Marsh Bulletin 7(2)(2012)162-168

MARSH BULLETIN

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Use Polymerase Chain Reaction for Molecular Determination of Phenol Degrading Bacteria isolated from Shatt AL- Basra / Iraq

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Abstract

Phenol is one of the organic pollutants in various industrial wastewaters especially petrochemical and oil refining. Biological treatment is one of the considerable choices for removing of phenol from these wastewaters. Identification of effective microbial species is considered as one of the important priorities for bacteria producting biomass in order to achieve desirable kinetic of biological reactions. Basic purpose of this research is identification of phenol-degrading *Pseudomonas putida* by polymerase chain reaction (PCR) that has high speed and specificity . Amplification gene coding the N fragment in *Pseudomonas putida*-derived methyl phenol operon (DmpN gene) was used for specific identification of phenol-degrading *Pseudomonas putida*,According to the results of this study 7 of 10 isolated bacteria *Pseudomonas putida* showed a 199 bp PCR product by DmpN primers.

***Key Word**:* PCR ,phenol Degrading Bacteria, Molecular Determination .Basra-Iraq.

1- Introduction

Phenols are aromatic compounds that are characteristic pollutants in wastewater and effluents from chemicals, petrochemicals, pharmaceuticals, textiles, and steel industries [Rocha,*et al* 2007]. The unwholesome and environmentally unacceptable pollution effects of the phenolic effluent have been reported worldwide [Ruiz-Qrdaz, *et al*., 2001]. Phenol contaminants are relatively soluble in water and accumulate in soil, resulting in extensive surface water, ground

water, and soil contamination owing to its severe toxicity [Liu, *et al*,2005]. Currently removal of phenol effluents from contaminated sites has been a major environmental concern. Different techniques have been applied to remove phenolic compounds from polluted areas [Wu, *et al* 2000 and Carmona ,*et al* 2006]. However, among all biodegradation processes, the more opportunities to complete the destroy of pollutants if possible or at least to transform them to innocuous substance [Vidali,2001], therefore the biodegradation processes possess relatively low cost, no chemicals used, and high public acceptance [Atlas and Unterman 1999]. Research on microbial degradation on phenols has intensified in recent years because it is the sustainable ways to clean-up contaminated environments [Diaz, 2010]. Microbes will adapt quite rapidly and grow at extreme condition using hazardous compounds as carbon and energy sources, in waste streams. The Important examples include phenol, chlorophenol, chlorobenzene, chloroalkanes, atrazine, and nitro aromatics [Agarry *et al*, 2008].

A wide variety of microorganisms are known to be capable of metabolizing or mineralizing phenol under aerobic and/or anaerobic conditions. Metabolic processes are governed by the action of enzymes Many microbes belonging to the genus of *Pseudomonas* have been reported as good degraders of phenol [Dowling, and O'Gara 1994]. *Pseudomonas* strains are often utilized for metabolic pathway studies evaluating the degradation of many aromatic compounds involving phenol [Timmis *et al* , 1994]. In *Pseudomonas*, many induced enzymes are nonspecific, and the metabolic pathway contains a high degree of convergences. The convergence of catabolic pathway allows for the efficient utilization of a wide range of growth substrates, while the non specificity of the induced enzymes allows for the simultaneous utilization of several similar substrates without redundant genetic coding for enzyme induction more over,In many cases, the bacterial aerobic catabolism of phenol (alkyl) is initiated by a multicomponent phenol hydroxylase (mPH) that hydroxylases phenolic substrates to the corresponding catechols in the presence of O_2 and NAD(P)H. The mPH was first identified in phenol and (di)methylphenol (*dmp*) degradation pathways in *Pseudomonas* sp. In this study, the first aim was to isolation and describe molecular characterization of phenol-degrading bacteria obtained from oil-contaminated water, the second aim was to detect catabolic genes related to degradation of phenol [Hutchinson and Robinson 1988]. .

Materials and Methods

Samples Collection and Culture media

Five water samples were collected from various sites of Shatt Al Basra by sterilized bottles sealed and transport immediately to the laboratory. The synthetic phenol broth medium used was based on the mineral salts medium containing (per liter): 2.25g of KH2PO4, 2.25 g of K2HPO4, 1 g of (NH4)2SO4, 0.2g of MgCl2.6H2O, 0.1g of NaCl , 0.02 g of FeCl3.6H2O, 0.06 g of bromothymolblue and 0.01 g of CaCl2 with pH6.8-7. These synthetic medium were supplemented with phenol as the sole carbon source at a concentration of 3 mM [Watanabe, et al 1998a]. Phenol broth was solidified with 15 g/l agar to obtain phenol agar medium (fisher). All media were sterilized at 121°C for 15minutes

Isolation of Phenol-degrading Bacteria

One milliliter of water sample was inoculated into phenol broth medium. After7 days at room temperature with shaking ,this medium was sub cultured into phenol agar medium and nutrient agar medium then incubated at 25°C .

DNA extraction:

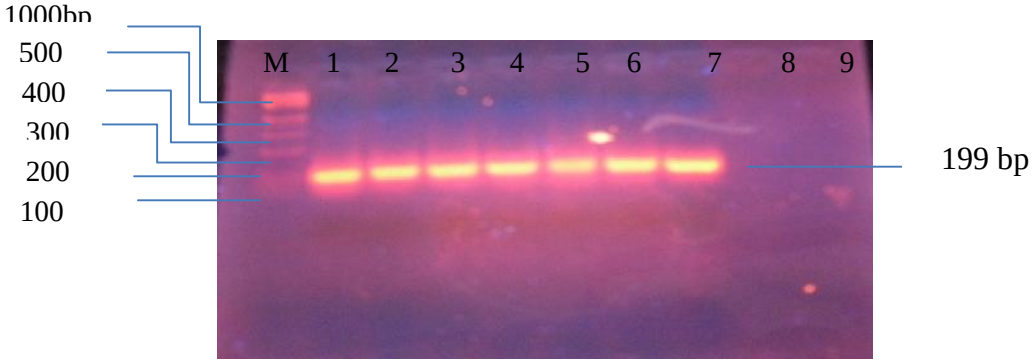
DNA was extracted from the pure culture of each isolates by boiling method. In this method, bacteria were dissolved in double distilled water and boiled for 1 min, then centrifuged at 10000 × g for 10min and 2 µl of aliquots phase was removed as template. The extracted DNA was checked by measuring UV absorption spectrum [Sambrook, et al.1989].

PCR condition:

For the detection of *P. putida* among isolates a 199-bp band located within the *dmpN* gene (a gene from the *dmp* operon) was amplified using two primers [Watanabe, et al 1998b].This operon encodes phenol hydroxylase enzyme. The sequences of primers and protocol depended on [Selvaratnam,et al.1997]. owere as follow: DMPN1 :(5′-ATC ACC GAC TGG GAC AAG TGG GAA GAC C-3′)DMPN2: (5′-TGG TAT TCC AGC GGT GAA ACG GCG G-3′)A total volume of 25 µl of PCR was amplified in a thermal cycler (Applied Biosystems) with initial denaturation of the target DNA at94 °C for 2 min, follow by 40 cycles of three-step PCR amplifications consisting of denaturation at 94 °C for 1min, primer annealing at 50 °C for 1 min and primer extension at 72 °C for 1 min. Samples were reacted at72 °C for 5 min at the end of amplification cycles to complete the extension reaction [Selvaratnam,et al.1997]. PCR products were electrophoresed on a 2 % horizontal agarose gel (Fisher). Gels solution were stained by ethidium bromide and visualized with a UV transilluminator.

3- **Results and Discussion:**

In this study ,10 various colonies were Gram negative rods which isolated based on specific morphologic characteristcs in order to study phenol degradation and molecular detection. Identification of phenol-degrading *P. putida* by using specific PCR primers (DMPN1 and DMPN2) was the aim of this study [Selvaratnam,et al 1997].The *dmpN* gene codes for phenol hydroxylase, an enzyme involved in the conversion of phenol to catechol [Nordlund,et al 1990]. Amplification of template DNA by DmpN primers indicated that seven bacteria out of ten isolates had a199-bp PCR product considered as *P.putida*. .Fig(1). This isolated phenol-degrading bacterium based on DmpN gene are encode to phenol hydroxylase in this species. It is clear that by using phenol degrading bacteria may lead to the promotion of phenolic waste water treatment. (Selvaratnam, et al.,1995).



Fig(1) The PCR product of *dmpN* gene visualized under UV light after electrophoresis in 2%) agarose gel(

M=Marker(100-1000bp), **1-7**= ***dmpN* gene** (199bp)

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أستخدام التفاعل متعدد السلسلة(PCR) في التشخيص الجزيئي للبكتريا المحطمة للفينول والمعزولة من مياه شط البصرة-العراق

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الخلاصة

يعتبر الفينول احد اهم الملوثات العضوية في مياه الصرف الصحي الصناعية المختلفة وخصوصا البتروكيماويات وتكرير النفط. وتعد المعالجة البيولوجية هي واحدة من الخيارات الكبيرة لإزالة الفينول من مياه الصرف الصحي. ويعتبر تحديد أنواع الجراثيم امرفعل باعتبارها واحدة من الأولويات الهامة لإنتاج الكتلة الحيوية لغرض التفاعلات البيولوجية.أن الغرض الأساسي من هذا البحث هو تحديد بكتريا الزائفة المحطمة للفينول *Pseudomonas putid* بواسطة تفاعل البلمرة المتسلسل (PCR) الذي يتميز بسرعة وخصوصية عالية . وقد استخدام جين الترميز لجزء N في اوبرون البكتريا الزائفة المحطمة للفينول (DmpN) من خلال تفاعل البلمرة المتسلسل (PCR) لتحديد الهوية العامة لهذه البكتيريا حيث كانت النتيجة وجود 7 من 10من البكتيريا المعزولة *Pseudomonas putida* الزائفة لأنها تمتلك الجين (DmpN) المسؤول عن ذلك التحطيم والمقدر بحجم 199زوج قاعدي وفقا لنتائج تفاعل البلمرة المتسلسل(PCR) في هذه الدراسة.