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The horizontal gene transfer in culturable bacteria isolated from industrial fluid

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Abstract
The hundred bacterial isolates were isolated from industrial fluid enriched in ethoxylated nonylphenol. These isolates were grown in selective medium containing nonylphenol ethoxylates as sole source of carbon and energy. The biodiversity analysis at the species level was performed by comparing the *Alu1* restriction patterns of the 16S ribosomal DNA amplified by PCR. The R-DNA restriction analysis enable us to cluster the isolates in to 15 groups. They represented about 80 percent of the community.

Keywords: bacteria isolated, industrial fluid, ethoxylated nonylphenol

Introduction

The studies carried out in the last two decades have established that the knowledge of the microbial diversity in natural and artificial ecosystems is still very limited. The biodiversity of microbial communities could be influenced by stressful conditions, such as the presence of pollutants, a common occurrence in sewage treatment plants, particularly in those receiving industrial wastes. In these artificial environments the horizontal gene transfer is one of the most important mechanisms for microbial adaptation to environmental changes. The organisms can be critical to the survival of microbial populations responsible for the degradation of pollutants (Singh AK 2021 and Van der Meer JR *et al* 1992) [1, 8]. The nonylphenol poly ethoxylates are non-ionic surfactants which despite their potential toxicity are still used for industrial purposes particularly in the textile industry because of their versatility and low cost (Patoczka J and Pulliam GW 1990) [7]. The information concerning about microorganisms and genes involved in the degradation of nonylphenol poly ethoxylates remains limited. The aim of this work was to study the diversity of culturable bacteria involved in nonylphenol ethoxylate degradation isolated from a sewage treatment plant collecting nonylphenol poly ethoxylates enriched wastes (John DM and White GF 1998) [5].

The bacteria from activated sludge of a plant collecting mainly wastes from textile industries were cultivated and characterized using a combination of molecular methods. This included restriction analysis of polymerase chain reaction amplified 16S R-DNA with the enzyme *Alu1* which often enables the division of bacterial strains into groups corresponding to different species the sequencing of amplified 16S ribosomal DNA (Vaneechoutte M *et al* 1992) [9].

2. Materials and Methods

2.1. Sampling and Cultivation

The industrial fluid collected from waste water mainly from textile industries which contain nonylphenol ethoxylates as the main non-ionic surfactants contained in the influent.

2.2 Media and Growth Conditions

The fluid was immediately subjected to serial dilutions and plated on LB plate and on selective plates which contained 15 mg l^{-1} nonylphenol ethoxylates. After three days of incubation at 30°C several colonies were randomly chosen streaked on the same medium and grown in the same conditions. The incubation of the original plates for three days led to the growth of new colonies only on selective plates. Fifty colonies randomly chosen among the newly grown medium were also re streaked. A total 100 isolates, 50 from LB medium, 25 from selective medium at three days of incubation and 25 from selective medium at six days of incubation were repeatedly streaked in the same growth conditions and examined.

2.3. Amplification of 16S R-DNA

The crude cell lysates were the source of template DNA for amplification. After an overnight growth on LB plates at 20°C a single colony was picked and suspended in 20 µl of sterile distilled water. The cell suspension was heated at 95°C for 10 min and shifted at 4°C. The amplified 16S R-DNA was obtained following the procedure described by Di Cello *et al* (1997) [4]. The primers 27 forward 5’-GAGAGTTTGATCCTGGCTCAG3’and1495reverse5’CTA CGGCTACCTTGTTACGA-3’which enabled amplification of nearly all of the 16S R-DNA were synthesized by standard phosphonamidite chemistry, deprotected, dried, dissolved in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) and used without further purification. Two microliters of each amplification mixture were analysed by one percent agarose gel electrophoresis in TAE buffer (0.04 M Tris-acetate; 0.001 M EDTA) containing 0.5 ug $ml^{-1}$of ethidium bromide.

2.4. Restriction Analysis of Amplified 16S R-DNA

The restriction analysis of the amplified 16S R-DNAs was performed with the enzyme *Alu1* as described Di Cello F *et al* (1997) [4]. The enzyme was inactivated by heating the reaction mixture to 65°C for 15 min. The reaction products were analysed by two percent of agarose gel electrophoresis in TAE buffer (0.04 M Tris-acetate: 0.001 M EDTA) containing 0.5 ug ml^{-1} ethidium bromide.

Results and Discussion

3.1. Isolation of Bacteria

The bacteria, sampled collecting mainly surfactant enriched wastes, were grown on LB rich medium and on selective medium containing nonylphenol ethoxylates. The sample studied was represented by 100 isolates, half from each medium.

3.2. Phenotypic Analysis

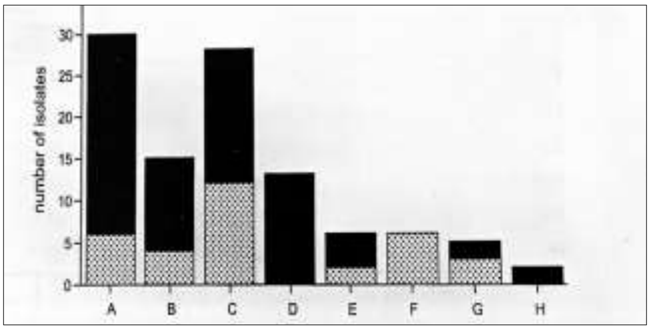

Fig 1: The distribution of bacterial strains. The dotted areas represent the utilization of nonylphenol poly ethoxylates by each group.

The 50 isolates from LB medium were checked for the ability to grow on selective medium and results showed that eighteen isolates were died. The effect of nonylphenol ethoxylates concentrations lower or higher than 15 mg l^{-1} each on the isolates they were screened on agar plates. All isolates were able to grow in the presence of both the lower and the higher concentration of nonylphenol ethoxylates. Some isolates showed more abundant growth with respect to others and had no clear preference for one or the other of the two surfactants.

3.3. Restriction Analysis of 16S R-DNA

The 16S R-DNA was successfully amplified by PCR from 100 isolates. The restriction analysis of each of the amplified 16S R-DNAs with the enzyme *Alu1* revealed 15 different patterns (Figure 2). Most isolates belonged to the groups, namely A, B, C and D, containing 25, 15, 10 and 12 isolates, respectively. The groups E, F, G and H contained 10, 6, 5 and 5 isolates, respectively, whereas profiles I to Q were characteristic of single strains. The restriction profiles of A and B were very similar, suggesting that these two groups might be phylogenetically very close. The groups D, H. N, O. P and Q were composed of isolates picked up at six days of incubation.

The overall variability found in the present study appears to be rather high and is in agreement with the results obtained by in situ hybridization (Amann R *et al* 1996) [2]. The isolates picked up at six days of incubation represented six of the ten uncharacterized groups D, H, N, O, P, and Q. The16S R-DNA sequencing, strains belonging to five of these groups were placed within the *Aeromonas* sp, *Acinetobacter* sp, *Shewanella* sp, *Aeromonas* sp and *Proteus sp.* All these strains belong to the gamma subclass of the *Proteobacteria* sp the most frequently isolated by culture dependent methods (Kampfer P *et al* 1996) [6].

To our knowledge this is the first time that bacteria of the genus *Aeromonas sp* and *Shewanella* sp have been associated with nonylphenol ethoxylates degradation. The involvement of *Acinetobacter* sp in nonylphenol ethoxylates degradation has already been suggested by Barberio C and Fani R (1998) [3].

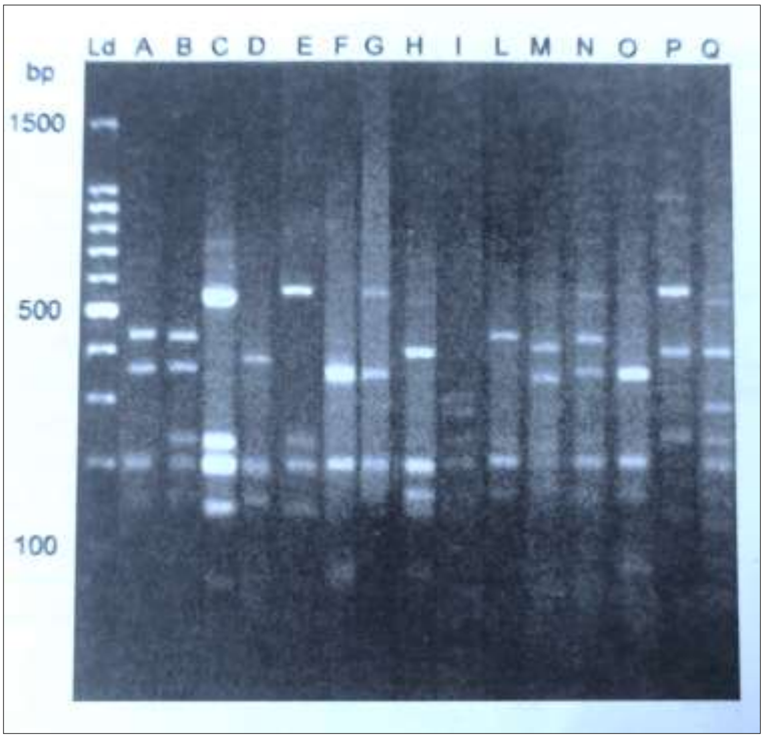

Fig 2: Agarose gel electrophoresis of amplified 16S R-DNA digested with restriction enzyme *Alu I.* The 15 bacterial strains representing the restriction profile.

These results suggest the occurrence of horizontal gene transfer at intra, inter specific and inter generic level (Singh AK 2021) [1]. On the other hand, it is not possible to draw any inference concerning the involvement of these plasmids in NPE degradation.

The overall results provide additional information on the diversity of bacteria involved in the degradation of nonylphenol ethoxylates isolated from activated sludge. Furthermore, they suggest that in natural microbial communities the decrease of biodiversity due the selective pressure of the environment is efficiently counteracted by the occurrence of gene flow mediated by plasmid transfer.

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