Genetic characterization of environnemental and clinical Pseudomonas aeruginosa strains degrading the n-alkanes

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Abstract- Environmental and hospital-sampling allowed us to isolate and identify one hundred of *Pseudomonas aeruginosa* (*P. aeruginosa*) strains. The study of the degradation capacity of alkanes has shown that almost all isolates grew in the presence of long chain n-alkanes, while no growth was recorded in the presence of short chain n-alkanes, isoalkanes or cycloalkanes. The kinetics of growth in the presence of hexadecane, as a sole carbon source, enabled recording various optical densities (OD) depending on the strain of *P. aeruginosa*. The polymerase chain reaction (PCR) in the presence of ERIC (Enterobacterial repetitive intergenic consensus) primers has shown genetic diversity within isolates. The search for *alkB* and *alkB1* genes, which are respectively responsible for the degradation of short chain n-alkanes and long chain n-alkanes, has shown the absence of *alkB* gene; however, the *alkB1* gene, strongly present within the population of *P. aeruginosa* isolated, is absent in a few strains despite their ability to degrade long chain n-alkanes. The nucleotide sequencing of a *alkB1* gene fragment for 4 *P. aeruginosa* strains as well as the reference strain *P. aeruginosa* PAO-1 has shown a highly conserved nucleotide sequences in spite of their heterogeneity origin.

Index Terms- Alkanes, *alkB1*genes, Biodegradation, optical density, *Pseudomonas aeruginosa*

I. Introduction

Alkanes are major constituents of crude oil (Marín *et al.,* 2003). They are also present at low concentrations in diverse non-contaminated areas because many living organisms produce them as protecting agents against water loss. They are saturated hydrocarbons of different sizes and structures. Many micro organisms, capable of utilizing them as a carbon and energy source, have been isolated and characterized (van Beilen *et al.,* 2003; Wentzel *et al.,* 2007, Rojo, 2009; Whyte *et al.,* 2002). However, some bacterial species are highly specialized for hydrocarbon degradation. These species play a key role in the removal or biodegradation of hydrocarbons from polluted and non-polluted environments (Harayama *et al.,* 2004; Head *et al.,* 2006; Yakimov *et al.,* 2007; Wang, *et al.,* 2010a; Wang *et al.,* 2010b). Among these species, *Pseudomonas aeruginosa* take up a preponderate place (Marín *et al*., 2003). The biodegradation of alkanes consists first on their oxidation by an oxygenase, which is under the control of an *alk* operon (Peterson *et al.,* 1966).This later includes a group of genes whose the first are *alkB* or similar genes.

This paper reports a set of *P. aeruginosa* strains isolated in Meknes city (Morocco) from environmental and Hospital samples. These strains were characterized by their growth profile in presence of alkanes and genes involved in their biodegradation. We report evenly the genetic diversity of these strains by the determination of their Enterobacterial repetitive intergenic consensus (ERIC) profil.

II. Materials and Methods

1 – Sampling

The samples studied are of two types: The first correspond to environment water and soil, the second correspond to Hospital samples. The environmental samples are carried along Boufekrane River which crosses the city of Meknes. The second corresponds to pathological samples from various regional hospital services (burned, resuscitation ...) of Meknes city; these sampling were performed using sterile swabs. All sampling are realized during the year 2012.

2 – Culture medium

The culture mediums used are Luria-Bertani agar, King A, King B medium and citrimide agar. The mineral medium (M.M) is containing in g/l: KH_2PO_4: 0.68; $MgSO_4.7H_2O$: 0.35; Na_2HPO_4: 1.7; $CaCl_2$: 0.02; NH_4NO_3: 1; $FeSO_4$: 0.004. The medium was supplemented with a solution (0, 01% final concentration) of trace elements containing in g/l: $CuSO_4$: 0.05; H_3BO_4: 0.1; $MnSO_4$: 0.1; $ZnSO_4$: 0.1; Na_2MoO_4: 0.1; $CoCl_2$: 0.1. The pH was adjusted to 7.

3-Samples preparation, inoculation and identification: Water samples were inoculated directly in Petri dishes containing King A, King B medium or citrimide agar. For soil samples, we performed a suspension of 10% (w/v) in sterile distilled water, and then the supernatant is inoculated as before. Swabs containing the pathological specimens are suspended in1ml of sterile physiologic water; this later is inoculated as the water samples.

All inoculated medium were incubated at 30 ° C during 24h to 48h.

The bacterial colonies obtained were examined to identify those belonging to the species *P. aeruginosa*. The identification tests are primarily morphology, Gram, oxidase enzyme, production of pigments pyoverdin and pyocyanin and growth at 42° C.

4- Biodegradation of alkanes

All bacterial strains identified as *P. aeruginosa* are tested towards short and long chains n-alkanes. Thus, each strain was inoculated into a 250 ml Erlenmeyer flask containing 100 ml of

MSM supplemented with one of the following HC as sole carbon source: n-hexane, n-heptane, n-octane, n-decane, n-dodecane, n-hexadecane, pristane and cyclohexane with concentrations ranging from 0.1% to 0.5%. Incubation is carried out at 30 ° C in agitation at 150 rpm for 72-96 h.

Strains of *P. aeruginosa* have shown a growth in the presence of one of the HC were tested again to measure changes in their optical density (OD) versus time. The OD measurements were performed regularly once a day at 580nm for a period of 8 days using the M.M as mineral medium.

5- Molecular biology and PCR amplification techniques

5-1- Preparation of DNA template for PCR: Total DNA was extracted by suspending 4-5 colonies of overnight culture of *P. aeruginosa* isolates growing on Luria Bertani agar (Bio-Rad, Marnes-la-Coquette, France) in 500µl of DNase- and RNase-free water (Invitrogen, Paisley, UK). The suspension was boiled at 100°C for 10min in thermal block (Polystat 5, Bioblock Scientific, France), then centrifuged at 19000 x *g* for 5min. An aliquot of 1 µL of the supernatant was used as DNA template for PCR.

5-2- Enterobacterial repetitive Intergenic Consensus (ERIC): ERIC analysis was performed using the primers ERIC-2 and ERIC 1R (Table I) as previously reported (Versalovic *et al.*, 1991). Each PCR reaction was carried out in a 25 µL volume using 1.5 U of *Taq* DNA polymerase (Promega, Madison, Wis, USA) in the reaction buffer provided by the manufacturer containing 2.5 mM of $MgCl_2$, 50 µM of each deoxynucleoside triphosphate, 10% of dimethyl sulfoxide, 1.7mg/ml of bovine serum albumin, 2 µM of the selected primer and 5 µl of the DNA template. Aliquots (10µl) of each PCR product were subjected to an electrophoresis on 1.5% agarose gel.

The similarity of the ERIC-PCR banding patterns was analyzed by the Dice coefficient, and the data obtained were analyzed by the unweighted pair group method with arithmetic average (UPGMA) clustering using the Pearson correlation coefficient (Biochemistry and Biotechnology Department, Rovira i Virgili University, Tarragona, Spain) (http://genomes.urv.cat/UPGMA/index.php).

5-3- Detection of alkane monooxygenases encoding genes: *P. aeruginosa* isolates were screened by PCR for the detection of genes involved in the biodegradation of n-alkanes as described by Whyte *et al.* (1996). Primers used are shown in Table I.

Table I: Oligonucleotides primers used in this study

Gene or location	Sequences Primers (5' to 3')	Fragment size (bp)	Reference
alk B	*alkB*-F TGGCCGGCTACTCCGATGATCGGAATCTGG	870	Kok et *al.* 1989
	alkB- R CGCGTGGTGATCCGAGTGCCGCTGAAGGTG		
alkB1	*alkB1*–F CGGGGTTCAAGGTCGAGCAT	434	Smits et *al.*,1999
	alkB1-R CAGGACCAGGTTGGTGAAGA		
ERIC	ERIC-2 AAGTAAGTGACTGGGGTGAGCG	variable	Versalovic et *al.* 1991
	ERIC 1R ATGTAAGCTCCTGGGGATTCAC		

PCR products were detected on 1.5 % agarose gel (FMC Bioproduct, Rockland, ME) after ethidium bromide staining and UV illumination, photographed with an Olympus digital camera and analyzed using the Digi-Doc-it software (UVP, Upland, CA).

5-4- Sequencing of alkane monooxygenases encoding genes: The amplified products obtained were sequenced to validate their identities. Both strands of the purified amplicons were sequenced with a Genetic Analyzer 3130x1 sequencer (Applied Biosystems, Foster City, CA, USA), with the same primers used for PCR amplification. The nucleotide and deduced protein sequences were analyzed with software available over the Internet at the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov).

III. Results and Discussion

1 - Isolation and identification of bacterial strains

Among bacterial strains isolated, a total of 100 strains are Gram-negative, oxidase positive, showing growth at 42 ° C and producing pyoverdine and pyocyanin on king A and king B as well as on citrimide agar mediums. They were capable of denitrification and were identified as *P. aeruginosa* strains using classical tests. 58 of them are from the environment and 42 are from hospitals. Theses strains are indicated Pa in the text and preceded with the letter E or H in case of Environmental or Hospital origins.

2- Alkanes biodegradation

All strains of *P. aeruginosa* isolated have not shown growth in the presence of short chain n-alkanes (n-hexane, n-heptane, n-octane or n-decane), isoalkanes (pristane) or cyclohexane; however, nearly all of them have grown in dodecane and hexadecane (long chain n-alkane) in concentrations ranging from 0.1 to 0.5%. This suggests that these strains are probably devoid of genes responsible for the degradation of these types of alkanes, but possess the ones responsible for the degradation of long chains n-alkanes.

We have opted for studying the kinetics of growth in the presence of hexadecane (0, 5%). We have chosen seven strains of *P. aeruginosa*. The choice of these strains was done taking into account those that show both a good and a moderate growth in the presence of hexadecane. Thus, we were able to identify two groups of strains, one containing four strains (EPa10, EPa45, HPa59 and EPa11) degrading effectively hexadecane and the other of 03 strains (EPa22, HPa32 and HPa24) degrading moderately hexadecane (Figure 1).

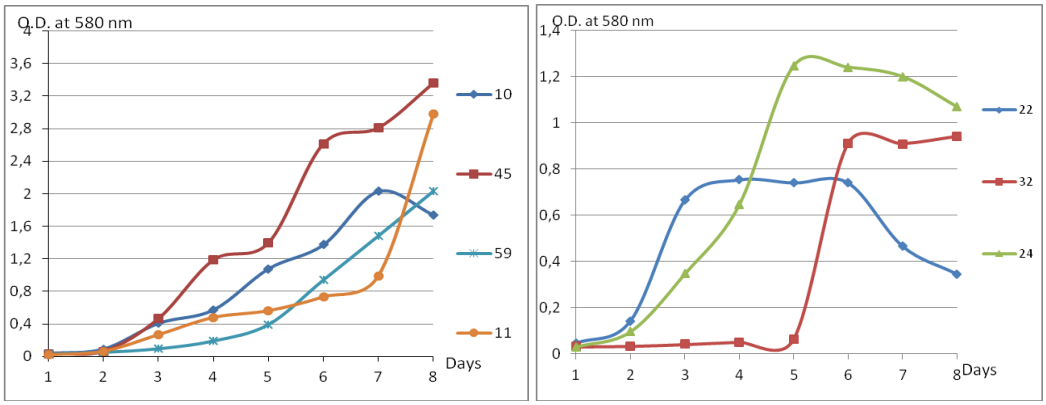

Figure 1: Growth of *P. aeruginosa* EPa10, EPa45, HPa59, EPa11 (group1), EPa22, HPa32 and HPa24 (group 2) in the presence of hexadecane at 0.5%.

Indeed, group 1 strains exhibits a high OD between 2 and 3.3, while those in group 2 show low OD with a maximum ranging between 0.8 and 1.3. In addition, the OD for group1 continues to increase until the 8th day, while the strains of group2 started decrease from the 6th day or before.

It should be noted that strain HPa32, which have a hospital provenance, unlike other strains of *P. aeruginosa*, present a large latency phase.

3- ERIC Migration Profile

The amplification patterns of total DNA using ERIC primers is illustrated by figure 2. The interpretation of patterns was based on the criteria of Tenover *et al.* (1995). Briefly, strains showing more than three DNA fragment variations and a similarity of <80% at dendrogram analysis were considered to represent different ERIC-PCR types, while one- to three-fragment differences and a similarity of >80% upon dendrogram analysis were considered to represent ERIC-PCR pattern subtypes (Figure 2).

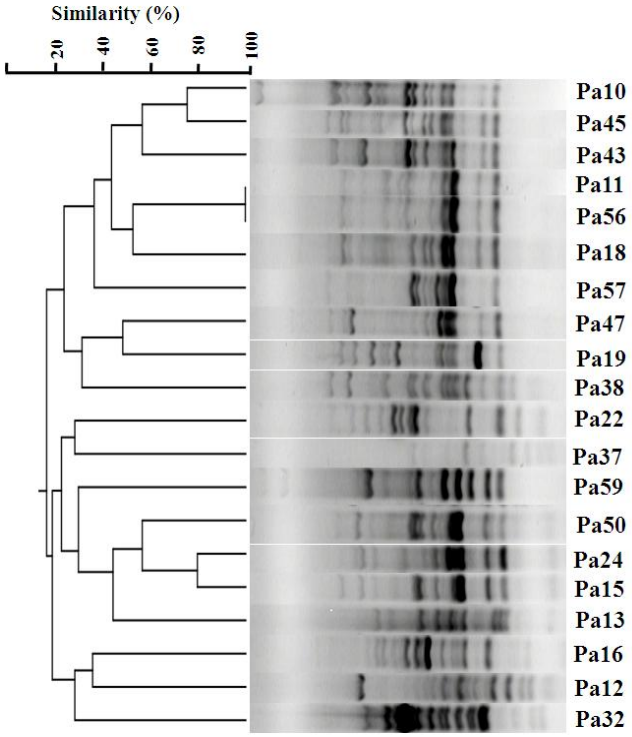

Figure 2: ERIC profile for twenty *P. aeruginosa*, the strains EPa10, EPa45, EPa43; EPa11, EPa56, EPa18, EPa57, EPa47,EPa19, EPa22 and EPa16 are environmental *P. aeruginosa*, the strains HPa59, HPa50, HPa12, HPa13, HPa38, HPa37, HPa32, HPa24 and HPa15 are hospital *P. aeruginosa*.

The patterns amplification showed a genetic diversity within strains of *P. aeruginosa* isolated. The similarity percentage seems varied with the origin of strains; it reached below 20% when the strains compared are from environmental and Hospital origins but increase when strains are from the same origin (strains EPa11 and EPa56, which are environmental strains, show 100% of similarity) (figure 2). Taken as whole, and except for these two latest strains, the *P. aeruginosa* strains isolated are not similar and belong at variables subtypes.

4 – Search for alkane monooxygenase encoding genes

Among the 100 *P. aeruginosa* strains isolated, we have studied a set of 50 strains for the presence of *alkB* and *alkB1* genes. The strains chosen covered that these having an environmental and hospital origin. The PCR in presence of *alkB* primers doesn't amplify the fragment of the expected size (870 bp) for all *P. aeruginosa* strains. These results concord with those indicating the failure growth in presence of short chains alkanes. However, many strains gave unspecific bands of 600 bp. Figures N° 3 shows the results of amplification for some of these strains.

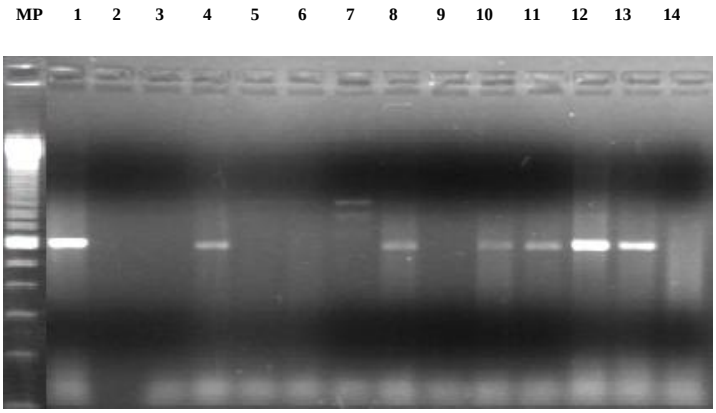

Figure 3: Amplification patterns on agarose gel 1.5% using *alk B* primers. MP: 100 bp DNA Ladder, lane 1: HPa20, lane 2: EPa19, lane 3: EPa18, lane 4: EPa17, lane 5:EPa27, lane 6:EPa26, lane 7: EPa14, lane 8: HPa 13, lane 9: HPa24, lane 10: EPa23 lane 11: EPa45, lane 12: EPa10, lane 13: EPa30 and lane 14: HPa12.

The band of 600bp was amplified with a frequency of 68% (Diagram 1), the latter is non-specific since the strains showed no growth towards short chain n-alkanes; besides, further research works (McCoy., 2000, Belhaj et *al*., 2002) have reported similar bands (600pb) for strains not degrading short chains n-alkanes. Therefore, the isolates do not possess the *alkB* gene. The *alkB* primers were possibly amplifying DNA fragments with no homology to *P. putida* GP01 (*P.oleovorans*) *alkB*. (McCoy., 2000). This suggestion seems very likely. Indeed, *P. aeruginosa* strains were isolated from the slightly contaminated site by hydrocarbon; the probability of finding the *alkB* gene at its stem is too low or even neglected (Gerhard 1986; Kok *et al.*, 1989b; Smith *et al.*, 1999, van Beilen, *et al.*, 1994; Whyte *et al*., 1996). In addition, research in this field reported that the *alkB* gene is in a mobile form and it is; therefore, very unstable (Gerhard 1986). Some alkane degradation genes have been found either on transposons (van Beilen *et al.*, 2001; Belhaj *et al.*, 2002) or on plasmids (Sekine *et al.*, 2006; van Beilen *et al.*, 1994), which clearly facilitate their horizontal transfer (Rojo, 2010).

Contrary to a*lkB*, the PCR in the presence of primers *alkB1* gave an amplification product of 434 bp for strains of *P. aeruginosa*. But strangely, 05 strains did not give this product; nevertheless, they perfectly grew in presence of long chain n-alkanes. At this purpose, the frequency amplification of the 434pb fragment is of 90% (diagram N°2). Figure N° 4 show the results of amplifications obtained for some of these strains.

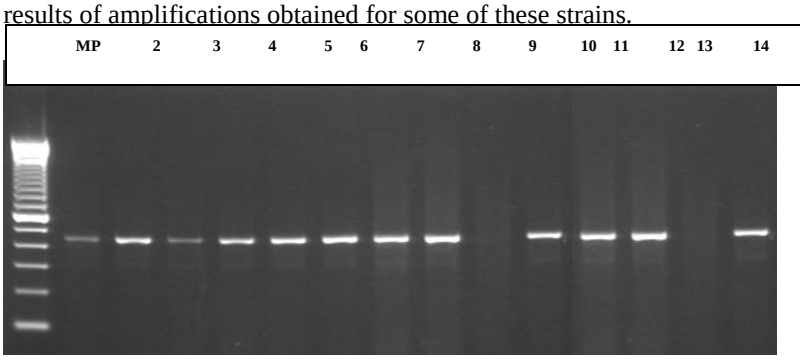

Figure 4: Migration on 1.5% agarose gel profile after PCR amplification in the presence of *alkB*1 primers.

MP: 100 bp DNA Ladder, lane 1: HPa20, lane 2: EPa19, lane 3: EPa18, lane 4: EPa17, lane 5:EPa27, lane 6:EPa26, lane 7: EPa14, lane 8: HPa 13, lane 9: HPa24, lane 10: EPa23 lane 11: EPa45, lane 12: EPa10, lane 13: EPa30 and lane 14: HPa12.

Diagrams N° 1 and N° 2 allow accounting the frequency of *alkB* and *alkB1* genes fragments amplification for isolated *P. aeruginosa* strains.

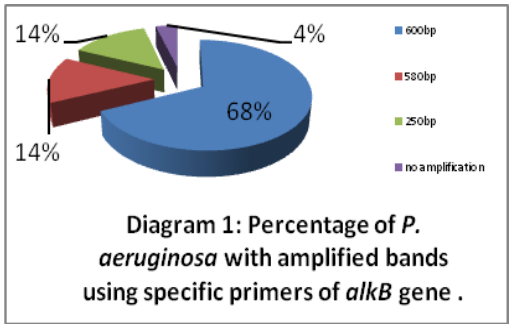

Diagram 1: Percentage of *P. aeruginosa* with amplified bands using specific primers of *alkB* gene .

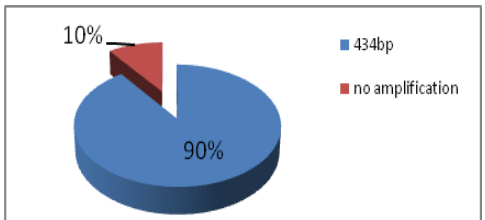

Diagram 2: Percentage of *P. aeruginosa* with amplified bands using specific primer of *alkB 1* gene.

Most *P. aeruginosa* strains isolated from both hospital and natural environments can degrade n-alkanes (Alonso *et al.*, 1999, van Beilen *et al.*, 1998**,** Yuste *et al.*, 2000). The ability of clinical *P. aeruginosa* strains to grow on long chain n-alkanes may be explained by the fact that *P. aeruginosa* strains are not committed to a pathogenic lifestyle: patients may be infected by clinical strains as well as bacteria picked up from soil or water. Apparently, *P. aeruginosa* frequently encounters alkanes as a carbon source, and normally retains the genes required for their biodegradation (Smits et *al*., 2003). Indeed, long chain alkanes remains in hydrocarbon contaminated soils for long periods of

time (Stroud *et al.,* 2007; Throne-Holst et *al.,* 2007), and short chain alkanes are especially difficult for microorganisms to degrade in nature (Matsumiya and Kubo , 2007). Interestingly, only a few of alkB enzymes oxidize C_5–C_{13} alkanes, as does *P. putida* GPo1, whereas most members of this family prefer alkanes larger than C_{10} **(**Rojo *et al*., 2010**)**.

In order to identify *alkB1* genes involved in the biodegradation of long chain n-alkanes, the fragments amplified from strains EPa10, EPa45, HPa38 and HPa59 were purified and nucleotides sequences established. The four strains chosen have hospital or environmental origins. The sequences obtained were compared to that of *P. aeruginosa* PAO-1 (reference strain).The results obtained are shown in Figure N° 5.

5 - Nucleotide Sequencing

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          ....|....| ....|....| ....|....| ....|....| ....|....|
          1       10        20        30        40     50
alkB1,Pa10   CGGGGTTCAA GGTCGAGCAT GTGCGCGGCC ACCATGTGCA TGTGTCTACG
alkB1,Pa45   CGGGGTTCAA GGTCGAGCAT GTGCGCGGCC ACCATGTGCA TGTGTCTACG
alkB1,Pa38   CGGGGTTCAA GGTCGAGCAT GTGCGCGGCC ACCATGTGCA TGTGTCTACG
alkB1,Pa59   CGGGGTTCAA GGTCGAGCAT GTGCGCGGCC ACCATGTGCA TGTGTCTACG
alkB1,PAO-1  CGGGGTTCAA GGTCGAGCAT GTGCGCGGCC ACCATGTGCA TGTGTCTACG

          ....|....| ....|....| ....|....| ....|....| ....|....|
                60        70        80        90        100
alkB1,Pa10   CCGGAGGACG CTTCGTCGGC GCGTTTCGGC CAGTCGGTCT ACCAGTTCCT
alkB1,Pa45   CCGGAGGACG CTTCGTCGGC GCGTTTCGGC CAGTCGGTCT ACCAGTTCCT
alkB1,Pa38   CCGGAGGACG CTTCGTCGGC GCGTTTCGGC CAGTCGGTCT ACCAGTTCCT
alkB1,Pa59   CCGGAGGACG CTTCGTCGGC GCGTTTCGGC CAGTCGGTCT ACCAGTTCCT
alkB1,PAO-1  CCGGAGGACG CTTCGTCGGC GCGTTTCGGC CAGTCGGTCT ACCAGTTCCT



          ....|....| ....|....| ....|....| ....|....| ....|....|
                110       120       130       140       150
alkB1,Pa10   GCCGCATGCC TACAAGTACA ACTTCCTCAA CGCCTGGCGC CTTGAAGCGG
alkB1,Pa45   GCCGCATGCC TACAAGTACA ACTTCCTCAA CGCCTGGCGC CTTGAAGCGG
alkB1,Pa38   GCCGCATGCC TACAAGTACA ACTTCCTCAA CGCCTGGCGC CTCGAAGCGG
alkB1,Pa59   GCCGCATGCC TACAAGTACA ACTTCCTCAA CGCCTGGCGC CTCGAAGCGG
alkB1,PAO-1  GCCGCATGCC TACAAGTACA ACTTCCTCAA CGCCTGGCGC CTTGAAGCGG
          ....|....| ....|....| ....|....| ....|....| ....|....|
                160       170       180       190       200
alkB1,Pa10   TGCGGCTGCG CAAGAAGGGC CTGCCGGTGT TCGGCTGGCA GAACGAACTG
alkB1,Pa45   TGCGGCTGCG CAAGAAGGGC CTGCCGGTGT TCGGCTGGCA GAACGAACTG
alkB1,Pa38   TGCGGCTGCG CAAGAAGGGC CTGCCGGTGT TCGGCTGGCA GAACGAACTG
alkB1,Pa59   TGCGGCTGCG CAAGAAGGGC CTGCCGGTGT TCGGCTGGCA GAACGAACTG
alkB1,PAO-1  TGCGGCTGCG CAAGAAGGGC CTGCCGGTGT TCGGCTGGCA GAACGAACTG
          ....|....| ....|....| ....|....| ....|....| ....|....|
                210       220       230       240       250
alkB1,Pa10   ATCTGGTGGT ACCTGCTGAG CCTGGCGTTG CTGGTCGGTT TCGGTTGGGC
alkB1,Pa45   ATCTGGTGGT ACCTGCTGAG CCTGGCGTTG CTGGTCGGTT TCGGTTGGGC
alkB1,Pa38   ATCTGGTGGT ACCTGCTGAG CCTGGCGTTG CTGGTCGGTT TCGGTTGGGC
alkB1,Pa59   ATCTGGTGGT ACCTGCTGAG CCTGGCGTTG CTGGTCGGTT TCGGTTGGGC
alkB1,PAO-1  ATCTGGTGGT ACCTGCTGAG CCTGGCGTTG CTGGTCGGTT TCGGTTGGGC
          ....|....| ....|....| ....|....| ....|....| ....|....|
                260       270       280       290       300
alkB1,Pa10   GTTCGGCTGG CTGGGGATGG TTTTCTTCCT TGGCCAAGCG TTCGTCGCGG
alkB1,Pa45   GTTCGGCTGG CTGGGGATGG TTTTCTTCCT TGGCCAAGCG TTCGTCGCGG
alkB1,Pa38   TTTCGGCTGG CTGGGGATGG TTTTCTTCCT TGGCCAGGCG TTCGTCGCGG
alkB1,Pa59   TTTCGGCTGG CTGGGGATGG TTTTCTTCCT TGGCCAGGCG TTCGTCGCGG
alkB1,PAO-1  TTTCGGCTGG CTGGGGATGG TTTTCTTCCT TGGCCAAGCG TTCGTCGCGG
          ....|....| ....|....| ....|....| ....|....| ....|....|
                310       320       330       340       350
alkB1,Pa10   TGACCCTGCT GGAGATCATC AACTACGTCG AGCACTACGG CCTGCATCGG
alkB1,Pa45   TGACCCTGCT GGAGATCATC AACTACGTCG AGCACTACGG CCTGCATCGG
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alkB1,Pa38   TGACCCTGCT GGAAATCATC AACTACGTCG AGCACTACGG CCTGCATCGG
alkB1,Pa59   TGACCCTGCT GGAAATCATC AACTACGTCG AGCACTACGG CCTGCATCGG
alkB1,PAO-1  TGACCCTGCT GGAGATCATC AACTACGTCG AGCACTACGG CCTGCATCGG
          ....|....| ....|....| ....|....| ....|....| ....|....| ....|....|
                360       370       380       390       400
alkB1,Pa10   CGAAAGGGCG AGGACGGGCG CTACGAGCGG ACCAACCATA CCCACTCCTG
alkB1,Pa45   CGAAAGGGCG AGGACGGGCG CTACGAGCGG ACCAACCATA CCCACTCCTG
alkB1,Pa38   CGGAAGGGCG AGGACGGGCG CTACGAGCGG ACCAACCATA CCCACTCCTG
alkB1,Pa59   CGGAAGGGCG AGGACGGGCG CTACGAGCGG ACCAACCATA CCCACTCCTG
alkB1,PAO-1  CGAAAGGGCG AGGACGGGCG CTACGAGCGG ACCAACCATA CCCACTCCTG


          ....|....| ....|....| ....|....| ....|....| ....
                410       420       430
alkB1,Pa10   GAACAGCAAC TTCGTCTTCA CCAACCTGGT CCTG
alkB1,Pa45   GAACAGCAAC TTCGTCTTCA CCAACCTGGT CCTG
alkB1,Pa38   GAACAGCAAC TTCGTCTTCA CCAACCTGGT CCTG
alkB1,Pa59   GAACAGCAAC TTCGTCTTCA CCAACCTGGT CCTG
alkB1,PAO-1  GAACAGCAAC TTCGTCTTCA CCAACCTGGT CCTG
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Figure 5: Comparison of Nucleotides sequences of *alkB1* gene with that of *P. aeruginosa* PAO-1. *P. aeruginosa* strains EPa10 and EPa45 are from environmental origin; HPa38 and HPa59 are from clinical origin.

It appears that *alkB1*genes fragments of strains EPa10 and EPa45 (environmental strains) present a high identity with that of *P. aeruginosa* PAO-1; however, strains HPa38 and HPa59 (clinical strains) differ by 4 nucleotides with those of PAO-1 and the environmental strains. Relying on the aforementioned facts, we notice that high DNA sequence similarity exist between the four strains and *P. aeruginosa* PAO-1. Furthermore, as we could not detect a plasmid in those strains, it's very probable that *alkB1* genes, like that of PAO-1 strain, are carried not by a plasmid but by the chromosome. In brief, DNA sequence conservation has been reported by several authors who have dealt with *P. aeruginosa* strains degrading long chain n-alkanes (Smits *et al*., 1999, Guerra-santos *et al*., 1986).

IV. Conclusion

The *P. aeruginosa* strains isolated in Morroco show heterogeneity in both biodegradation of n-akanes and the presence of *alk* genes. No strains degrade short chains n-alkanes or posses the *alkB* gene. However, they degrade the long chains n-alkanes, but the biodegradation kinetics and the presence of *alkB1* genes vary according to strains; furthermore, a few strains did not posses this latest gene. The heterogeneity of *P. aeruginosa* strains is confirmed with their patterns amplification with primers ERIC.

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