PHYSIOLOGICAL AND GENETIC CHARACTERIZATION OF A *N*-ALKANE-DEGRADING STRAIN OF *ACINETOBACTER CALCOACETICUS* ISOLATED FROM LAGUNA VENETA

R. FANI[1], A. GRIFONI[1], L. DA ROS[2], V.U. FOSSATO[2], A. GUIDONI[3], M. PEPI[3] and F. BALDI[3]

[1] Dipart. di Biologia Animale e Genetica, Univ. di Firenze, Via Romana 17, Firenze, Italia
[2] Istituto di Biologia del Mare, C.N.R., Castello 1364/A. 30122 Venezia, Italia
[3] Dipart. di Biologia Ambientale, Univ. di Siena via Mattioli, 4, 53100 Siena, Italia

The presence of petroleum into the oceans represents one of the most important cause of the marine pollution. The major contribution of petroleum into the oceans has been attributed to deliberate releases of oil from marine operations followed by accidental spills in connection with oil transport. The role of oil degrading bacteria is known since 1950, but only later the molecular genetics of oil degradation was studied (CHAKRABARTY *et al.*, 1973). In spite of the enumeration of oil degrading bacteria carried out frequently in many parts of the world, the identification at genus, species and strains level of these bacteria is poorly investigated. The presence of oil degrading bacteria was investigated in water samples collected throughout the Laguna Veneta (GALASSI and CANZONIER, 1978) and the effect of phosphate and nitrate concentration on the kinetics of oil biodegradation in the lagoon water were also investigated.

Sixty three strains of oil degrading bacteria were isolated from three different stations of the Laguna Veneta; one of these strains, VE-C3, was isolated from station C. The *n*-alkane degrading activity of this strain was tested by gas chromatography equipped and the degradation rate of respiratory activity was measured under laboratory condition by oxygen consumption determined by Clark's electrode.

Physiological tests allowed to assign this strain to the species *Acinetobacter calcoaceticus* subsp. *haemoliticus*. In this study we also report the molecular characterization of this strain including determination of 16S rDNA sequence, plasmid profile and identification of genes homologous to *Pseudomonas oleovorans alk* genes. The 16S rDNA from strain VE-C3 was obtained via PCR by amplifyng the genomic DNA with ad hoc oligonucleotides designed on the basis of conserved prokaryotic 16S rDNA. The nucleotidic sequence obtained showed the highest degree of sequence homology with the *A. calcoaceticus* 16S rDNA, showing a correspondence in the genus assignment performed by physiological and molecular methods.

Since in *P. oleovorans* the *alk* genes that are responsable for *n*-alkane degradation are localized on OCT plasmid (GRUND *et al.*, 1975; EGGINK *et al.*, 1987), the presence of plasmid(s) was investigated by agarose gel electrophoresis of the DNA extracted. The electropherogram showed the presence of two plasmids of different dimensions, 10 and 20 kb (figure 1). To verify if the ability to degrade *n*-alkanes possessed by VE-C3 strain could be due to the presence of genes homologous to the *P. oleovorans alk* genes, a Southern blotting experiment was carried out using as probe the 4.58 PstI-PstI fragment containing *P. oleovorans alk* BFGH genes.

The targets were represented by total DNA and plasmid DNA previously digested by appropriate restriction enzymes. The hybridization signals obtained were found on chromosomal and plasmid DNA of VE-C3 strain showing the presence of genes homologous to the *P. oleovorans alk* genes with genomic and plasmidic location; this fact suggested that the organization of the *alk* genes in *A. calcoaceticus* could be different from that of *P. oleovorans*.

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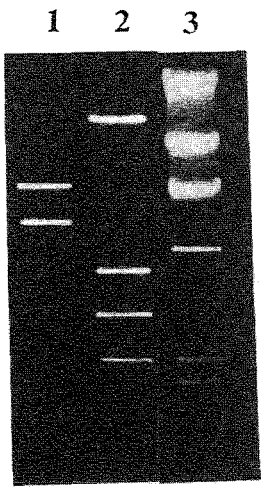

Figure 1. Plasmid profile of *A.c alcoaceticus* strain VE-C3. Lanes: 1)VE-C3 plasmid DNA; 2) VE-C3 plasmid DNA digested with EcoRI restriction enzyme; 3) molecular weight marker II, λDNA/HindIII.