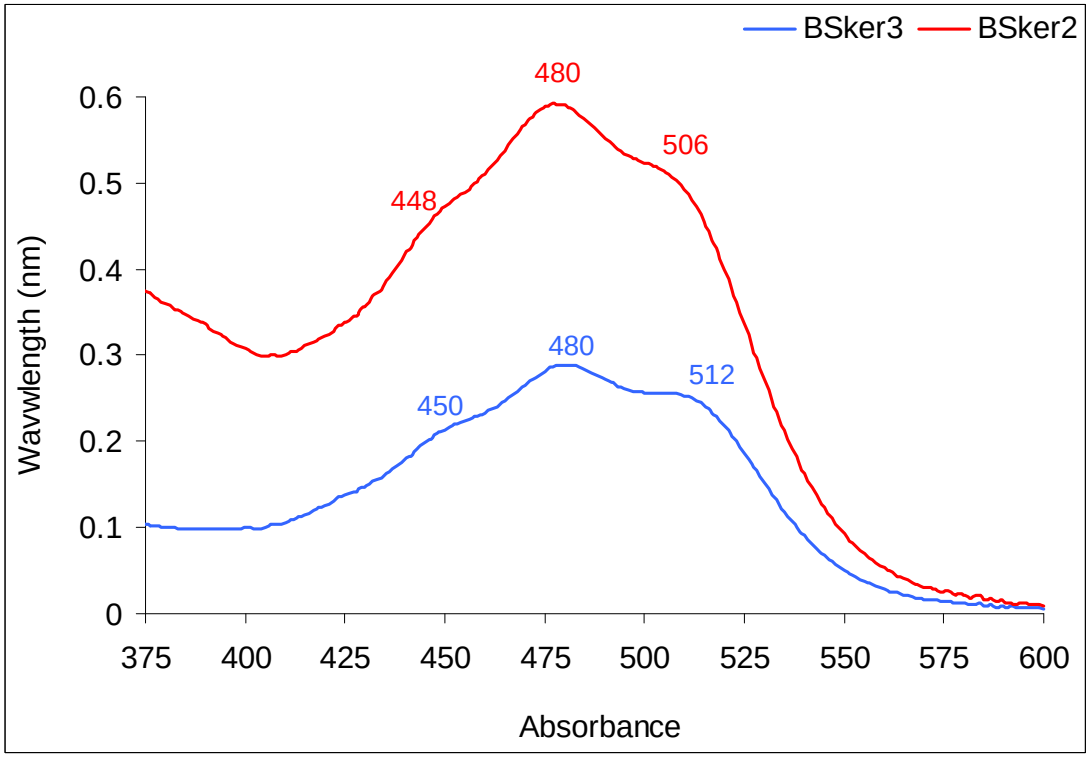
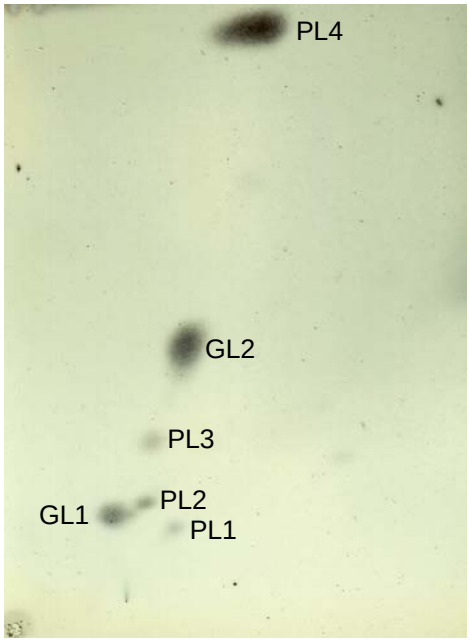
Content:



Supplementary Fig.S1. Absorption spectra of carotenoids extracted with acetone/MeOH (1:1) from the cells of extremely haloalkaliphilic proteolytic bacteria from hypersaline alkaline lakes



Supplementary Fig.S2. Polar lipid profile (2D TLC) of extremely haloalkaliphilic proteolytic bacterium BSker2 from hypersaline alkaline lakes (DSMZ Identification Service). PL – phospholipid, GL - glycolipid

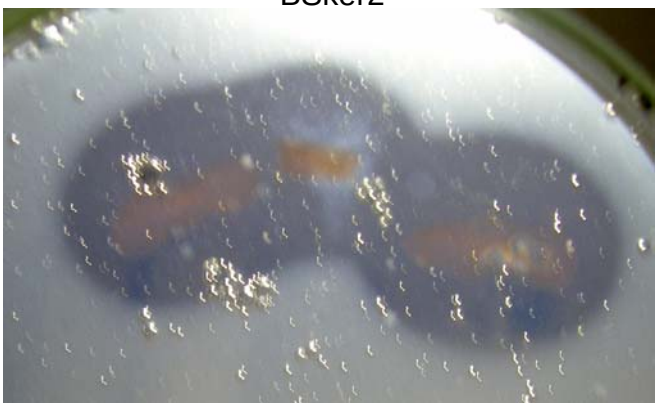
Supplementary table S1. Comparative composition of PLFA in BSker strains and related type species within the *Rhodothermaeota*. Compounds above 5% are in bold. BSker strains were grown with casein at 4 M total Na^+, pH 9.5 (BSker2) or pH 9 (BSker3), 37°C until late exponential growth phase.

Compound	**BSker2**	**BSker3**	*Longimonas halophila*[a]	*Salisaeta longa*[b]
12:0		0.8		
14:0	1.2	1.9	0.9	2.4
i15:0	2.7	2.5	**9.4**	**10.9**
ai15:0	0.7			
15:0			0.6	2.3
i16:0	**10.9**	**9.9**	**13.1**	**8.5**
16:0	**6.9**	**7.7**	**7.1**	**22.1**
16:1 ω7c	**21.9**	**25.8**	**23.9**	**27.9**
OH16:0	1.1			
i17:1ω9c	3.0	**6.8**	1.4	1.7
ai17:1ω9c		0.5		
i17:0	**11.7**	**7.4**	1.6	3.1
ai17:0		**9.8**	**6.2**	**5.1**
17:1ω6c	1.1			
3-OH i17:0	**5.3**	4.8	2.7	2.9
i18:1ω9c	3.8			
18:1ω9c		**5.0**		
18:1ω7c	**18.2**	**14.4**	**11.5**	1.2
18:0	1.6	2.7		

Xia J, ZhouYX, Zhao LH, Chen GJ, Du ZJ. *Longimonas halophila* gen. nov., sp. nov., isolated from a marine solar saltern. *Int J Syst Evol Microbiol* 2015; 65: 2272-2276.

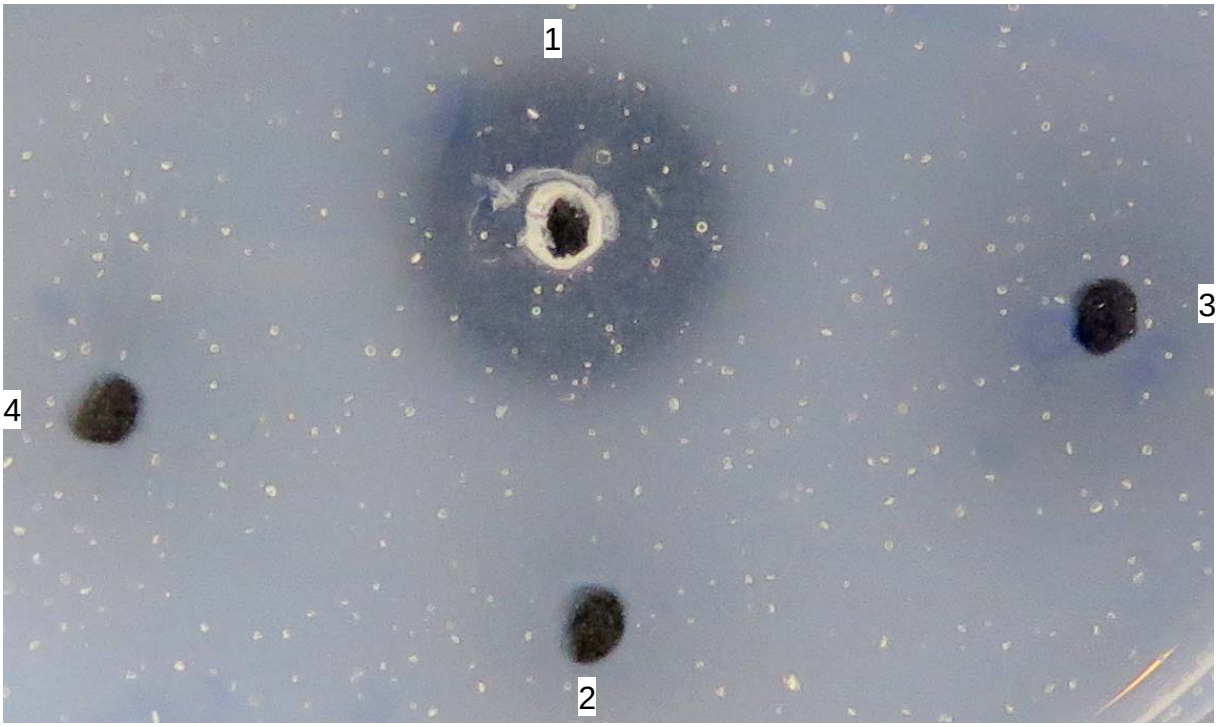
Vaisman N, Oren A. *Salisaeta longa* gen. nov., sp. nov., a red, halophilic member of the *Bacteroidetes*. *Int J Syst Evol Microbiol* 2009; 59, 2571-2574.

BSker2



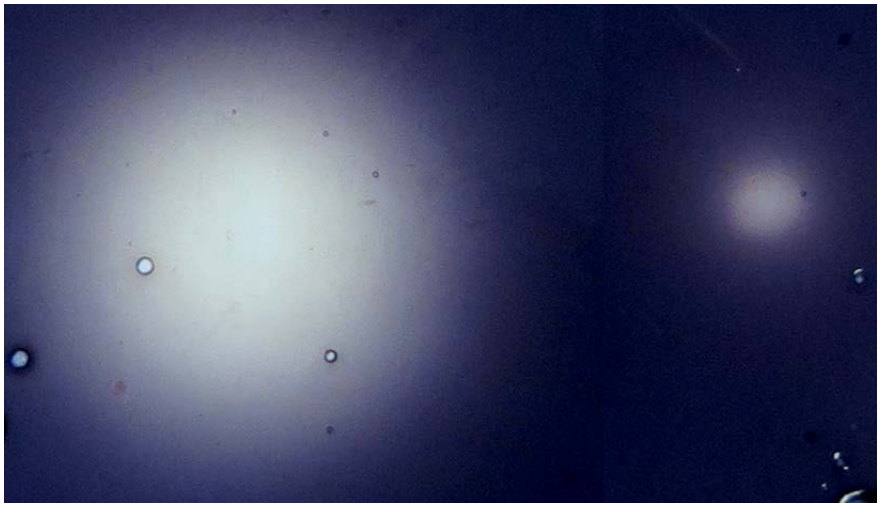
BSker3

Colonies of extremely haloalkaliphilic proteolytic strains on casein agar at 4 M total Na^+ and pH 9.2. The halos around the colonies indicate the zone of casein hydrolysis



Qualitative measurement of proteilytic activity in cell fractions of strain BSker2 by the agar diffusion approach (4 M total Na^+, pH 9.5; 37°C, 48 h).
1 – cells lyzate;
2 – culture supernatant;
3 – supernatant fraction x10 concentrated> 30 kDa;
4 – supernatant x10 concentrated 10-30kDa

Supplementary fig.S3. Proteolytic activity of extremely haloalkaliphilic isolates from hypersaline alkaline lakes



Supplementary fig.S4. Detection of alpha-amylase activity BSker isolates Single colony was grown on agar medium (4 M Na^+, pH 9.1) containing 0.1 g/L casein hydrolysate and 1 g/L soluble starch. After incubation for 5 days at 37^{o}C, The plate was flooded with 50 mM J_2 solution. Left – BSker2; right – BSker3