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**SHORT COMMUNICATIONS**

# Isolation of Strain *Pseudomonas* sp. ASA2 from a Methanogenic Community Degrading Aminobenzoate and Aminosalicylate

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Aminoaromatic compounds are the products of anaerobic degradation of azo dyes and microbial and abiotic reduction of homocyclic nitroaromatics. In recent decades, such xenobiotics, due to their mutagenic and carcinogenic properties, have caused severe environmental problems. These compounds accumulate in bottom sediments of water bodies and soils near varnish-and-dye, textile, and pharmaceutical plants and crucially affect the trophic chains and microbial diversity in these environments. Anaerobic microbial treatment is the main method of eliminating these pollutants, since, under aerobic conditions, certain toxic intermediates may form, and aminoaromatic compounds may polymerize in the presence of oxygen to persistent macromolecules. The behavior of microbial populations in waste treatment plants, as well as in natural environments, is poorly understood. In this connection, it was of interest to study anaerobic microbial communities degrading aminoaromatic substrates and to isolate bacteria responsible for the first stages of this process. The degradation of isomers of aminobenzoic (ABA) and aminosalicylic (ASA) acids was shown in methanogenic sludges and enrichment cultures [1–3]. Anaerobic degradation of ABA by pure bacterial cultures has been investigated [2, 4–6], whereas anaerobic degradation of ASA is still insufficiently studied [7].

Enrichment cultures capable of degrading isomers of ABA and ASA to methane were obtained from mesophilic anaerobic sludge. Cultivation was performed anaerobically in bottles flushed with $N_2$ in mineral medium [8] supplemented with ABA or ASA (3 to 8 mM) and yeast extract (50 mg/l) at 30°C. The pH of the medium was adjusted to 7.3; sodium sulfide (0.8 mM) was used as the reducing agent. The medium contained no exogenous electron acceptors. The consumption of the aminoaromatic substrate was evaluated from the UV spectrum of the culture liquid or measured by high-pressure liquid chromatography (HPLC). The amount of ammonium was determined with the Nessler reagent [9].

One of the obtained enrichment cultures converted both 5-ASA and 2-ABA to methane; acetate and $CO_2$, were revealed by gas chromatography as intermediates. The observed increase in the amount of $NH_4^+$ in the medium suggested deamination of aminoaromatic substrates. It was of interest to elucidate whether one or different bacterial species degraded ABA and ASA in this enrichment.

To isolate the bacterium responsible for the primary transformation of aminoaromatics, the enrichment culture degrading 5-ASA and 2-ABA was cultivated anaerobically on agarized medium (1.9% agar) supplemented with 2 mM 2-ABA and devoid of yeast extract. The 5-ASA was not applied as a substrate since it is easily polymerized, both under heating and in the presence of trace amounts of oxygen. Anaerobic bottles with agar medium were inoculated with serial dilutions of active enrichment culture that had been subjected to several passages on aminoaromatic-containing medium. Individual colonies were transferred to liquid medium and incubated anaerobically. In the cases where consumption of 2-ABA was observed, the culture was transferred to fresh agar medium once again. The purity of the culture was checked microscopically and by its cultivation on nutrient agar both aerobically and anaerobically. After repeated passages of the culture, uniform colonies were obtained.

The isolated strain ASA2 was a facultative anaerobe: it was able to grow on nutrient agar under both aerobic and anaerobic conditions. The strain grew aerobically on agar medium with 2-ABA as the sole source of carbon and energy. Cells were short, thin, motile, gram-negative, rod-shaped. Colonies grown on nutrient agar aerobically produced a nonfluorescent blue–green pigment. According to the conventional microbiological characteristics, strain ASA2 apparently belongs to the genus *Pseudomonas*.

To study the utilization of various aminoaromatic substrates by strain ASA2, it was grown under anaerobic conditions in liquid media with different electron acceptors (table). Nitrate concentrations were 10 and 20 mM; sulfate, sulfite, and thiosulfate were added to a concentration of 10 mM. The removal of aminoaromatic compounds from the medium was stimulated by yeast extract. The transformation of ABA and ASA isomers by the bacterium was accompanied by the forma-

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Anaerobic growth of *Pseudomonas* sp. ASA2 in media with aminoaromatic substrates

| Aminoaromatic substrates | Electron acceptors | |
|---|---|---|
| | nitrate | sulfate, sulfite, thiosulfate |
| 2-ABA | + | – |
| 3-ABA | + | – |
| 4-ABA | + | – |
| 4-ASA | + | – |
| 5-ASA | + | – |

Note: Concentration of yeast extract in the medium was 0.5 g/l; "+" and "–" denote the presence and absence of the substrate transformation, respectively.

tion of an unidentified product, which, according to HPLC analysis, seemed to be of an aromatic nature. The production of $H_2$ was not observed; the formation of $CO_2$ most probably resulted from yeast extract decomposition rather than from the transformation of the benzene ring of ABA and ASA isomers. It can be assumed that strain *Pseudomonas* sp. ASA2 grown anaerobically on medium with an aminoaromatic substrate utilized yeast extract as the carbon source.

To our knowledge, this study is the first to demonstrate the conversion of ABA and ASA to methane by one and the same microbial consortium. In this consortium, only one bacterium, *Pseudomonas* sp. ASA2, seems to be responsible for the primary transformation of aminoaromatic compounds. Since this strain does not form substrates for methanogenesis, the trophic chain for the complete mineralization of aminoaromatics to methane should contain at least one microorganism converting the primary product of the substrate transformation to acetate, $CO_2$, and, possibly, hydrogen. At present, the study of the intermediate members of the trophic chain involved in the degradation of aminoaromatic compounds is in progress.

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