EPAS n.v.
Technologiepark-Zwijnaarde 3
9052 Zwijnaarde
Fax: 32 9221 82 18
Tel: 32 9241 56 18

Laboratory of Microbial Ecology
Faculty of Agricultural & Appl. Biol. Sciences
University of Gent
Coupure L. 653, 9000 Gent
Fax: 32 9264 62 48
Tel: 32 9264 59 76

Melissa

ESA/YCL Contract 130808

Taxonomic study of a thermophilic, proteolytic anaerobic isolate I8 obtained from a Dry Anaerobic Composting process

TN 20.1

september 1993

ir. L. Meheus

Melissa

TN 20.1

Table of contents

Taxonomic study of a thermophilic, proteolytic anaerobic isolate I8 obtained from a Dry Anaerobic composting process.

1 INTRODUCTION

An anaerobic thermophilic proteolytic strain I8 was isolated from a Dry Anaerobic Composting Proces. Such a strain could be used in the first or liquefying compartment of the MELISSA-cycle. The growth on and degradation of proteïns was demonstrated in earlier experiments. The aim of this study was a complete identification of the isolated strain. Different techniques were used. A comparison was made between a reference strain achieved from ATCC, Thermobacteroides proteolyticus ATCC 35245, and our I8.

2 MATERIALS AND METHODS

Biomass production

Biomass was obtained by growing cells (I8 / T. proteolyticus) for two days in bottles containing 100 ml basal MS medium as described in previous experiments (Kersters, 1992). About 0.3 g gelatin was added. Cells were seperated from the liquid phase by centrifugation at 10000 g for 20 minutes.

SDS-page of whole cell proteins

Cellular proteins were extracted and electrophorised in SDS containing polyacrylamide gels according to procedures described by Vauterin et al. (1991).

Determination of DNA base composition

The DNA was isolated and purified by the method of Marmur (1961). The average guanine-plus-cytosine (G + C) content of the DNA was determined by the thermal denaturation method and was calculated by using the equation of Marmur and Doty (1960) as modified by De Ley (1970).

Gaschromatographic analysis of cellular fatty acids

Extraction and quantitative analysis of methyl esters of the cellular fatty acids

(FAMEs) using the Hewlett Packard model 5890A gas-liquid chromatograph were done as described by Vauterin et al. (1991).

Microscopy

Phase-contrast microscopy was performed using a Polyvar microscope.

3 RESULTS and DISCUSSION

The cells of I8 were straight rods, sometimes slightly curved and Gram negative. They were non-motile, non-sporeforming and occurred mostly single and sometimes in pairs. Exponential growing cells were 0.2 - 0.5 μm in width and 3 - 5 μm in length. The DNA base composition of strain I8 was 43.5 mol% G + C.

Strain I8 failed to grow aerobically on gelatin agar plates. It is a strict anaerobic strain. The optimum temperature for growth was 70 °C and temperature ranges for growth were 40 to 70 °C. The optimum pH for growth was 6.8 and pH ranges for growth were 5.4 to 7.9.

From the above mentioned properties could be deducted that strain I8 can be classified in the genus Thermobacteroïdus. Three species belong to this genus: T. acetoethylicus (31 % G + C by thermal denaturation; Bassat & Zeikus, 1981), T. proteolyticus (45 % G + C by boyant density; Ollivier et al., 1985) and T. leptospartum (43 % G + C by HPLC, Toda et al., 1988). There was a high similarity between base compositions determined by thermal denaturation for strain I8 and T. proteolyticus BT^T (43.5 % vs. 43.1 %). The proteïn electrophoretic patterns of strain I8 and T. proteolyticus were also highly similar.

To confirm the previously mentioned similarity of the two strains, the cellular fatty acids were analysed. The results are presented in Table 1. The most important fractions were $C_{16:0}$(74.6 %) and $C_{18:0}$(5.8 %). A major compound (10.43 %) with an equivalent chain length of 15.36 could not be identified. There is only a slight variation in the fatty acids pattern of the reference strain and I8. This strain could certainly not be identified as T. leptospartum due to the difference in the fatty acids composition of the two strains (Toda et al., 1988).

Table 1. Composition of cell fatty acids of strain I8 and T. proteolyticus BT^T

Fatty acid	Strain I8 (%)	T. Proteolyticus BT^T (%)
10:0	1.03	1.44
12:0	0.98	0.89
14:0	3.71	3.66
Un 15360	10.43	9.61
16:0	74.61	72.44
18:1 U9C	0.99	1.19
18:0	5.82	6.95
Un 19705	1.61	1.30

4. REFERENCES

BASSAT, A. B. & ZEIKUS, J. G. (1981) Thermobacteroides acetoethylicus gen. nov. and spec. nov., a new chemoorganotrophic, anaerobic, thermophilic bacterium. Arch. Microb., **128**, 365-370.

DE LEY, J. (1970). Reexamination of the association between melting point, buoyant density and chemical base composition of the deoxyribonucleic acid. J. Bacteriol., **101**, 738-754.

KERSTERS, I. (1992) Melissa TN 15.3. ESA/YCL Contract 8125/NL/FG.

MARMUR, J. (1961). A procedure for the isolation of deoxyribonucleic acid from the microorganisms. J. Molec. Biol., **3**, 208-218.

MARMUR, J. and DOTTY, P. (1960). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Molec. Biol., **5**, 109-118.

OLLIVIER, B. M., MAH, R. A., FERGUSON, T. J., BOONE, D. R., GARCIA, J. L. and ROBINSON, R. (1985). Emendation of of the genus Thermobacteroides proteolyticus sp. nov., a proteolytic acetogen from a methanogenic enrichment. Int. J. System. Bacteriol., **35** (4), 425-428.

TODA, Y., SAIKI, T., UOZOMI, T., and BEPPU, T. (1988). Isolation and characterisation of a protease producing, thermophilic anaerobic bacterium, Bacteroides leptospartum sp. nov. Agric. Biol. Chem., 52, 1339-1344.

VAUTERIN, L;, SWINGS, J. and KERSTERS, K. (1991). Grouping of Xanthomonas campestris pathovars by SDS-page of proteins. J. Gen. Microbbiol., 137, 1677-1687.