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Diversity of cultivated aerobic poly-hydrolytic bacteria in saline alkaline soils

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Alkaline saline soils known also as “soda solonchaks” represent a natural soda habitat which differed from soda lake sediments by higher aeration and lower humidity. The microbiology of soda soils, in contrast to the more intensively studied soda lakes, remains poorly explored. In this work we present information on the diversity of culturable aerobic haloalkalitolerant bacteria with various hydrolytic activities from soda soils at different locations in Central Asia and Africa. In total, 180 isolates were obtained by using media with various polymers at pH 10 and 0.6 M total Na^+. According to the 16S rRNA gene sequences analysis, most of the isolates belonged to *Firmicutes* and *Actinobacteria*. Most isolates possessed multiple hydrolytic activities, including endoglucanase, xylanase, amylase and protease. The pH profiling of selected representatives of actinobacteria and endospore-forming bacteria showed, that the former were facultative alkaliphiles, while the latter were mostly obligate alkaliphiles. The hydrolases of both groups were active at a broad pH range from 6 to 11. Overall, this work demonstrated the presence of a rich hydrolytic bacterial community in soda soils which might be explored further for production of haloalkalistable hydrolases.

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Diversity of cultivated aerobic poly-hydrolytic bacteria in saline alkaline soils

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Abstract

Alkaline saline soils known also as "soda solonchaks" represent a natural soda habitat which differed from soda lake sediments by higher aeration and lower humidity. The microbiology of soda soils, in contrast to the more intensively studied soda lakes, remains poorly explored. In this work we present information on the diversity of culturable aerobic haloalkalitolerant bacteria with various hydrolytic activities from soda soils at different locations in Central Asia and Africa. In total, 180 isolates were obtained by using media with various polymers at pH 10 and 0.6 M total Na^+. According to the 16S rRNA gene sequences analysis, most of the isolates belonged to *Firmicutes* and *Actinobacteria*. Most isolates possessed multiple hydrolytic activities, including endoglucanase, xylanase, amylase and protease. The pH profiling of selected representatives of actinobacteria and endospore-forming bacteria showed, that the former were facultative alkaliphiles, while the latter were mostly obligate alkaliphiles. The hydrolases of both groups were active at a broad pH range from 6 to 11. Overall, this work demonstrated the presence of a rich hydrolytic bacterial community in soda soils which might be explored further for production of haloalkalistable hydrolases.

Keywords: soda solonchak soils, aerobic hydrolytics, haloalkaliphilic, *Actinobacteria*, *Bacillus*

Running title:

poly-hydrolytic bacteria from solonchaks

Introduction

For a long time already alkaliphilic aerobic hydrolytic bacteria have attracted attention as sources of alkali-stable hydrolases for various industrial applications, primarily enzymatic laundry detergents (reviewed by: Horikoshi 2004; 2006; Fujinami & Fujisawa, 2010; Grant & Heaphy, 2010; Sarethy et al., 2011; Zhao et al., 2014; Mamo & Mattiasson, 2016). Most of this research was done with non-halotolerant *Bacillus* species producing alkalistable proteases, amylases and endoglucanases. In contrast, only a few salt tolerant alkaliphilic hydrolytics have been isolated and characterized from saline alkaline (soda) lakes. So far, the majority of known soda lake hydrolytics are found among anaerobic fermenters. A low salt-tolerant *Clostridium alkalicellulosi* is so far the only truly anaerobic cellulolytic bacterium able to grow on crystalline cellulose found in soda lakes (Zhilina et al., 2005). Pectin utilization for growth at haloalkaline conditions has been demonstrated in two fermentative anaerobic haloalkaliphiles: *Natronoflexus pectinovorans* (*Bacteriodetes*) and *Natranaerovirga hydrolytica* (*Clostridia*) at moderate and high salinity, respectively (Sorokin et al., 2011; 2012a). Two groups of fermentative haloalkaliphilic bacteria narrowly specialized in the utilization of chitin as a growth substrate have been found in hypersaline soda lakes. They formed two classes, *Chitinivibrionia* (high salt-tolerant) and *Chitinispirilla* (low salt-tolerant) assigned into the phylum *Fibrobacteres* (Sorokin et al., 2012b; 2016). A single example of an anaerobic proteolytic natronophile isolated from soda lake decaying phototrophic biomass, *Proteivorax tanatarense* (*Clostridiales*), has been recently published (Kevbrin et al., 2013).

Very few examples of aerobic hydrolytic haloalkaliphiles have been characterized from soda lakes, with most of the work done on alkaline protease producers. The low to moderately salt-tolerant organisms are represented by a well-studied salt-tolerant gammaproteobacterium *Alkalimonas amylolytica* producing amylase (Ma et al., 2004), *Alkalibacillus* sp. (*Firmicutes*), *Nesterenkonia* sp. (*Actinobacteria*) and *Salinivibrio* sp. (*Gammaproteobacteria*) producing haloalkalitolerant serine proteases (Abdel-Hamed et al., 2016; Gessesse et al., 2003; Lama et al, 2005), gammaproteobacteria from the genus *Marinimicrobium* and actinobacteria utilizing chitin (Sorokin et al., 2012b).

Furthermore, a unique group of aerobic extremely halo(natronophilic) hydrolytic *Euryarchaeota* is also present in hypersaline soda lakes. The previous findings characterized highly haloalkalistable protease-producing *Natronococcus occultus*, *Natrialba magadii, Natronolimnobius innermongolicus* (Studdert et al., 2001; de Castro et al., 2008; Selim et al., 2014) and amylolytic *Natronococcus amylolyticus* (Kobayashi et al., 1992). Recently we also demonstrated a presence of four novel genus-level groups of natronoarchaea in soda lakes capable of growth on insoluble celluloses and chitin (Sorokin et al., 2015).

However, another type of mainly aerobic soda habitats, saline alkaline soils, also called soda solonchaks, remains practically unexplored as a potential source of aerobic haloalkaliphilic hydrolytics. These habitats differ significantly from soda lakes in that, in contrast to the mostly anoxic soda lake sediments, soda soils are well aerated and dry during most of the year. Such conditions should favour domination by aerobic spore-forming *Firmucutes* and *Actinobacteria*, as has been shown in our recent exploration of bacterial nitrogen fixation in such habitats (Sorokin et al., 2008). Soda solonchaks are located in patches in dry steppe and semi-desert areas, such as south Siberia, north-eastern Mongolia, northern China, Egypt, India, Pakistan, Hungary and North American Steppes. In many cases they are hydromorphic and associated with high-standing saline, alkaline ground waters and often occur in the vicinities of saline alkaline (soda) lakes (Basilevich, 1970; Kondorskaya, 1965).

In this paper we describe a previously unexplored culturable diversity of aerobic haloalkalitolerant hydrolytic bacteria recovered from saline alkaline soils of several regions in Central Asia, Africa and North America.

Materials and Methods

Sample characteristics

Surface soil samples (0-5 cm depth) were collected into sterile plastic Petri dishes at 5 locations in Central Asia, Egypt and California. Each individual sample comprised a composite of 4 pseudo-replicate soils in a 3-5 m^2 area. Samples from Kenya and Tanzania were collected in sterile plastic bags (Whirl-Pak®) and vials using disposable sterile tongue depressors as described previously (Duckworth et al., 1996). The samples were kept at 4^oC before analysis. At most locations, the top soil layer was desiccated at the sampling time with a 20% maximum content of moisture. The selection of the samples was based on an immediate measurement of pH of a 1:5 water extract using a field pH-conductivity meter (model WTW 340i, Weilheim, Germany). Only those soils showing the pH of the water extract above 9.5 were selected for sampling. In total, more than 70 saline alkaline soil samples were obtained. Some of their characteristics are presented in **Table 1**. The content of total soluble salts was estimated in the laboratory by gravimetry after extraction of 2 g dry soil homogenized with 5 ml water followed by filtration through 0.2 µm filter and drying at 105^oC. Carbonate alkalinity in the soluble fraction was determined by acid titration monitored by a pH meter, using 5 g dry soil extracted with 20 ml water and after centrifugation at 10,000 x g for 10 min a 10 ml aliquot was titrated to pH 4.5 with 0.1 M HCl providing the value of total soluble carbonate alkalinity ($NaHCO_3$+Na_2CO_3).

Enrichment, isolation and cultivation of pure cultures of haloalkaliphilic aerobic hydrolytic bacteria

The general methods for the cultivation of aerobic alkaliphiles have been described elsewhere (Grant, 2006). The basic sodium carbonate mineral medium for cultivation of moderately salt-tolerant alkaliphiles contained 0.6 M total Na^+ and 1 g l^{-1} K_2HPO_4 and was strongly buffered at pH 10. After sterilization, the medium was supplemented with 1 mM $MgSO_4 \cdot 7H_2O$ and trace metal solution (Pfennig & Lippert, 1966). The enrichments were performed in 20 ml medium contained in 100 ml bottles closed with rubber septa (to prevent evaporation during prolonged incubation) inoculated with 1 g soil. Incubation was performed on a rotary shaker at 100 rpm and 28°C. After achieving growth and polymer degradation, the cultures were plated on solid media of the same composition. Five different polymers were used as substrates at concentration 1 g l^{-1}: CMC, soluble starch, casein, powdered alpha-keratin and emulsified olive oil prepared according to Sorokin and Jones (2009). Testing of pure cultures also included 3 additional polymers: beech xylan, amorphous cellulose and chitin prepared as described by Sorokin et al. (2015). In the case of CMC, xylan and olive oil, the solid medium was supplemented with 0.2 g l^{-1} and in the case of chitin and starch - with 20 mg l^{-1} yeast extract. Growth of the xylanase-positive cultures on xylan was also tested in liquid culture containing 20 mg l^{-1} yeast extract. The pure cultures were isolated from individual colonies and checked for purity by repeated re-inoculation on to solid media. The culture purity and endospore formation was also checked by phase contrast microscopy (Zeiss Axioplan Imaging 2, Göttingen, Germany) and, finally, by nucleotide sequencing. The pH profiling of growth and hydrolytic activities was performed on solid media containing 0.6 M total Na^+ in the form of either NaCl (for pH 5-8) or $NaHCO_3$-Na_2CO_3 (for the pH range 8 to 11). The media at pH range 5 to 8 were buffered with a mixture of potassium phosphates (50 mM) and HEPES (50 mM).

Detection of hydrolytic activities

All activities were detected using plate assays. Beta-1,4-endoglucanase and endoxylanase activities were visualized by using sequential flooding of the plates with 0.1% (w/v) Congo Red and 1 M NaCl each with 30 min incubation. The hydrolysis of keratin, emulsified olive oil, and amorphous chitin and cellulose was directly observed by formation of clarification halos around the colonies. The hydrolysis of casein was visualized by flooding the plates with 10% (w/v) trichloroacetic acid. For several strains the pH profile and thermotolerance of endoglucanase activity were measured in culture supernatant by agar diffusion approach and measurements of reducing sugar release with DNS (Miller, 1959).

16S rRNA gene sequence and analysis

Genomic DNA was extracted from colony biomass using alkaline SDS cell lysis at 65°C for 30 min followed by pH neutralization and DNA purification using the Wizard MaxiPreps Purification resin (Promega). The 16S rRNA gene was amplified with eubacterial forward primer 11f and the reverse universal primer 1495r. The obtained sequences were analyzed using

SILVAngs web interface (Quast et al., 2013) on 07.03.2017. The Project summary and settings are shown in Supplementary Table S1. The 16S rRNA gene sequences of 13 isolates, possibly representing novel taxa, together with the most identical sequences from the Genbank, verified by BLASTn, were aligned in MAFFT 7 (Katoh et al., 2002). The Maximum Likelihood phylogenetic analysis with General Time Reversible model (*G+I*, 4 categories, Nei & Kumar, 2000) was performed in MEGA 6 (Tamura et al., 2013).

Results

Isolation and identification of pure cultures of aerobic hydrolytics from saline alkaline soils

A total of 179 strains with one of five polymer degrading activities have been isolated. From the general colony morphology and microscopy, the isolates were obviously dominated by two large groups - actinomycetes (formation of aerial or substrate mycelium) and endospore-forming bacilli. Furthermore, isolates obtained with proteins as substrate also included Gram negative bacteria. The identification by 16S rRNA gene sequencing generally confirmed this conclusion. The two largest groups of isolates from the saline soda soils are typical hydrolytics belonging to the phyla *Actinobacteria* and *Firmicutes* (**Fig. 1, Table 2**) which may reflect a combination of the specific habitat (**Suppl. Table S**2), sampling methods and culture conditions (Duckworth et al., 1996).

The general phylogenetic distribution of the isolates is shown on a Krona diagram, obtained in the course of SILVAngs analysis (**Fig. 1**) and in the sample-dependent taxa clustering (**Suppl. Table S1**). The *Actinobacteria* were mostly represented by two genera -

Nocardiopsis and *Streptomyces*, and they were closely related to halotolerant alkaliphilic strains and species of these two genera found previously in haloalkaline habitats, in particular in Kenyan and Chinese soda lakes and saline alkaline soils (Grant & Jones, 2016). The relatively low diversity within the otherwise extremely diverse genera of these *Actinobacteria* indicates that haloalkaline conditions are rather selective for a few highly adapted species. Only two isolates from this group were distantly related to known species. One strain might represent a new genus in the *Micromonosporacea* with a closest relative from the genus *Salinispora*, while the second isolate is a distant member in the family *Glycomycetaceae* (**Suppl. Fig. S1A** and **S1B,** respectively).

Same low genetic diversity was also observed in the second largest group represented by the genus *Bacillus*. Most of the isolates were closely related to the known alkaliphilic (*B. pseudofirmus*, *B. horokoshii* and *B. akibai*), or haloalkaliphilic (*B. halodurans*, *B. daliensis*, and *B. alkalisediminis*) species. The only exception was a single isolate only distantly related (95% sequence similarity) to *B. mannanilyticus* - a low salt-tolerant alkaliphilic species producing beta-mannanase (Akino, Nakamura & Horikoshi, 1987; Nogi, Takami & Horikoshi, 2005). (**Suppl. Fig. S1C**).

A relatively minor group of isolates enriched with proteins belonged to the proteobacterial class *Gammaproteobacteria*. A subgroup of three isolates was closely related (99% sequence similarity) to species of the genus *Alkalimonas*, a known amylolytic haloalkaliphile (Ma et al., 2004). Four isolates were closely related to a haloalkaliphilic member of the genus *Aliidiomarina*, *A. soli*, isolated from a soda soil in Inner Mongolia (Xu et al., 2017). The third gammaproteobacterial subgroup is represented by 4 proteolytic strains distantly related to organisms in the genus *Lysobacter* in the *Xanthomonadaceae* (95-96% sequence similarity).

Three out of four strains of this subgroup clustered with an undescribed haloalkaliphilic isolate from Mono Lake (ML-122, 99% similarity), while the fourth strain was distant (96% similarity to ML-122). Therefore, this subgroup probably consists of two novel species and together with the Mono Lake strain ML-122 might represent a new genus in the family *Xanthomonadacea* (**Suppl. Fig. S1D**).

Finally, a significant group of actinobacteria with strong polyhydrolytic potential belonged to the *Cellulomonas/Isoptericola* clad within the family *Promicromonosporaceae* (**Suppl. Fig. S1E**). The *Cellulomonas* species are known for their cellulolytic activity and include a haloalkaliphilic isolate from a Kenyan soda lake (Jones et al., 2005), while the genus *Isoptericola* mostly include halotolerant representatives, although the described neutrophilic species apparently have only a limited hydrolytic activity (Schumann & Stackebrandt, 2014).

Hydrolytic spectrum of the soda soil isolates

Most of the actinobacteria and bacilli isolates enriched with CMC or starch, were polyhydrolytic, being able to degrade all tested polymers, except for the insoluble native cellulose and chitin (**Table 3**). Only three actinobacterial isolates showed the ability to hydrolyse amorphous cellulose on the plate assay and only one of the three (DS33), a relative of *Salinispora*, was actually capable of growth with cellulose as substrate. Six isolates showed a potential to grow with amorphous chitin (**Table 3**). On the other hand, most of the endo-glucanase and endoxylanase positive actinobacteria and bacilli isolates utilized beech xylan as the growth substrate, which indicates that they are rather specialized in the mineralization of soluble hemicelluloses.

The isolates enriched with proteins belonged to the *Gammaproteobacteria* and *Firmicutes.* All of them, as expected, showed highest hydrolytic potential with against casein, and many of them did not have endoglucanase, endoxylanase or lipase activities (**Table 3**). So, they can be considered as dedicated proteolytics. Indeed, proteolytics are the most well-studied group of alkaliphilic hydrolytics.

For the pH profiling, four strains from actinomycetes and from bacilli were selected for test on solid medium containing 0.6 M total Na^+ with CMC+yeast extract as substrate. The solid medium is not optimal for the profiling but it was chosen for two reasons: 1) the mycelium-forming actinomycetes do not grow homogenously in liquid media and their growth is often estimated by radial colony increase; 2) test on solid medium permitted simultaneous estimation of both growth and endoglucanase activity. The results (**Table 4**) demonstrated that the tested actinomycetes are facultative moderate alkaliphiles, while the bacilli isolates are obligate alkaliphiles. The endoglucanase activity of both groups had a very broad pH range from 6 to 11 with an optimum for actinomycetes from 8 to 10 and for the bacilli from 9 to 10.5.

Overall, the results of this study demonstrated that saline alkaline soils represent a potentially valuable resource of aerobic haloalkaliphilic bacteria capable of producing multiple alkalistable hydrolytic enzymes. Most of the haloalkaliphilic polyhydrolytic isolates belong to *Actinobacteria* (genera *Streptomyces* and *Nocardiopsis*) and the genus *Bacillus*.

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Figure legend

Figure 1. Distribution of 179 sequences of hydrolytic haloalkaliphilic bacterial isolates, created by SILVAngs service.

Figure 1(on next page)

Figure 1

Distribution of 179 sequences of hydrolytic haloalkaliphilic bacterial isolates, created by SILVAngs service.

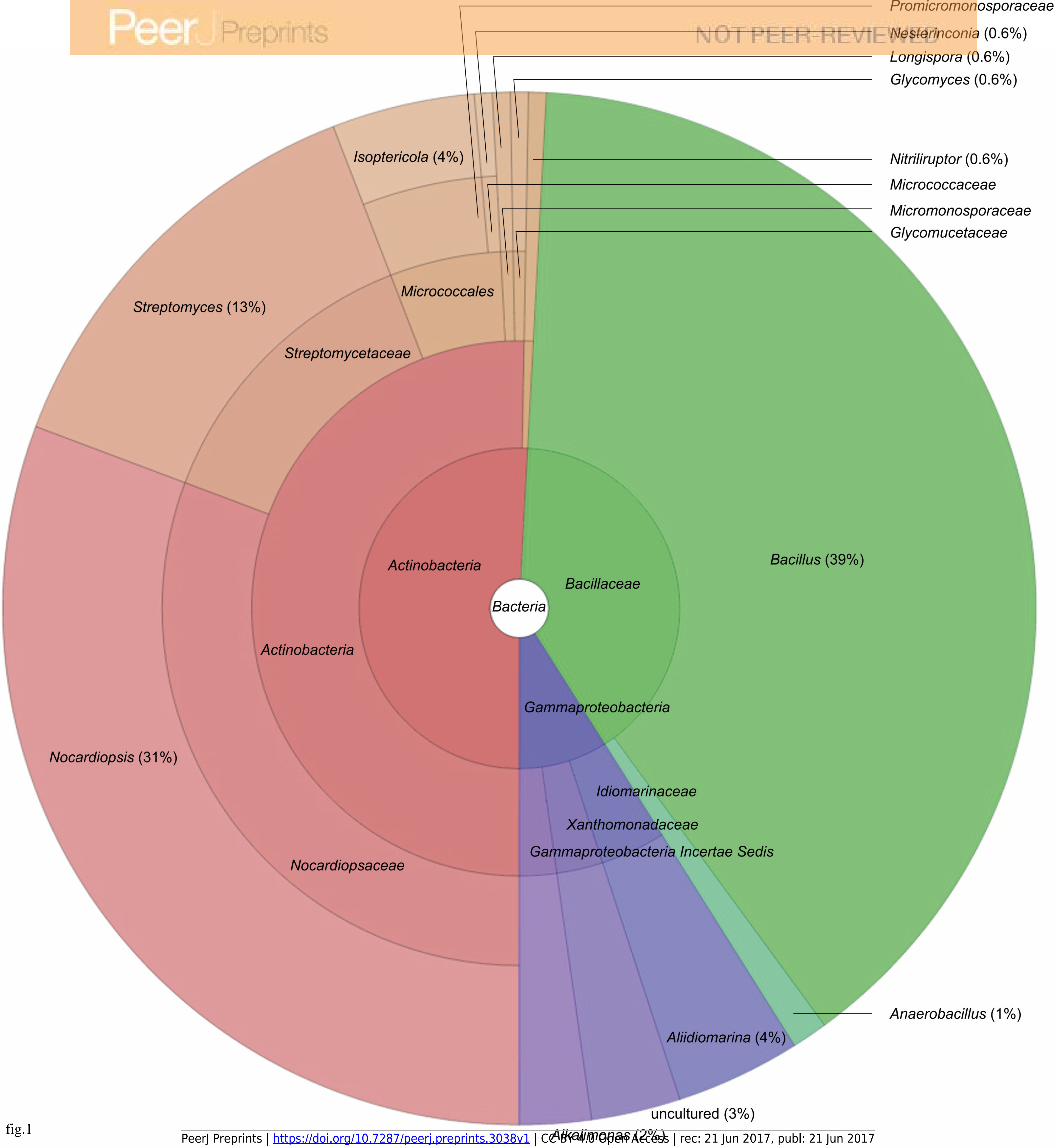
Promicromonosporaceae
Nesterinconia (0.6%)
Longispora (0.6%)
Glycomyces (0.6%)
Nitriliruptor (0.6%)
Micrococcaceae
Micromonosporaceae
Glycomucetaceae
Isoptericola (4%)
Micrococcales
Streptomyces (13%)
Streptomycetaceae
Actinobacteria
Bacteria
Bacillaceae
Bacillus (39%)
Actinobacteria
Gammaproteobacteria
Idiomarinaceae
Xanthomonadaceae
Gammaproteobacteria Incertae Sedis
Nocardiopsis (31%)
Nocardiopsaceae
Anaerobacillus (1%)
Aliidiomarina (4%)
uncultured (3%)
Alkalimonas (2%)

fig.1

Table 1(on next page)

Tables 1-4

Table 1. Characteristics of soda solonchak soils and lacustrine dry soda mad samples. Sample code: **AA** - Ararate valley, Armenia; **BS** – Barabinskaya Steppe, Novosibirsk region, Russia; **KUS** - Kunkurskay steppe, Buriatia, RF; **KS** – Kulunda Steppe, Altai region, Russia; **MS** – north-eastern Mongolia, Choibalsan province; **EWN** – Wadi al Natrun valley, Libyan Desert, Egypt; **MLC** - Mono Lake, California; **KT** - Kenya-Tanzania. Sample type: SS - continental soda solonchak soil; SLM - dry soda mad near soda lakes.

Table 2. Strains of hydrolytic aerobic haloalkaliphilic bacteria isolated from soda solonchak soils. Potential new species are highlighted in bold (<97-98% 16S-rRNA gene identity) and in red (<97% identity).

Table 3: Polymer hydrolysis and utilization for growth by aerobic haloalkaliphiles from soda soils. CMCase - 4 d, Xylanase, protease, amylase - 3 d; lipase - 10d; amorphous cellulose and chitin - 30 d; *positive on amorphous cellulose; **growth on amorphous cellulose; $^{\#}$growth on chitin. ϕ col - colony diameter, mm; ϕ zone - hydrolysis zone diameter, mm. Highlights: on the basis of activity to colony diameter ratio: highly active -in bold; most active - in red.

Table 4. Influence of pH on growth and endoglucanase activity of soda solonchak alkaliphiles : average profiles estimated from individual results for eight isolates: actinomycetes - *Nocardiopsis* DS50, 51; *Streptomyces* DS8,9; *Bacillus*: DS85, 100, 101, 102.

Table 1. Characteristics of soda solonchak soils and lacustrine dry soda mad samples. Sample code: **AA** - Ararate valley, Armenia; **BS** – Barabinskaya Steppe, Novosibirsk region, Russia; **KUS** - Kunkurskay steppe, Buriatia, RF; **KS** – Kulunda Steppe, Altai region, Russia; **MS** – north-eastern Mongolia, Choibalsan province; **EWN** – Wadi al Natrun valley, Libyan Desert, Egypt; **MLC** - Mono Lake, California; **KT** - Kenya-Tanzania. Sample type: SS - continental soda solonchak soil; SLM - dry soda mad near soda lakes.

General information				pH of 1:5 water extract	Total soluble salts (g/kg)	Soluble carbonate alkalinity (mM)
Sample code	Number of samples	Year of sampling	Sample type			
AA	10	1988	SS	9.45-10.2	12-388	20-1870
KUS	4	1998	SS	9.2-9.9	26-96	23-40
BS	2	1998	SS	9.71-10.70	25-60	10-502
KS	14	2003	SS	9.60-10.21	53-385	150-1520
MS	24	1999	SS	9.70-10.80	12-128	10-1140
EWN	3	2000	SS	10.05-10.30	85-102	750-1740
MLC	4	2001	SLM	9.2-9.8	30-43	130-240
KT	16	1988; 1996; 1999	SLM	9.6-10.7	43-160	45-890

Table 2. Strains of hydrolytic aerobic haloalkaliphilic bacteria isolated from soda solonchak soils. Potential new species are highlighted in bold (<97-98% 16S-rRNA gene identity) and in red (<97% identity).

Isolate code	Source	Colony morphology			Phylogeny	
	Sample code	mycelium	pigment aerial/ substrate	Endo-spores	Closest relative	% similarity
DS47	**AA**	+		-	*Nocardiopsis alba*	99
DS48					*Nocadiopsis alba*	98
DS49					*Nocadiopsis sinuspersici*	99
DS50					*Nocardiopsis* sp. YIM 80133 (haloalkaliphile)	99
DS51					*Nocardiopsis* sp. E-143 (haloalkaliphile)	99
DS53					*Nocardiopsis* sp. E-143 (haloalkaliphile)	99
DS54			-/red		*Nocardiopsis* sp. E-143 (haloalkaliphile)	99
DS55					*Streptomyces sodiiphilis* (haloalkaliphile)	**97**
DS56					*Nocardiopsis alba*	99
DS63					*Nocardiopsis* sp. E-143 (haloalkaliphile)	99
DS64					*Nocardiopsis* sp. E-143 (haloalkaliphile)	99
DS65			gray		*Streptomyces alkaliphilus* (haloalkaliphile)	99
DS66					*Nocardiopsis* sp. YIM 80130 (haloalkaliphile)	99
DS67					*Nocardiopsis sp.* AACh2 (haloalkaliphile)	99
DS68					*Nocardiopsis* sp. YIM 80130 (haloalkaliphile)	99
DS69					*Nocardiopsis* sp. E-143 (haloalkaliphile)	99
DS70					*Streptomyces alkalithermophilus* (alkaliphile)	**97**
DS71			gray/red		*Streptomyces alkaliphilus* (haloalkaliphile)	99
DS72	**KUS**	-		+	*Bacillus sp. E-141* (haloalkaliphile)	99
DS73		+		-	*Nocardiopsis sp.* AACh2 (haloalkaliphile)	99
DS74					*Nocardiopsis sp.* AACh2 (haloalkaliphile)	99
DS75					*Nocardiopsis* sp. E-143 (haloalkaliphile)	99
DS76					*Nocardiopsis* sp. E-143 (haloalkaliphile)	99
DS78					*Nocardiopsis* sp. YIM 80130 (haloalkaliphile)	99
DS79					*Nocardiopsis sp.* AACh2 (haloalkaliphile)	99
DS1		+	-	-	*Streptomyces sodiiphilus* (haloalkaliphile)	**97**
DS11		-			*Nitriliruptor alkaliphilus* (haloalkaliphile)	98
DS2		+			*Nocardiopsis exhalans* VTT E-063001	99
DS3					*Nocardiopsis* sp. YIM 80251 (haloalkaliphile)	99
DS4					*Nocardiopsis* sp. E-143 (haloalkaliphile)	99
DS180			reddish		*Nocardiopsis ganjiahuensis* (haloalkaliphile)	100
DS181			-		*Nocardiopsis sp.* AACh2 (haloalkaliphile)	99
DS13					*Nocardiopsis* sp. E-143 (haloalkaliphile)	99
DS115		-			*Alkalimonas amylolytica* (haloalkaliphile)	99
DS6	**BS**	+		+	*Bacillus horikoshii* (alkaliphile)	100
DS7			gray	-	*Streptomyces sodiiphilus* (haloalkaliphile)	**97**
DS8					*Streptomyces sodiiphilus* (haloalkaliphile)	**97**
DS9			gray		*Streptomyces alkaliphilus* (haloalkaliphile)	99
DS10					*Nocardiopsis exhalans* VTT E-063001	99
DS124		-	-	-	*Aliidiomarina maris*	99
DS125			-	-	*Alkalimonas collagenimarina* (haloalkaliphile)	99
DS126			-	+	*Bacillus pseudofirmus* (alkaliphile)	99
DS127			orange	+	*Bacillus pseudofirmus* (alkaliphile)	99
DS128			orange	+	*Bacillus pseudofirmus* (alkaliphile)	99
DS129			-	+	*Bacillus pseudofirmus* (alkaliphile)	99
DS130			-	-	*Alkalimonas amylolytica* (haloalkaliphile)	99
DS131			orange	-	*Bacillus pseudofirmus* (alkaliphile)	100
DS36	**KS**	+	gray	-	*Streptomyces sodiiphilus* YIM 80305 (haloalkaliphile)	99
DS37			gray		*Streptomyces alkaliphilus* (haloalkaliphile)	99
DS38			biege/red		*Nocardiopsis* sp. E-143 (haloalkaliphile)	99
DS39			-/brown		*Streptomyces* sp. E-070 (haloalkaliphile)	99
DS40			beige		*Nocardiopsis* sp. YIM 80129 (haloalkaliphile)	99
DS41			beige		*Nocardiopsis sp.* AACh2 (haloalkaliphile)	99
DS42			beige		*Streptomyces alkalithermotolerans* (haloalkaliphile)	**97**
DS43			beige		*Streptomyces sodiiphilus*	99
DS44			-		*Nocardiopsis* sp. E-143 (haloalkaliphile)	99
DS45			-		*Nocardiopsis* sp. YIM 80129 (haloalkaliphile)	100
DS46			gray		*Streptomyces* sp. E-070 (haloalkaliphile)	99

DS57			beige		*Nocardiopsis* sp. YIM 80133 (haloalkaliphile)	99
DS58			-		*Streptomyces sodiiphilus* YIM 80305 (haloalkaliphile)	**97**
DS59			-		*Streptomyces sodiiphilus* YIM 80305 (haloalkaliphile)	**97**
DS60		+	-	-	*Isoptericola halotolerans*	99
DS61			beige		*Streptomyces sunnurensis*	98
DS62			olive		*Nocardiopsis* sp. E-143 (haloalkaliphile)	99
DS174	**KS**	-	-/red	-	*Nocardiopsis* sp. E-143 (haloalkaliphile)	99
DS175			-		*Nocardiopsis* sp. E-143 (haloalkaliphile)	99
DS176			-		*Nocardiopsis* sp. E-143 (haloalkaliphile)	99
DS177			gray/viol		*Streptomyces alkaliphilus* (haloalkaliphile)	99
DS178			-		*Nocardiopsis* sp. YIM 80034 (haloalkaliphile)	100
DS179			-		*Aliidiomarina soli* (haloalkaliphile)	98
DS88			yellow		*Isoptericola halotolerans*	99
DS89			-	+	*Bacillus daliensis* (haloalkaliphile)	99
DS113			orange	-	*Bacillus daliensis* (haloalkaliphile)	99
DS160			yellow	+	*Bacillus horokoshii* (alkaliphile)	99
DS161			-	+	*Bacillus horokoshii* (alkaliphile)	99
DS162			yellow	-	*Xanthomonadacea* ML-122 (haloalkaliphile) ***Rehaibacterium terrae***	**97** **95**
DS163			-	+	*Bacillus pseudofirmus* (alkaliphile)	100
DS164			yellow	-	*Isoptericola halotolerans*	99
DS165			-	+	*Bacillus pseudofirmus* (alkaliphile)	99
DS166			-	+	*Bacillus pseudofirmus* (alkaliphile)	99
DS167			-	-	*Aliidiomarina soli* (haloalkaliphile)	99
DS168			-	+	*Bacillus pseudofirmus* (alkaliphile)	99
DS169			-	+	*Bacillus pseudofirmus* (alkaliphile)	99
DS170			-	-	*Xanthomonadacea* ML-122 (haloalkaliphile)	99
DS171			-	-	***Lysobacter*** spp.	96
DS172			-	+	*Bacillus pseudofirmus* (alkaliphile)	99
DS173			yellow	-	*Xanthomonadacea* ML-122 (haloalkaliphile) ***Lysobacter*** spp.	99 **95**
DS17	**MS**	+	beige	-	*Nocardiopsis sp.* E-143 (haloalkaliphile)	99
DS18			beige		*Nocardiopsis sp.* E-143 (haloalkaliphile)	99
DS19			gray		*Nocardiopsis sp.* E-143 (haloalkaliphile)	99
DS20			-		***Glycomycetacea* (halophiles)**	**92**
DS21			olive		*Nocardiopsis sp.* E-143 (haloalkaliphile)	99
DS22			-		*Nocardiopsis sp.* E-143 (haloalkaliphile)	99
DS23			beige		*Nocardiopsis* sp. YIM 80251 (haloalkaliphile)	99
DS24			beige		*Nocardiopsis* sp. YIM 80251 (haloalkaliphile)	99
DS25			beige		*Nocardiopsis sp.* E-143 (haloalkaliphile)	99
DS26			beige		*Nocardiopsis* sp. YIM 80133 (haloalkaliphile)	99
DS27			beige		*Nocardiopsis sp.* E-143 (haloalkaliphile)	99
DS28			-/brown		*Nocardiopsis* sp. YIM 80133 (haloalkaliphile)	99
DS29			-		*Nocardiopsis* sp. YIM 80133 (haloalkaliphile)	99
DS30			-		*Nocardiopsis sp.* E-143 (haloalkaliphile)	99
DS96		-	orange		*Bacillus halodurans* (haloalkaliphile)	99
DS97			yellow		*Isoptericola halotolerans*	99
DS99			-		*Isoptericola halotolerans*	99
DS100			orange	+	*Bacillus daliensis* (haloalkaliphile)	98
DS101			-		*Bacillus akibai* (alkaliphile)	99
DS102					*Bacillus alkalisediminis* (haloalkaliphile)	98
DS103					*Bacillus akibai* (alkaliphile)	99
DS104					*Bacillus alkalisediminis* (haloalkaliphile)	98
DS105					*Bacillus akibai* (alkaliphile)	99
DS106					*Bacillus alkalisediminis* (haloalkaliphile)	98
DS107					*Bacillus akibai* (alkaliphile)	99
DS108					*Bacillus alkalisediminis* (haloalkaliphile)	98
DS109					*Bacillus alkalisediminis* (haloalkaliphile)	98
DS110					*Bacillus akibai* (alkaliphile)	99
DS111			yellow	-	*Isoptericola halotolerans*	98
DS112			-	+	*Bacillus pseudofirmus* (alkaliphile)	99
DS144				+	*Bacillus pseudofirmus* (alkaliphile)	99
DS145				-	*Aliidiomarina soli* (haloalkaliphile)	99
DS146				-	*Aliidiomarina soli* (haloalkaliphile)	99
DS147				-	*Xanthomonadacea* ML-122 (haloalkaliphile) ***Lysobacter*** spp.	99 **95**

DS148				+	*Bacillus alkalinitrilicus* (haloalkaliphile)	99
DS149			orange	-	*Nesterenkonia xinjiangensis*	100
DS150			orange	+	*Bacillus daliensis* (haloalkaliphile)	98
DS151			-	+	*Bacillus halodurans* (haloalkaliphile)	100
DS152			-	+	*Bacillus horokoshii* (alkaliphile)	99
DS153			-	+	*Bacillus pseudofirmus* (alkaliphile)	99
DS154			greenish	-	*Alkalimonas amylolytica* (haloalkaliphile)	99
DS155			-	+	*Bacillus pseudofirmus* (alkaliphile)	99
DS156		-	-	-	*Aliidiomarina soli* (haloalkaliphile)	99
DS157				-	*Aliidiomarina soli* (haloalkaliphile)	99
DS158				+	*Bacillus pseudofirmus* (alkaliphile)	99
DS159				+	*Bacillus akibai* (alkaliphile)	99
DS31	**EWN**	+	gray	-	*Streptomyces* sp. E-070 (alkaliphile)	99
DS32			-		*Streptomyces* sp. E-070 (alkaliphile)	99
DS33			pink		***Salinispora arenicola*** NPS11684	**94**
DS34	**MLC**	+	gray	-	*Streptomyces* sp. YIM 80244 (haloalkaliphile)	97
DS35			beige		*Streptomyces* sp. E-070 (alkaliphile)	99
DS182	**KT**	+	olive	-	*Streptomyces alkaliphilus* (haloalkaliphile)	99
DS183			-		*Streptomyces* sp. E-070 (haloalkaliphile)	97
DS12					*Nocardiopsis* sp. YIM 80129 (haloalkaliphile)	99
DS14					*Nocardiopsis sp.* E-143 (haloalkaliphile)	99
DS15					*Nocardiopsis sp.* E-143 (haloalkaliphile)	99
DS16					*Streptomyces alkalithermotolerans* (haloalkaliphile)	98
DS62					*Nocardiopsis sp.* E-143 (haloalkaliphile)	99
DS81		-	-	+	*Bacillus okhensis* (haloalkalitolerant)	99
DS82			yellow		*Isoptericola halotolerans*	99
DS83					*Bacillus sp.* ABCh1 (haloalkaliphile	98
DS84			yellow		*Bacillus cellulolyticus* (alkaliphile)	99
DS85					*Bacillus cellulolyticus* (alkaliphile)	99
DS86			cream		Bacillus *pseudofirmis (*alkaliphile)	100
DS87					*Bacillus polygoni* (haloalkaliphile)	99
DS90					*Bacillus halodurans (*haloalkalitolerant)	100
DS91			yellow	-	*Isoptericola halotolerans*	99
DS92			yellow	-		
DS184			-	+	*Bacillus halodurans* (haloalkaliphile)	100
DS93					*Bacillus cellulolyticus* (alkaliphile)	100
DS94					*Bacillus vedderi* (alkaliphile)	98
DS95					*Bacillus akibai* (alkaliphile)	98
DS114			-	+	*Bacillus bogoriensis* (haloalkaliphile)	97
DS116					*Bacillus* sp. Z24-11 (haloalkaliphile)	100
DS117			orange	-	*Anaerobacillus alkalidiazotrophicus* (haloalkaliphile)	97
DS118			-	+	*Bacillus polygoni* (alkaliphile)	99
DS119					*Bacillus pseudofirmus* (alkaliphile)	100
DS120					*Bacillus pseudofirmus* (alkaliphile)	99
DS121					*Bacillus pseudofirmus* (alkaliphile)	99
DS122			cream		*Bacillus pseudofirmus* (alkaliphile)	98
DS123			-		*Anaerobacillus alkalidiazotrophicus* (haloalkaliphile)	97
DS132			cream		*Bacillus polygoni* (haloalkaliphile)	99
DS133			-		*Bacillus halodurans* (haloalkaliphile)	100
DS134			cream		*Bacillus clarkii* (alkaliphile)	99
DS135			-		*Bacillus polygoni* (haloalkaliphile)	99
DS136			cream		*Bacillus* sp. Z24-11 (haloalkaliphile)	99
DS137					*Bacillus pseudofirmus* (alkaliphile)	99
DS138					*Bacillus* sp. Z24-11 (haloalkaliphile)	99
DS139					*Bacillus polygoni* (haloalkaliphile)	100
DS140			-		*Bacillus alkalisediminis* (haloalkaliphile)	99
DS141			yellow		*Bacillus alkalinitrilicus* (haloalkaliphile)	99
DS142			-		*Bacillus alkalinitrilicus* (haloalkaliphile)	99
DS143			-		***Bacillus mannanilyticus*** (alkaliphile)	**96**

Table 3: Polymer hydrolysis and utilization for growth by aerobic haloalkaliphiles from soda soils. CMCase - 4 d, Xylanase, protease, amylase - 3 d; lipase - 10d; amorphous cellulose and chitin - 30 d; *positive on amorphous cellulose; **growth on amorphous cellulose; #growth on chitin. ϕ col - colony diameter, mm; ϕ zone - hydrolysis zone diameter, mm. Highlights: on the basis of activity to colony diameter ratio: highly active -in bold; most active - in red.

Strain code	Enriched with:	**CMC**		**Xylane**			**Starch**		**Casein**		**Olive oil**	
		activity		growth	activity		growth/activity		growth/ activity		activity	
		ϕ col	ϕ zone		ϕ col	ϕ zone	ϕ col	ϕ zone	ϕ col	ϕ zone	ϕ col	ϕ zone
Ds1	**CMC**		-		-		3	19	4	**30**	4	8
Ds11		4	16		-		5	20	-	-	-	-
Ds2		7	20	+	**6**	**30**	8	22	7	32	10	12
Ds3		8	24	+	**4**	**22**	4	25	6	30	8	11
Ds4		2	18	+	**6**	**27**	8	25	8	30	10	12
Ds180		7	19		**6**	**32**	8	28	9	30	8	13
Ds181		7	23		6	22	5	24	10	**35**	9	13
Ds6		1	12		-		3	20	4	25		-
Ds7		2	14	weak	**2**	**18**	3	24	5	22	8	13
Ds8*		2	14	+	2	15	4	20	3	20		-
Ds9		4	12	+	**5**	**25**	5	20	5	**35**	10	13
Ds10		6	17	+	**5**	**28**	7	24	10	30	15	17
Ds182#		3	16		**3**	**24**	3	28	5	**30**	5	8
Ds183		2	10		3	12	3	20	5	28		-
Ds12		6	18	+	**5**	**25**	7	24	10	25	12	14
Ds13		7	19	+	**5**	**26**	7	25	6	25	12	14
Ds14		5	17	+	**5**	**30**	9	25	5	25	12	14
Ds15		5	20			-	3	17		-	5	7
Ds16*		5	20	+	5	22	4	15	2	23	8	13
Ds17		6	21	+	**6**	**28**	8	24	6	22	10	12
Ds18		5	14	+	**5**	**25**	7	22	5	24	7	9
Ds19		7	16			-	7	25	4	**28**	10	12
Ds20		5	14			-		-	4	18		-
Ds21		7	17	+	2	18	9	32	5	27		-
Ds22		4	13			-	4	15	4	**25**	**2**	**10**
Ds23		6	16	+	**7**	**26**	6	30	5	20	10	12
Ds24		4	14	+	5	18	6	30	4	**28**	8	10
Ds25		4	12	+	**7**	**30**	9	27	5	22	10	12
Ds26		2	13			-	2	10	3	**25**	-	-
Ds27		5	15	+	**7**	**26**	10	26	4	**25**	10	11
Ds28		4	14	+	6	21	8	15	5	25	7	10
Ds29		2	9			-	4	9	3	24		-
Ds30		6	17		**7**	**26**	9	28	5	20	12	14
Ds31		8	17		**2**	**25**	5	23	6	22	10	13
Ds32		4	17		**3**	**23**	6	22	2	20	5	9
Ds33**		5	20	+	**2**	**28**	2	16	2	20		-
Ds34		3	12		**6**	**40**	5	30	5	23	6	10
Ds35		4	18	weak	4	20	3	20	6	22	**5**	**13**
Ds36		3	22	+	**4**	**23**	4	30	4	25	7	12
Ds37		3	10	+	3	12	6	25	6	28	6	9
Ds38		5	15	+	**4**	**25**	7	24	6	28	13	14
Ds39		2	12			-	6	25	2	12	10	10
Ds40		5	15	+	**7**	**23**	7	27	4	23	9	11
Ds41		6	16	+	**7**	**23**	5	23	5	27	9	11
Ds42		2	14			-		-	3	**27**	7	10
Ds43#		2	24		2	14	4	28	3	**32**	6	10
Ds44		5	20	+	**7**	**30**	8	27	5	22	9	12
Ds45		3	15	+	**5**	**30**	7	25	4	20		-
Ds46		2	10	+	**2**	**20**	4	22	3	20	8	10
Ds47		5	21	+	**5**	**23**	7	27	8	28	10	14
Ds48		3	15	+	4	17	4	20	4	20	8	10
Ds49		2	13	+	4	17	5	23	10	**35**	8	10
Ds50		3	15		**7**	**26**	5	14	6	17	8	10
Ds51		3	15	+	**5**	**23**	7	30	8	30	10	13
Ds53		3	18		-	-	2	20		-		-
Ds54		2	12	+	**6**	**24**	9	29	8	30	10	13

Ds55		4	15		**1**	**23**	4	22	4	25	5	7
Ds56		4	17	+	**5**	**23**	9	29	7	26	7	9

Ds81	**CMC**	2	10		**2**	**24**	5	24		-		-
Ds82		3	21	+	**4**	**24**	6	28	5	**30**	7	8
Ds83		2	15	weak	2	16	5	32		-		-
Ds84		3	19	weak	3	15	4	24	4	20		-
Ds85		3	14	weak	4	15	5	25	3	20		-
Ds86		**1.5**	**20**			-	4	28	5	**30**		-
Ds87		2	16	weak	**2**	**21**	4	17	3	12		-
Ds88		4	22		4	20	6	22	3	20		-
Ds89		3	12	+	**2**	**23**	3	25		-		-
Ds90		4	15	+	**3**	**27**	4	25		-		-
Ds91#		5	20	++	**3**	**29**	5	24	7	20	**8**	**15**
Ds92#		5	23	+	**6**	**28**	7	32	7	30	3	6
Ds93		3	18		3	15	4	10	7	15	3	7
Ds94		2	14			-	4	9	3	15	3	5
Ds95		2	8	+	**4**	**30**	4	23	4	11		-
Ds96		2	20	++	**3**	**26**	5	24	3	10		-
Ds97		3	22		5	14	5	28	4	15		-
Ds98		5	23	+	**6**	**24**	5	25		-	11	14
Ds99		2	21		3	14	4	20	2	8		-
Ds100		2	24	+	**2**	**27**	3	29		-		-
Ds101		3	22	+	**3**	**22**	6	32	1	12		-
Ds102		5	23		3	8	5	18		-		-
Ds103		3	28	+	**4**	**22**	5	28	1	10		-
Ds104		2	18		3	10	4	19	4	12		-
Ds105		3	27	+	**3**	**22**	**5**	**34**	4	21		-
Ds106		3	25		4	11	6	18	4	20		-
Ds107		3	27	weak	3	18	4	28	4	20		-
Ds108		3	28			-	5	18	5	23		-
Ds109		2	25		4	11	4	18	5	22		-
Ds110		2	27	+	**3**	**20**	**4**	**35**	7	25		-
Ds111#		3	26	+	**4**	**20**	3	25	5	17	7	7
Ds112		3	25	+	4	21	4	25	7	20		-
Ds113		2	13	weak	2	15	4	23		-		-
Ds184		5	12		**9**	**34**	6	25	4	25	**10**	**16**
Ds57	**casein**	5	20	+	4	19	8	26	5	**28**	12	14
Ds58			-	+	4	17		-	4	22		-
Ds59		4	17			-		-	2	16		-
Ds60		3	0	weak	4	23	5	24	5	17		-
Ds61		4	0			-	3	10	2	20		-
Ds62		1	7		2	14	3	24	3	20		-
Ds114			-	+	4	27	5	28	4	20		-
Ds115			-			-	5	30	4	20	4	10
Ds116			-		2	17	4	16	2	15		-
Ds117			-		2	10	5	20	3	20		-
Ds118			-		3	12		-	2	18	6	11
Ds119			-			-	3	**30**	4	18		-
Ds120			-		2	10	4	30	2	24		-
Ds121			-	+	6	29	3	**30**	3	24		-
Ds122			-		5	-		-	2	22		-
Ds123			-	+	4	17	4	15	4	20		-
Ds124			-			-		-	4	22		-
Ds125			-			-	5	20	5	24		-
Ds126			-			-	5	25	2	18		-
Ds127			-			-	4	28	2	12		-
Ds128			-			-	5	32	2	22		-
Ds129			-	+	3	13	4	32	3	20		-
Ds130			-			-	3	**40**	5	23	4	8

Ds131	casein		-	weak	2	10	3	**33**	4	15		-
Ds132			-		3	14		-	3	12		-
Ds133		4	20		3	**20**	7	25	5	15		-
Ds134			-	weak	2	19		-	2	20		-
Ds135			-		2	15		-	3	15		-
Ds136			-			-	4	20	3	17		-
Ds137			-			-	5	29	3	15		-
Ds138			-			-	4	28	2	14		-
Ds139			-		3	18		-	3	14		-
Ds140			-	weak	2	11	4	**33**	5	22		-
Ds141			-			-		-	2	14		-
Ds142			-			-		-	3	17	5	15
Ds143			-			-		-	3	22		-
Ds144			-			-	5	30	5	23		-
Ds145			-			-		-	5	19		-
Ds146			-			-		-	5	24		-
Ds147			-			-		-	4	22		-
Ds148			-		8	0		-	3	20		-
Ds149			-			-	3	28	3	20		w
Ds150			-	++	4	**31**	5	25	3	14		-
Ds151		4	23	weak	3	17	7	25	4	24		-
Ds152			-			-	3	20	3	23		-
Ds53			-			-	3	23	2	20		-
Ds154			-			-	4	**30**	6	20	1	5
Ds155		5	17		3	12	6	28	3	15	9	11
Ds156			-			-		-	5	15		-
Ds157			-			-		-	5	17		-
Ds158			-		5	9	5	29	4	10		-
Ds159		5	28	+	5	**30**	5	30	2	12		-
Ds160			-		2	0	5	30	3	22		-
Ds161			-				4	25	2	**28**		-
Ds162			-		3	15		-	1	17		-
Ds163			-				3	25	2	25		-
Ds164		3	22	+	3	18	6	26	4	25		-
Ds165			-			-	3	28	3	20		-
Ds166			-			-	5	26	3	15		-
Ds167			-			-	5	27	4	20		-
Ds168			-			-	5	26	3	20		-
Ds169			-			-	5	30	2	22		-
Ds170	**keratin**		-			-		-	4	20		-
Ds171			-			-		-	5	23		-
Ds172			-			-	5	32	2	20		-
Ds173			-			-		-	3	18		-
Ds174		3	20			-	5	25	9	25	12	14
Ds175			-			-	3	**30**	8	30	10	12
Ds176			-	+	8	**35**	9	25	9	30	10	12
Ds177		1	7	+	2	24	5	19	4	25	7	9
Ds178		5	22			-	7	22	8	30	9	10
Ds179		5	14	+	8	**34**	8	25	10	30	10	13
Ds63	**starch**	2	10	+	5	24	7	26	7	25	10	12
Ds64		5	13	+	6	28	8	24	10	33	11	16
Ds65		2	13		4	**27**	5	24	6	30	6	9
Ds66		2	12			-	5	20	5	25	10	15
Ds67			-	+	5	23	5	28	3	22	6	12
Ds68		3	10	+	6	25	6	25	5	25	12	15
Ds69		5	15		3	20	6	28	6	29	11	15
Ds70			-			-		-	2	15	2	6
Ds71		1	8	+	4	20	5	25		-	8	11
Ds72		5	12		4	30		-	10	30	8	14
Ds73		2	8	+	5	24	6	20	10	32	8	13
Ds74	**olive oil**	3	13	+	8	18	7	30	10	32	10	14
Ds75		8	20	+	6	**35**	10	30	11	30	10	14
Ds76		5	18	+	7	28	7	25	8	30		-
Ds78		4	10			-	6	20	5	12	12	13
Ds79		2	13	+	4	21	6	24	8	15	6	9

Table 4. Influence of pH on growth and endoglucanase activity of soda solonchak alkaliphiles : average profiles estimated from individual results for eight isolates: actinomycetes - *Nocardiopsis* DS50, 51; *Streptomyces* DS8,9; *Bacillus*: DS85, 100, 101, 102.

pH	% of maximum			
	Actinomycetes		*Bacillus* ACB	
	growth	activity	growth	activity
5	0		0	
6	20-70	30-70	0	
7	40-100	70-100	0-10	0-40
8	80-100	90-100	20-60	40-100
9	90-100	90-100	70-100	90-100
10	80-100	90-100	100	90-100
10.5	40-90	70-100	80-100	100
11	10-40	40-80	30-70	50-90

Solid medium 0.6 M total Na^+ buffered with: pH 5-8 - 0.1 M HEPES/NaCl/$NaHCO_3$; pH 8-11 - $NaHCO_3$/Na_2CO_3. Substrate: 0.1% CMC+yeast extract 0.2 g/l. Growth and activity was estimated from the colony and zone of hydrolysis, respectively, after 4 days of plate incubation at 30°C.