DIESEL FUEL DEGRADATION : PHYSIOLOGICAL AND MOLECULAR CHARACTERIZATON OF ACINETOBACTER VENETIANUS STRAINS ISOLATED FROM VENICE LAGOON

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Abstract

The bacterial strain VE-C3, belonging to the new species *Acinetobacter venetianus*, isolated from Venice Lagoon and involved in diesel fuel degradation, was characterized by a combination of molecular and physiological techniques. *A. venetianus* VE-C3 cells were able to grow in the presence of n-hydrocarbons by forming cell aggregates which adhere to hydrocarbon drops. A 23.8 KDa protein appeared to be involved in the formation of cell aggregates. The oxidation of n-hydrocarbons was carried out by Alk proteins homologous to the *Pseudomonas oleovorans alk*BFGH gene products and whose genes were located on the VE-C3 chromosome and in plasmid pAV2.

Keywords: diesel fuel, Acinetobacter venetianus, alk genes

Twenty-five bacterial strains isolated from Venice Lagoon and able to degrade *n*-alkanes, *n*-alkanols, *n*-alkanals and *n*-alkanoates, were previously characterized at the molecular and physiological level (1, 2). Only strains belonging to the new species *A. venetianus* grew in minimal medium containing *n*-alkanes (C_{10}, C_{14}, and C_{20}) (2) and their respective oxidation products as the sole carbon and energy source. The other strains thrived on n-alkane oxidation products, giving rise to a sort of "functional complementation", in that single members of the community perform different step(s) of the degradation process. The *A. venetianus* VE-C3 cells initiate diesel fuel degradation by oxidizing n-alkanes, providing organic residues suitable for the other bacteria of the community.

The analysis of plasmid content revealed that *A. venetianus* VE-C3 cells harboured two plasmid molecules, pAV1 and pAV2, of 10Kb and 15Kb, respectively. Hybridization experiments also showed that pAV2 contained sequences homologous to the *Pseudomonas oleovorans* alkBFGH genes.

The *A. venetianus* VE-C3 cells were able to degrade diesel fuel by a mechanism requiring the formation of cell aggregates and their further adhesion to diesel fuel drops, via the synthesis of a polysaccharide capsule (3). This process was studied in *A. venetianus* mutants, obtained through the conjugal transfer of pRK290, a 20 kb broad-host range plasmid, from *Escherichia coli* to VE-C312 (a spontaneous Rif^r mutant) cells. Most of ex-conjugants showed large rearrangements in plasmid profile, mainly consisting in loss of plasmid pAV1 (such as in mutant C312/3); other mutants showed a molecular rearrangement of this plasmid (mutant C312/30). Mutants C312/3 and C312/30 were further characterized at physiological and molecular level; they showed a reduced ability to grow in the presence of diesel fuel or hexadecane as the sole carbon and energy source. This was probably due to a reduced ability of aggregation and the further adhesion to hydrocarbons, as shown in figure 1. Moreover in cell-to-surface adhesion experiments, performed by MATH test, VE-C3 and VE-C312 cells appeared to be hydrophilic when grown in a complex medium but became hydrophobic when incubated in mineral medium with diesel fuel as the sole carbon and energy source (not shown). The same test, carried out on mutants C312/3 and C312/30, showed that both of them were always hydrophobic, even when grown in a complex medium without diesel. The mutant phenotype could be due to the loss (or mutation) of genes (very likely located on plasmid pAV1) encoding proteins involved in cell to cell aggregation and that might be localized on cell envelope. To check this hypothesis, membrane proteins were extracted from VE-C3 cells grown in the complex medium and in the mineral medium with diesel fuel as sole carbon and energy source. PAGE analysis of these proteins showed the presence of a 23.8 KDa protein only in A. venetianus VE-C3 cells grown in the presence of diesel fuel. This protein was absent in cell extracts of VE-C3 cells grown in the absence of hydrocarbons. The 23.8 KDa protein is induced by hydrocarbons and is probably involved in adhesion of bacteria to hydrocarbons. Studies are in progress in order to understand the role of the protein in the cell adhesion to hydrocarbon process.

The study of hydrocarbon degradation in A. venetiuanus VE-C3 was also carried out by cloning of alk genes. For this purpose polymerase chain reaction (PCR) experiments were carried out in order to amplify alk sequences from both VE-C3 chromosome and plasmids. PCR reactions were performed using two primers previously designed for the amplification of alk sequences from different microorganisms. We obtained the amplification of a 600-bp DNA fragment from the VE-C3 chromosome, whose nucleotide sequence was determined. The comparative analysis of this sequence with those available in databases revealed that it shared a high degree of sequence similarity with alkM genes from different hydrocarbon-degrading bacteria. This suggested that, at least some genes involved in diesel fuel degradation in A. venetianus are located on the bacterial chromosome. Studies of this gene, as well as the nucleotide sequencing of plasmids pAV1 and pAV2, are in progress.

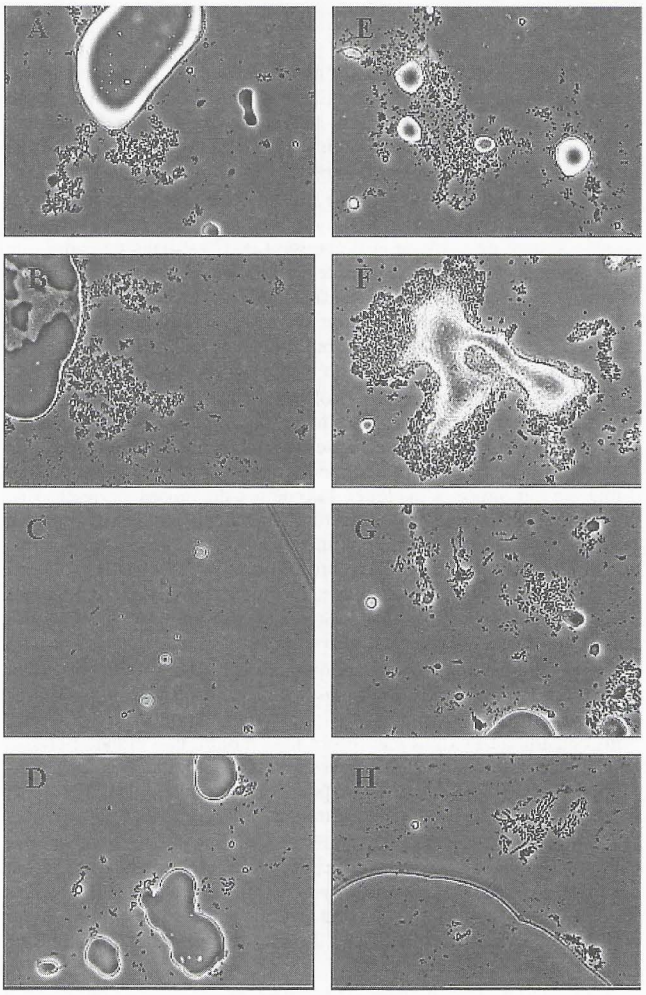

Figure 1. Analysis of cell aggregation of *A. venetianus* strains grown on diesel fuel (A-D) or on *n*-hexadecane (E-H). (A, E) *A. venetianus* VE-C3; (B, F) *A. venetianus* C312; (C, G) *A. venetianus* C312/3; (D, H) *A. venetianus* C312/30.

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