Natronoflexus pectinivorans gen. nov. sp. nov., an obligately anaerobic and alkaliphilic fermentative member of Bacteroidetes from soda lakes

Sorokin, D.Y.; Panteleeva, A.N.; Tourova, T.P.; Kaparullina, E.N.; Muijzer, G.

Published in:
Extremophiles

DOI:
10.1007/s00792-011-0399-7

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Natronoflexus pectinivorans gen. nov. sp. nov., an obligately anaerobic and alkaliophilic fermentative member of Bacteroidetes from soda lakes

D. Y. Sorokin · A. N. Panteleeva · T. P. Tourova · E. N. Kaparullina · G. Muyzer

Received: 22 July 2011 / Accepted: 26 August 2011 / Published online: 14 September 2011
© The Author(s) 2011. This article is published with open access at Springerlink.com

Abstract Anaerobic enrichment with pectin at pH 10 and moderate salinity inoculated with sediments from soda lakes of the Kulunda Steppe (Altai, Russia) resulted in the isolation of a novel member of the Bacteroidetes, strain AP1T. The cells are long, flexible, Gram-negative rods forming pink carotenoids. The isolate is an obligate anaerobe, fermenting various carbohydrates to acetate and succinate. It can hydrolyze and utilize pectin, xylan, starch, laminarin and pullulan as growth substrates. Growth is possible in a pH range from 8 to 10.5, with an optimum at pH 9.5, and at a salinity range from 0.1 to 2 M Na+.

Phylogenetic analysis based on 16S rRNA sequences placed the isolate into the phylum Bacteroidetes as a separate lineage within the family Marinilabilaceae. On the basis of distinct phenotype and phylogeny, the soda lake isolate AP1T is proposed to be assigned in a new genus and species Natronoflexus pectinivorans (=DSM24179T = UNIQEM U807T).

Keywords Natronoflexus pectinivorans · Pectin · Haloalkaliphilic · Soda lakes

Introduction

Pectin, a natural polymer of partially methylated galacturonic acid, is an important component of plant biomass acting as a glue for the cellulose fibrils. It is degraded by pectinolytic microorganisms to monomers, which are then utilized as a growth substrate. The pectinolytic organisms produce an extracellular enzymatic complex with a general term pectinases, the principal component of which is represented by pectate lyases (Jayani et al. 2005). The pectin hydrolysis is an important process, both for natural habitats and for industrial processing of food and textile (Kashyap et al. 2001; Sarethy et al. 2011). Most of the known pectinolytic microorganisms grow optimally at acidic and neutral pH, while evidences for pectinolysis at high pH and/or high salt are scarce and were never specifically investigated with respect to carbon cycling in soda lakes. However, due to a potential for application in the food and textile industry, pectinases with high alkali tolerance were specifically looked for in nonsalt-tolerant aerobes, such as various bacilli (Hoondal et al. 2002). There is only single evidence in the literature of a soda lake anaerobic fermentative bacterium, which is reported to grow with...
The isolation of the DNA and determination of the G+C content of DNA was performed according to Marmur (1961) and Marmur and Doty (1962), respectively. For molecular analysis, the DNA was extracted from the cells using alkaline SDS lysis at 60°C and purified with the Wizard Preps Kit (Promega, USA). The nearly complete 16S rRNA gene was obtained using the general bacterial PCR primers 11f and 1492r (Lane1991). The sequences were aligned with sequences from GenBank using CLUSTAL W and a phylogenetic tree was reconstructed using neighbor-joining algorithm in the TREECONW program package (van de Peer and de Wachter 1994).
Results and discussion

Enrichment and isolation of a pure culture of a pectinolytic alkaliphile

When apple pectin was used as a single substrate in enrichments from two soda lake sediments at pH 10, surprisingly, active growth was obtained only under anaerobic conditions. Most probably, the sediments were too reduced to support active aerobic communities. The enrichment was dominated by a morphotype represented by long thin flexible rods that was eventually isolated in pure culture by several rounds of serial dilutions. The purity of the isolate was verified by obtaining single colony morphotypes, homogenous morphology, and by 16S rRNA sequence analysis. The isolate was designated strain AP1\textsuperscript{T}.

Morphology and identification

The cells of AP1\textsuperscript{T} are long thin rods, 0.25–0.3 \times 3–10 \mu m, bending and gliding when solid surfaces (e.g., agar, pectin particles) are present. The cell wall is of the Gram-negative type and the cells are covered by a slime-like layer (Fig. 1). In young cultures, the cells were mostly single and suspended, while in the old cultures they started to aggregate and rapidly lysed. No cyst-like round bodies appeared during cell lysis in old cultures. Colonies were mucoid, convex, up to 2 mm, and pink. The concentrated cell biomass was red, due to the presence of carotenoids (Supplementary Fig. 1).

Phylogenetic analysis based on 16S rRNA sequences placed AP1 into the phylum Bacteroidetes as a novel, well separated lineage within the family Marinilabiliaceae (Ludwig et al. 2008) with 90–91% sequence similarity to the representatives of the genera Marinilabilia, Anaerophaga, Alkaliflexus, and “Geofilum” (Fig. 2). One of the non-described xylanolytic isolates from soda Soap Lake (State, Washington), annotated as “Alkalitalea” in GenBank (HQ191474), is a closest relative of strain AP1\textsuperscript{T} (ca. 98% sequence similarity) and probably belongs to the same genus.

A comparison of the polar lipid fatty acids composition of strain AP1\textsuperscript{T} with its relatives from the family “Marinilabiliacea” demonstrated a general trait of domination of 2–3 isomers of C\textsubscript{15} (Table 1). However, there are two complications that prevent more detailed comparison between the members of this family: firstly, the AP1 profile could be directly compared only with Alkaliflexus, since both were grown at high pH; secondly, the new profiles for Alkaliflexus and Marinilabilia appeared in a recent description of a novel member of the family (Geofilum) (Miyazaki et al. 2011), which differed substantially from the original profiles. It is difficult to judge which of these data are more trustworthy. We can only comment that in the Geofilum paper, neither the growth conditions nor the source of the reference strains are indicated.

The polar lipid profile of strain AP1 contained phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine (PS), two unidentified aminophospholipids, an aminolipid, and 4 different unidentified phospholipids (Supplementary Fig. 2).

Metabolic characteristics

Strain AP1 is an obligately anaerobic bacterium that grows by fermentation of a wide range of carbohydrates. The
fastest growth was observed with the pectin monomer galacturonic acid and with xylose (\( \mu_{\text{max}} \) at pH 10 and 30°C = 0.30 and 0.28 h\(^{-1}\), respectively). The final products of galacturonic acid fermentations were acetate and succinate. Apart from the monomeric sugars, the bacterium was able to grow with polysaccharides, such as pectin, xylan, pullulan, laminarin, starch and glycogen, thus being a typical representative of the hydrolytic \( \text{Bacteroidetes} \) (Thomas et al. 2011). Of the variety of pectic substrates, it grew with polygalacturonate, polypectate and pectate. It did not grow in the presence of oxygen in the gas phase and growth was stimulated by the addition of a reductant. The maximal growth temperature with galacturonic acid as substrate was 41°C. Catalase activity was present, but at a much lower level than in the aerotolerant fermentative alkaliophile \( \text{Natronobacillus} \) (Sorokin et al. 2008).

Influence of pH and sodium on growth and activity

With galacturonic acid as substrate, AP1 was able to grow at a pH between 8 and 10.5 with an optimum at 9.5 (Fig. 3a). The growth was chloride independent. In sodium carbonate buffer at pH 10, growth was possible between 0.1 and 1.5 M total Na\(^+\) with an optimum at 0.4–0.6 M (Fig. 3b). According to these characteristics, the organism belongs to the moderately salt-tolerant obligate alkaliophiles.
In the overall characteristics, the pectinolytic strain AP1\textsuperscript{T} isolated from the sediments of southeastern Siberian soda lakes resembles *Alkaliflexis imshenetskii*—a low salt-tolerant alkaliphilic saccharolytic bacterium isolated from a Transbaikal soda lake (Zhilina et al. 2004) (Table 2). However, the large phylogenetic distance and several phenotypic differences allow the novel isolate to be assigned to a separate genus and species for which the name *Natronoflexus pectinivorans* is suggested.

**Fig. 3** Influence of pH at 0.6 M Na\textsuperscript{+} (a) and of sodium carbonate at pH 10 (b) on anaerobic growth of strains AP1 with galacturonic acid

**Table 2** Phenotypic comparison of strain AP1 with the closest described relatives

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>AP1\textsuperscript{T}</th>
<th><em>Alkaliflexis imshenetskii</em>\textsuperscript{a}</th>
<th>“<em>Geofilum rubicundum</em>”\textsuperscript{b}</th>
<th><em>Marinilabilia salmonicolor</em>\textsuperscript{c}</th>
<th><em>Anaerophaga thermohalophila</em>\textsuperscript{d}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size (µm)</td>
<td>0.25 – 0.3 × 3 – 10</td>
<td>0.25 – 0.4 × 4 – 10</td>
<td>0.2 – 0.4 × 4.0 – 22.0</td>
<td>0.3 – 0.5 × 2 – 6</td>
<td>0.3 × 4 – 8</td>
</tr>
<tr>
<td>Motility</td>
<td>Gliding</td>
<td>Gliding</td>
<td>Gliding</td>
<td>Gliding</td>
<td>–</td>
</tr>
<tr>
<td>Pigmentation</td>
<td>Pink</td>
<td>Pink</td>
<td>Salmon pink</td>
<td>Yellow to orange</td>
<td>Orange – red</td>
</tr>
<tr>
<td>Fermentation products</td>
<td>Acetate, succinate</td>
<td>Acetate, succinate, propionate, formate</td>
<td>n.d.</td>
<td>Acetate, succinate, propionate, lactate, H\textsubscript{2}</td>
<td>Acetate, succinate, propionate</td>
</tr>
<tr>
<td>Aerobic growth</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Catalase activity</td>
<td>Weak</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Substrates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galacturonic acid</td>
<td>+</td>
<td>+</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Xylose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Fructose</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glucosamine</td>
<td>+</td>
<td>+</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>N-acetyl glucosamine</td>
<td>+</td>
<td>+</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Pectin, xylan, pullulan</td>
<td>+</td>
<td>+</td>
<td>xylan—</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Maximal growth temperature</td>
<td>41</td>
<td>45</td>
<td>36</td>
<td>n.d.</td>
<td>55</td>
</tr>
<tr>
<td>pH range (optimum)</td>
<td>8.0 – 10.5 (9.5)</td>
<td>7.2 – 10.2 (8.5)</td>
<td>6.9 – 9.3 (7.8)</td>
<td>Neutrophile</td>
<td>Neutrophile</td>
</tr>
<tr>
<td>Salt range (M Na\textsuperscript{+})</td>
<td>0.2 – 2.0</td>
<td>0 – 0.88</td>
<td>0 – 1.0</td>
<td>0.17 – 0.5</td>
<td>0.33 – 2.0</td>
</tr>
<tr>
<td>G+C content (mol %)</td>
<td>40.6</td>
<td>44.3</td>
<td>42.9</td>
<td>37</td>
<td>41.8</td>
</tr>
<tr>
<td>Habitat</td>
<td>Soda lakes</td>
<td>Soda lake</td>
<td>Marine</td>
<td>Marine</td>
<td>Marine oil field</td>
</tr>
</tbody>
</table>

\textsuperscript{a} (Zhilina et al. 2004), \textsuperscript{b} (Miyazaki et al. 2011), \textsuperscript{c} (Suzuki et al. 1999), \textsuperscript{d} (Denger et al. 2003)

**Acknowledgments** This work was supported by RFBR (10-04-00152). We are grateful to E. Detkova for the DNA analysis and G. Osipov for the cellular fatty acid analysis.

**Open Access** This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.
Appendix 1: Description of *Natronoflexus* gen. nov.

(Na.tro.no.flex’us; N.L. n. natron (arbitrarily derived from the Arabic n. natrum or natron) soda, sodium carbonate; N.L. pref. natrono-, pertaining to soda; L. masc. n. flexus a bending, N.L. masc. n. *Natronoflexus* bending/flexible cells living in soda.)


Appendix 2: Description of *Natronoflexus pectinivorans* sp. nov.

(N.L. n. *pectinum*, pectin; L. part. adj. vorans, devouring; N.L. part. adj. *pectinivorans*, pectin-devouring.)

Cells are long flexible rods, 0.25–0.3 × 3–10 μm, single or in bundles, capable of gliding movement on solid surfaces. Gram-negative, contain carotenoids with absorption peaks in methanol-acetone at 468 (shoulder), 492 (main), and 523 nm. Strictly anaerobic fermentative saccharolytic bacterium, utilizing the following carbohydrates: d-galacturonic acid, d-glucuronic acid, dextrose, fructose, glucose, α,α-trehalose, α-CH3-glycoside, 2-deoxyglucose, d-mannose, sucrose, d-maltose, d-cellobiose, d-glucosamine, N-acetyl glucosamine, galactose, xylose, glycogen, starch, pectin, laminarin, pullulan. Substrates not utilized: lactose, melibiose, melitose, arabinose, arabinine, glycerol, l-sorbose, m-erithitol, m-inositol, d-raffinose, dextrin, d-rhamnose, d-ribose, alginate, CMC, cellulose, agar. The final products of galacturonic acid fermentation are acetate and succinate. Obligately alkaliphilic with a pH range for growth between 8.0 and 10.5, and an optimum at pH 9.5. Moderately salt tolerant with a range from 0.1 to 2.0 M NaCl (optimum at 0.4–0.6 M). Mesophilic, with a maximum temperature for growth at 41°C and an optimum at 30°C. The polar lipids consist of unidentified phospho-, amino- and aminophospholipids, phosphatidylcholine, phosphatidylethanolamine, and phosphatidyserine. The predominant fatty acids in the polar membrane lipids are isomers of C15:0. The G+C content of the genomic DNA is 40.6 mol% (Tm). The type strain is AP1T (DSM24179T = UNIQEM U807T), isolated from sediments of soda lakes in southwestern Siberia. The GenBank 16S rRNA gene sequence accession number is GQ922844.

References


