Available online at www.sciencedirect.com
SCIENCE DIRECT®
Systematic and Applied Microbiology 28 (2005) 230–241
SYSTEMATIC AND APPLIED MICROBIOLOGY
www.elsevier.de/syapm

Isolation and characterization of heterotrophic bacteria able to grow aerobically with quaternary ammonium alcohols as sole source of carbon and nitrogen

Andres Kaech[a], Nathalie Vallotton[b], Thomas Egli[a,*]

[a]*Swiss Federal Institute for Water Resources and Water Pollution Control (EAWAG), Environmental Microbiology, P.O. Box 611, CH-8600 Duebendorf, Switzerland*
[b]*Swiss Federal Institute for Water Resources and Water Pollution Control (EAWAG), Water and Agriculture, P.O. Box 611, CH-8600 Duebendorf, Switzerland*

Received 6 July 2004

Abstract

The quaternary ammonium alcohols (QAAs) 2,3-dihydroxypropyl-trimethyl-ammonium (TM), dimethyl-diethanol-ammonium (DM) and methyl-triethanol-ammonium (MM) are hydrolysis products of their parent esterquat surfactants, which are widely used as softeners in fabric care. We isolated several bacteria growing with QAAs as the sole source of carbon and nitrogen. The strains were compared with a previously isolated TM-degrading bacterium, which was identified as a representative of the species *Pseudomonas putida* (Syst. Appl. Microbiol. 24 (2001) 252). Two bacteria were isolated with DM, referred to as strains DM 1 and DM 2, respectively. Based on 16S-rDNA analysis, they provided 97% (DM 1) and 98% (DM 2) identities to the closest related strain *Zoogloea ramigera* Itzigsohn 1868[AL]. Both strains were long, slim, motile rods but only DM 1 showed the floc forming activity, which is typical for representatives of the genus *Zoogloea*. Using MM we isolated a Gram-negative, non-motile rod referred to as strain MM 1. The 16S-rDNA sequence of the isolated bacterium revealed 94% identities (best match) to *Rhodobacter sphaeroides* only. The strains MM 1 and DM 1 exclusively grew with the QAA which was used for their isolation. DM 2 was also utilizing TM as sole source of carbon and nitrogen. However, all of the isolated bacteria were growing with the natural and structurally related compound choline.

Keywords: Biodegradation; Cationic surfactants; Esterquat; *Zoogloea ramigera*; Quaternary ammonium alcohol

Introduction

Today, esterquat surfactants are used in large quantities as softeners in washing detergents. The annual production of esterquats probably exceeds 100,000 tons worldwide with basically over 99% of the material applied in fabric care [16]. The three mainly used esterquat surfactants are shown in Fig. 1. They hydrolize rapidly, abiotically and/or biocatalysed, when reaching

Abbreviations: DM, dimethyl-diethanol-ammonium; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; MM, methyl-triethanol-ammonium; OD, optical density; OECD, Organization for Economic Cooperation and Development; QAA, quaternary ammonium alcohol; SM, synthetic medium; TM, 2, 3-dihydroxypropyl-trimethyl-ammonium; TSA, tryptic soy agar

*Corresponding author. Tel.: +41 1 823 5158; fax: +41 1 823 5547.
E-mail address: thomas.egli@eawag.ch (T. Egli).

0723-2020/$ - see front matter © 2004 Elsevier GmbH. All rights reserved.
doi:10.1016/j.syapm.2004.12.004

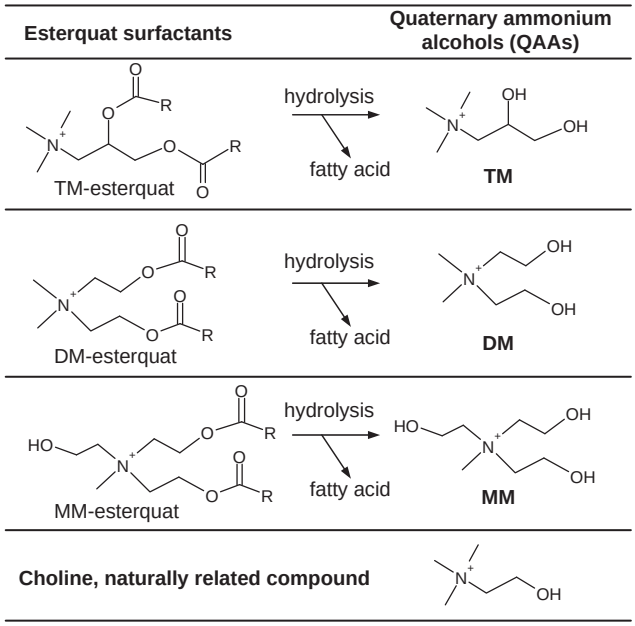

Fig. 1. Structures of the three main commercially used esterquat surfactants, their hydrolysis products, and the structure of the naturally related compound choline.

surface water or sewage treatment plants. The products are the corresponding fatty acids and quaternary ammonium alcohols (QAAs) 2,3-dihydroxypropyl-trimethyl-ammonium (TM), dimethyl-diethanol-ammonium (DM) and methyl-triethanol-ammonium (MM) as displayed in Fig. 1 [13,10,16,21,26,32]. The parent esterquats and the QAAs have been investigated extensively in standard biodegradation tests [19] mimicking degradation in complex systems. Based on these tests, both, the parent esterquats and the QAAs, are expected to be readily and ultimately biodegradable in the environment [10,16,21,17,26,32,33]. Their structural resemblance to the naturally widely occurring compound choline (Fig. 1) suggests that QAAs are degraded in a similar way and at rates comparable to that of choline. However, the three QAAs showed very different degradation patterns and different degradation rates in OECD die-away tests [12] despite their structural similarity. From the simple and choline-like structure of QAAs one would also expect that many different microorganisms would utilize the compounds as sole source of carbon and nitrogen and that no consortium is required for their degradation. However, no choline degraders, including reference strains from the German Culture Collection DSMZ (Deutsche Sammlung für Mikroorganismen und Zellkulturen), were able to metabolize these QAAs (own results). Moreover, up to now, no microorganisms degrading these QAAs as only source of carbon and/or nitrogen have been isolated and consequently the catabolic pathways are not elucidated yet. In view of the widespread application of the esterquats in laundry detergents, we have set out to isolate and enrich strains able to grow with TM, DM and MM.

Here we report the isolation and characterization of bacterial strains able to grow with the QAAs DM and MM as sole source of carbon and nitrogen. With DM two strains were isolated, referred to as strain DM 1 and DM 2. Only one strain, capable to degrade MM, designated as strain MM 1, was isolated. The isolate able to grow with TM (*Pseudomonas. putida* TM 1) has been described previously in detail [17]. All strains are deposited at the German Collection of Microorganisms and Cell Cultures, DSMZ, Braunschweig, under the following accession numbers: for strain DM 1, 16843; for pseudomonas putida TM 1, 16845; and for strain MM 1, 16851.

Materials and Methods

Chemicals

The QAAs (±)-2,3-TM, DM and MM were provided by Unilever (SEAC Safety and Environmental Assessment Center, Unilever Research, Port Sunlight, UK) as the iodide salts in aqueous solution (TM-iodide: dry weight 51.7%; DM-iodide: dry weight 85.1%, MM-iodide: dry weight 84.9%). The purity of all compounds was > 99% (w/w), the impurities consisting mainly of non-methylated tertiary amine. All other chemicals were purchased from Fluka unless indicated.

Isolation, growth and maintenance of organisms

For isolation of organisms (in batch and continuous enrichment cultures), for growth tests and batch experiments, a synthetic medium (SM) was used. It contained per litre of deionized water: $MgSO_4 \cdot 7H_2O$, 0.3 g; $CaCl_2 \cdot 2H_2O$, 0.02 g; $Na_2HPO_4 \cdot 2H_2O$, 2.05 g; KH_2PO_4, 1.30 g; 1 ml of trace element stock solution as described by Pfennig et al. [20], but three-times concentrated (containing per litre: $FeCl_2 \cdot 4H_2O$, 4.5 g; $MnCl_2 \cdot 4H_2O$, 0.3 g; $CoCl_2 \cdot 6H_2O$, 0.36 g; $ZnCl_2$, 0.21 g; $CuCl_2 \cdot 2H_2O$, 0.045 g; $Na_2MoO_4 \cdot 2H_2O$, 0.075 g; H_3BO_3, 0.18 g; $NiCl_2 \cdot 6H_2O$, 0.075 g; $Na_4EDTA \cdot 4H_2O$, 14.023 g); 1 ml of vitamin stock solution (that contained per litre: pyridoxin · HCl, 100 mg; 50 mg of each, thiamine · HCl, riboflavin, nicotinic acid, D-Ca-pantothenic acid, *p*-amino benzoic acid, lipoic acid, nicotinamide, vitamin B12; biotin 20 mg and folic acid 20 mg). The pH of the medium was always 7.0, except for experiments used for the determination of pH optima.