Marine Science Center-University of Basrah

Mesopotamian Journal of Marine Sciences
Print ISSN: 2073-6428
E- ISSN: 2708-6097
www.mjms.uobasrah.edu.iq/index.php/mms

The importance of *Pseudomonas aeruginosa* plasmids for the n-alkanes biodegradation ability

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Article info.
✓ *Received: 6 March 2023*
✓ *Accepted:18 April 2023*
✓ *Published: 29 June2023*

Key Words:
Biodegradation.
N-alkanes,
Plasmids,
Pseudomonas aeruginosa,

Abstract - The study was conducted to evaluate the importance of *Pseudomonas aeruginosa* plasmids in their efficiency for n-alkanes biodegradation. *Pseudomonas aeruginosa* was isolated and characterized using biochemical and genetic tests, then acridine orange was used as a chemical plasmid curing agent, the loss of plasmids was confirmed by profiling using agarose gel electrophoresis comparing with the wild type, the two bands appear in the gel which located between 3Kbp to 4Kbp refer to the two plasmids harbouring by wild type. The biodegradation efficiency of wild and plasmid cured bacteria was 85% and 81.63% respectively, the study demonstrated that the loss of *Pseudomonas aeruginosa* plasmids leads to a slight reduction effect of the n-alkanes biodegradation efficiency

أهمية بلازميدات بكتريا *Pseudomonas aeruginosa* في قابلية تكسير الالكانات الاعتيادية

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المستخلص - أجريت الدراسة لتقييم أهمية بلازميدات بكتريا *Pseudomonas aeruginosa* في كفاءتها على التحلل الحيوي للالكانات الاعتيادية مستقيمة السلسلة. تم عزل وتشخيص بكتريا *Pseudomonas aeruginosa* باستخدام الاختبارات البيوكيميائية والجينية ، بعد ذلك استخدمت مادة الأكريدين البرتقالي للتخلص من بلازميدات البكتريا قيد الدراسة ، وتم تأكيد فقدان البلازميدات عن طريق الترحيل الكهربائي لهلام الاكاروز مقارنة بالبكتريا الطبيعية قبل المعالجة ، أن ظهور الحزمتين في الجل الذي تقع بين 4000 – 3000 قاعدة نيتروجينية يشير إلى اثنين من البلازميدات التابعة للبكتريا الطبيعية. بلغت كفاءة التحلل الحيوي للبكتيريا الطبيعية والبكتيريا المزال منها البلازميد 85% و81.63% على التوالي ، وأظهرت الدراسة أن فقدان بكتريا *Pseudomonas aeruginosa* لبلازميداتها يؤدي إلى تقليل طفيف في كفاءتها على تحليل الالكانات الاعتيادية.

الكلمات المفتاحية: *Pseudomonas aeruginosa* ، بلازميدات ، الكانات ،تكسير حيوي

DOI: https://doi.org/10.58629/mjms.v38i1.327.,

Introduction

Pseudomonas aeruginosa is a widespread microorganism, opportunistic pathogenic G-ve bacterium, capable of cause serious diseases for human during weak body defence and causing severe pulmonary and nosocomial infections. Pathogenicity is characterized by host cell pathways adhesion, modification, or disturbance. The biofilm of *P. aeruginosa* has protected it against wide types of antibiotics as well as host immunity Alhazmi, (2015); Diggle and Whiteley, (2020).

According to the data of Lara Rodríguez, (2018); Smits *et al.*, (2003), a great number of clinical *P .aeruginosa* isolates can degrade the long-chain alkanes. On the other hand Alonso *et al.*, (1999) approved that the growth of the clinical strains is not well on the long-chain alkanes as the environmental strains, perhaps because certain factors necessary for growth on these substrates (for example putative uptake proteins, alkane-solubilizing factors such as PraA and rhamnolipids, or enzymes involved downstream-metabolism) are not optimally expressed in these strains.

Plasmids are independently replicate extrachromosomal DNA molecules that provide additional genetic material important for the survival and adaptation of the bacterial hostsAntipov *et al.*, (2019); Banu and Prasad, (2017); San Millan, (2018). Some particular plasmids play a significant role in the adaptation of wild microbial populations to the hydrocarbon compounds, several genes related to microbial catabolic pathways responsible for the hydrocarbon degradation are generally located on plasmids, ex; alk (C5 to C12 n-alkanes), nah (naphthalene) and xyl (toluene) Sayler *et al.*, (1990). There is extrachromosomal DNA as plasmids in many different bacterial genera Isiodu *et al.*, (2016); San Millan, (2018). Researchers found that the biodegradation capacity of aerobic heterotrophic bacteria, with or without their degradative plasmid was approved by the plasmid-cure study Isiodu *et al.*, (2016); and confirmed this by removing a plasmid from bacteria which resulted in some degradation capacity decreasing, but which did not cause the loss of the whole biodegradation potentials. The previously mentioned results were supported by the study carried out by Akpe who observed that plasmid curing of *Klebsiella pneumoniae* and *Serratia marscencens* did not result in total loss of degradative abilities while only leads to a lack in their degradation capability Akpe *et al.*, (2013).

Materials and Methods

Pseudomonas aeruginosa was isolated and characterized using biochemical and genetic tests by own previous study Al-kanany and Othman, (2020) from oil-polluted soil samples which were collected from five different sources as shown in Figure 1 , and detected as alkanes degrading bacteria.

Figure 1: Sampling stations: 1: Second refining unit / Shuaiba refinery 2: Southern Rumaila 3: Al-Toba and Al-Nakhaila 4: Nurhan Omar 5:Al Qurna.

To determine whether the bacterial isolate with the ability of alkanes biodegradation carries its n-alkanes gene in the plasmid or chromosomal DNA, the plasmids of *Pseudomonas aeruginosa* were removed by plasmid curing protocol, then an experiment was designed to compare the ability of biodegradation between the plasmid cured and uncured *Pseudomonas aeruginosa* by plasmid curing using acridine orange Churchill and Romanus, (2019); Isiodu *et al.*, (2016), to achieve that, *Pseudomonas aeruginosa* were grown in nutrient broth overnight then five ml of the mentioned medium supplemented with 0.1 mg/ml acridine orange was prepared, then the organisms were sub-cultured into the prepared medium and incubated at 37ºC for three days after that 0.5ml of the growth was plated on nutrient agar, the colonies able to grow were isolated and considered as cured, These colonies were subjected to plasmid profiling to confirm the loss of plasmids.

The plasmids were isolated following the Qiagen kit instructions and to confirm the loss of plasmid (plasmid profiling), gel electrophoresis was performed.

The predicted results as below:

A. No bands in the gel, this result indicate the loss of plasmids and successful plasmid curing procedure.
B. The appearance of bands in the gel and refers to the presence of plasmids and fails of the plasmid curing procedure.

To detection the effect of plasmid losing on the utilizing of alkanes, a biodegradation experiment was conducted by preparation of Bushnell and Haas (BHM) medium containing

crude oil at the concentration of 0.5% v/v and the final experiment comprised three set-ups as below:

A. Plasmid cured bacteria: were inoculated in the BHM medium containing crude oil (0.5% v/v).
B. Plasmid uncured bacteria: also were inoculated in the BHM medium containing crude oil (0.5% v/v).
C. Control**:** BHM medium containing crude oil (0.5% v/v) was incubated without inoculum.

After 7 days of the incubation period, the residual crude oil was extracted and separated into aliphatic and aromatic fractions, the aliphatic fraction was sent to the GC laboratory, GC charts were plotted and the percentage of biodegradation was calculated.

Results

Plasmid curing and profiling

Line 1 of Figure 2 shows the gel electrophoresis result for the extracted plasmids of the *Pseudomonas aeruginosa* after performing the curing of plasmid by acridine orange, on the other hand, it is obvious that there are two bands in lane number 2 which reveals the gel electrophoresis result of plasmid extraction of the wild *Pseudomonas aeruginosa*.

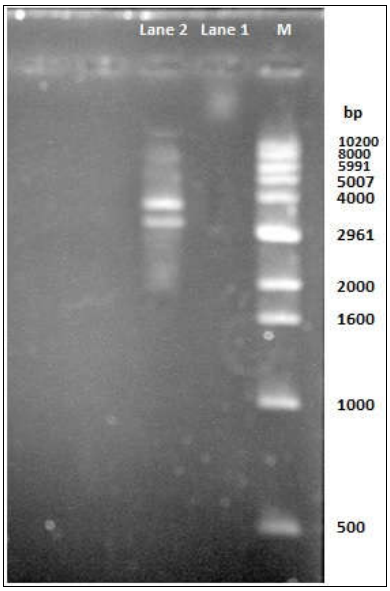

Figure 2:lane 1: the result of plasmid profiling by plasmid extraction method after plasmid curing, lane2: extraction of plasmids for wild *Pseudomonas aeruginosa* before plasmid curing, M: 1Kb ladder.

Evaluation of biodegradation percentage for the cured and wild bacterium

The blot of Figure 3 shows the aliphatic fraction residues of the extracted crude oil from the conical flask after the incubation period without any bacteria as a control part.

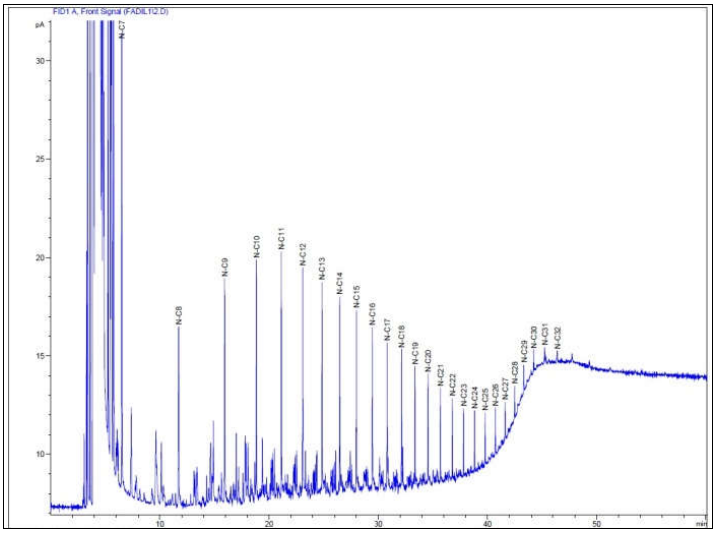

Figure 3: GC result of residual control crude oil (aliphatic fraction) 0.5% after the incubation period.

Figure 4 shows the GC result of aliphatic fraction residues after the incubation period of plasmid uncured bacteria with 5% crude oil, the degradation percentage was 83.87%, it calculated according to the GC report comparing to the control.

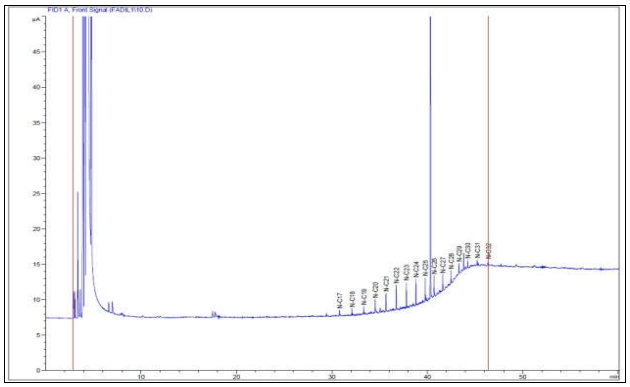

Figure 4:GC result of residual crude oil (aliphatic fraction) 0.5% after an incubation period of plasmid uncured bacteria**.**

Figure 5 shows the GC result of aliphatic fraction residues after an incubation period of plasmid cured bacteria with 5% crude oil, the percentage of degradation was calculated according to the GC report comparing with the control, it was 81.63%.

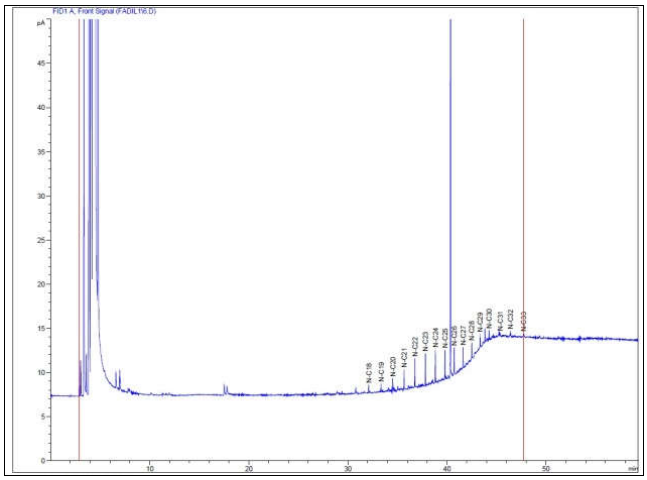

Figure 5: GC result of residual crude oil (aliphatic fraction) 0.5% after an incubation period of plasmid cured bacteria.

Discussion

The importance of plasmids for the ability of biodegradation

This study was conducted to determine whether the bacterial isolates with the ability of alkanes biodegradation carry their n-alkanes genes on the plasmid or chromosomal DNA, to ensure that, plasmid curing strategy was used to eliminate the plasmids of *Pseudomonas aeruginosa* and then biodegrading comparison study between the plasmid cured and uncured *Pseudomonas aeruginosa* was designed to prove this fact.

There are many chemical agents such as acridine orange, SDS and ethidium bromide, and physical agents were used in plasmid curing protocols Letchumanan *et al.*, (2015), in the current project acridine orange was used to remove the plasmids from the concerned bacteria. To confirm the loss of plasmids during the curing experiment, the plasmids of cured and uncured *Pseudomonas aeruginosa* was extracted and profiling in the gel to detect the presence of the plasmids, Figure 2 shows the presence of two bands located between 3Kbp to 4Kbp in the lane 2 which represented two plasmids related to the wild uncured bacterium while in the lane 2 there were no bands and that reflect the successful loss of plasmids during the curing process.

As shown in the Figure 3 , lots of the aliphatic compounds peaks (NC8-NC32) were detected and appear in the CG blot because this part of experiment was incubated without any bacterial inoculation and that refer to no biodegradation activity so that this part was considered as control, on the other hand the another part concerned with plasmid uncured *Pseudomonas aeruginosa*, the biodegradation percentage was 83.87% as shown in the Figure 4 when the wild bacteria (plasmid uncured bacteria) where incubated with the 5% crude oil, and that reflect the biodegradation ability of *Pseudomonas aeruginosa* and this agree with the study of Li *et al.*,

(2020) which was suggested that the capability of this bacterial species to utilized n-alkanes with chain lengths from 8 to 40 carbon atoms as a sole carbon source with a degradation efficiency ranges to about 85% on the other hand, Figure 5 shows the biodegradation percentage of the plasmid cured *Pseudomonas aeruginosa* after incubation with crude oil, the percentage was 81.63% and this also high degrading efficiency.

The data from the experiments demonstrate that the loss of plasmids had very slight negative and insignificant effects on the n-alkanes utilization ability which may be due to the experimental conditions, and that clarifies that the alkanes degrading genes in this bacterial species located in the chromosomal DNA, this result agrees with the study of Isiodu *et al.*, (2016) which was suggested that there is no significant change detected in the percentage of hydrocarbon degradation at the end of the incubation period between the plasmid cured and uncured bacteria, as well as the study of Akpe *et al.*, (2013) was proved that the plasmid curing leads to a little reduction in the degradation potential.

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