Some chemical effects on growth of Azotobacter chroococcum wild-type and it's mutant-44

** Talib, k. Al-mofraji,* *** sanaa, s.k. al-kubaisi*

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Abstract:

The effects of some chemicals (50 ppm of aniline, phenol, benzoic acid,different concentrations of NaCl, and different pH-values) on growth of two *Azotobacter chroococcum* strains, wild-type-3, and it' s Mutant-44 were studied. The results indicated that the presence of phenol and aniline caused significant increase of growth of the Mutant-44 compared with that in the control. Increase of growth of this mutant in the presence of benzoic acid was observed, whereas, growth of the Wild-type-3 has increase only in the presence of phenol but lower than the control. The Wild-type-3 tolerated NaCl concentrations up to 1.5%, while, growth of the Mutant-44 was inhibited at all NaCl concentrations.

Maximum growth of the Wild-type-3 and the Mutant-44 was observed at pH 7 and 9 respectively. Growth of these two tested strains was completely inhibited at highly-acidic and basic conditions.

Introduction:

Chemical compounds such as aniline, phenol and benzoic acid are intermediate products during the microbial degradation of numerous pesticides in the soil. These intermediate compounds accumulated in soils because of the progressive use of pesticides(1)

. Moreover, the simple phenolic and benzoic compounds are commonly found in the decaying plant residues(2). These chemical pollutants have different effects on soil microorganisms especially on N-fixing microorganisms(3,4,5,6,7,8).

* Basic Science Branch,Dentistry College, Al-Mustansirya University , Baghdad ,Iraq

** Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.

Soil N-fixing bacteria closely related to ***Pseudomonas*** can grow in N-free medium supplemented with 5mmol./L of each of the following compounds, P-hydroxybenzoate, protocatechuate and benzoate, which has found that these bacteria could reduce acetylene in the presence of these aromatic compounds (4). However, the effect of these chemical factors on growth of ***Azotobacter chroococcum*** has not been fully established. Some reports have been published on the distribution and densities of ***Azotobacter spp.*** in Iraqi soils .For example, it was found that ***Az. chroococcum*** and ***Az. Vinelandi*** were abundant in Iraqi soils(9), while other studies indicated that the majority of the isolates were identified as ***Az. Chroococcum*** **75.6 %,** ***Az.venelandii*** 15.6% and ***Beijerinkia*** **spp.**were of lesser frequency **8.8%** out of 90 isolates from different regions of Iraq (10).

Initial works 0n ***Az. chroococcum*** isolates indicated that these isolates were able to grow at different salinity levels in the soils (11,12,13). Some reports showed that ***Az. chroococcum*** strains were abundant in soil habitats at pH-range 7-8 (10,14).

The present work aims to study the effects of some factors, such as aniline, phenol and benzoic acid, salinity levels, and pH-values on growth of ***Az. chroococcum*** Wild-type-3 and its Mutant-44.

Materials and methods:

Organisms:

Bacterial strains of ***Az. chroococcum*** described in previous study (15) were used.These strains were maintained at 4°C on N-free agar medium desicribed previouly (14).

Growth experiments:

100 ml. of mineral salt medium described previously (14) was added to sterile 250ml. conical flasks and supplemented with the appropriate amounts of aniline, phenol and benzoic acid to obtain 50 ppm concentration. To other parts of this medium, different concentratios of NaCl were added to obtain 0.5%, 1%, 1.5% and 2% concentrations. The pH values (4, 6, 7, 8, 9 and 10) of required media were adjusted using 1N. HCl and lN. NaOH. Appropriate amounts of bacterial cell suspensions(72-hours old) were added to the above treated liquid media to get the desired optical densities of 0.03-0.05/ml. Untreated media were inoculated with desired amounts of cell suspensions of the two strains used as controls. Duplicate of all above treatments were incubated in an environmental-

orbital-shaker 80 rpm at 28°C. The optical densities of the growth cultures were determined at 578nm using spectrophotometer (Spectronic 20).

Results:

Az. chroococcum strains showed variable growth densities in the medium containing the chosen aromatic compounds. The Wild-type strain could grow in the presence of phenol, but it's growth density was less than that in the control. Presence of aniline or benzoic acid caused a significant growth reduction of this strain (Fig. 1). Growth of the Mutant showed an evidence that the presence of aniline and phenol caused significant increase, where as the benzoic acid did not result in such increase as compared with the control.

The various NaCl concentrations had different effects on growth of the two strains (Fig. 2). The Wild-type strain tolerated up to 1.5% of NaCl concentration. There were no clear differences in its growth in 0.5, and 1.5% NaCl. Growth of this strain was clearly inhibited by 2% NaCl. Growth of the mutant strain was completely differed, it was inhibited by all NaCl concentrations. pH effect indicated that the suitable value for growth of the two strains were in range 7-9 as shown in (Fig. 3). The Wild-type, apparently, was favored by neutral pH. It flourished at pH 7. It's growth at (pH 4 and 6) as well as at (pH 10) was inhibited. On the other hand the Mutant strain thrived at (pH 8 and 9). It's growth was partially reduced at (pH 6) and completely inhibited at(pH 4 and10).

Discussion:

The results revealed that aniline and benzoic acid had inhibition effects on the growth of the Wild-type strain. Therefore, it may be suggested that the expected induction of oxygenase enzyme system (OES) was not occurring in the presence of aniline and benzoic acid, and these compounds may completely suppress the already present nitrogenase enzyme in this strain,this suggestion is in agreement with the findings reported previously (4), which indicated that the presence of protocatechuate,P-hydroxybenzoate and benzoate caused adecrease in the nitrogenase activity of two tested diazotrophic ***Pseudomonas*** *spp.*

Furthermore, it is already known that the microbial transformation of mono-aromatic compounds into catechols depends on the type of the responsible OES. These systems are different due to the type of the additional group (s) and it's position on the benzene ring.(8,16). This previously evidence confirmed the ability of the Wild-type to grow only in presence

of phenol, while the Mutant was able to grow in the presence of all used aromatic compounds. Based on these results may suggest that production of OES was differed in the Mutant and have protected the nitrogenase enzyme from the O_2 activity repression. The present results also indicated that the two tested strains have different physiological growth mechanisms in the presence of NaCl.

NaCl concentration effect observed in this work and that reported previosly(10,11,12) is not supported by any valid interpretation other than appreciable salt tolerance of ***Az. chroococcum*** strains.

Both present and previous results (14,17,18) agree in terms of limited pH range (7-9) suitable for optimum growth of ***Az. chroococcum*** strains. This leads to the conclusion that both strains have comparable pH tolerance.

*Corresponding address: Assist. Prof. Dr. Talib,K. AL-Mofarji ,

Al-Karkh,AL-Dawoudi post office, P.O.Box No.28478

Postal code 12631, Baghdad, Iraq.

E-mail: talibmofarji@yahoo.com

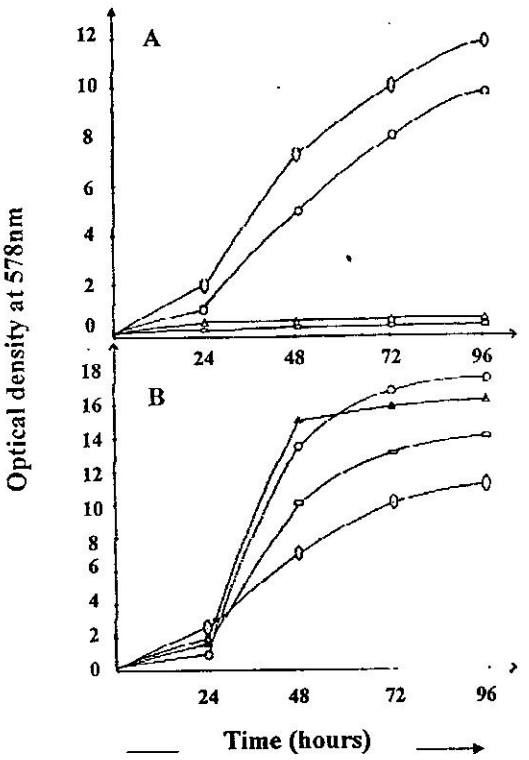

Figure (1): Growth of ***Azotobacter chroococcum*** wild-type-3 (A) and it's mutant-44 (B) control ; and in presence (50ppm) of phenol ; aniline ; and benzoic acid

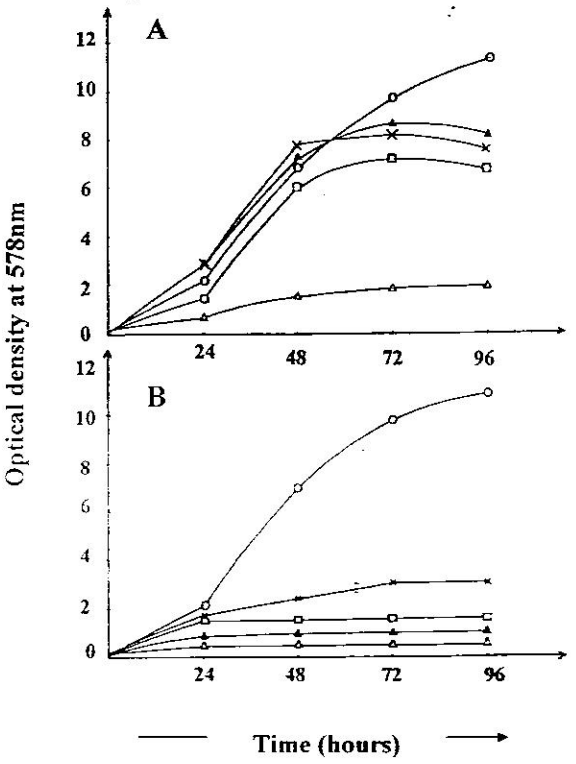

Figure (2): Growth of *Azotobacter chroococcum* wild-type-3 (A) and it's mutant-44 (B) control ; and in presence (0.5% and 2%

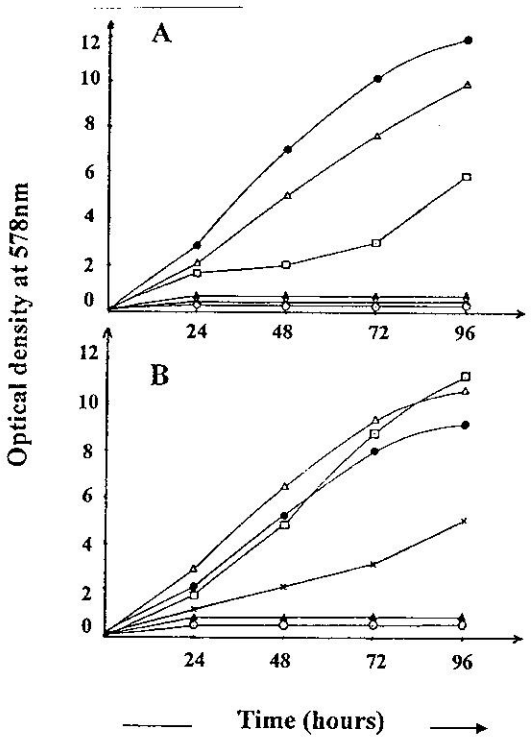

Figure (3): Growth of *Azotobacter chroococcum* wild-type-3 (A) and it's mutant-44 (B) mineral medium at pH-4 ; pH-6 ; pH-7 ; pH-8 ; pH-9 ; and pH-10

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بعض التاثيرات الكيمياوية في نمو *AZOTOBACTER CHROOCOCCUM* البرية وطافرتها-٤٤ .

طالب كاظم المفرجي * سناء سعود الكبيسي **

* استاذ مساعد، فرع العلوم الاساسية،كلية طب الاسنان، الجامعة المستنصرية ،بغداد،العراق.

** مدرس،قسم علوم الحياة،كلية العلوم،جامعة بغداد، بغداد، العراق.

الخلاصة:-

درست تاثيرات بعض المواد الكيمياوية(٥٠ جزء بالمليون من الأنيلين،الفينول،حامض البنزويك وتراكيزمن ملح الطعام،وقيم مختلفة من pH)في نمو بكتريا ***Az. chroococcum*** البرية-٣ وطافرتها-٤٤. دلت النتائج بان وجود الفينول والأنيلين قد سببا زيادة معنوية في نمو السلالة الطافرة-٤٤ مع زيادة طفيفة في نمو هذه السلالة بوجود حامض البنزويك، بينما كانت الزيادة في نمو السلالة البرية-٣ بوجود الفينول فقط ولكنها اقل من السيطرة. اظهرت النتائج ايضا بان ملح الطعام قد ثبط نمو السلالة الطافرة-٤٤ بوجود التراكيز المستخدمة، بينما لم يتاثر نمو السلالة البرية-٣ بتراكيز الملح لحد تركيز ١،٥%. بينت النتائج ايضا ان افضل نمو للسلالتين البرية-٣ والطافرة-٤٤ كان عند قيم PH ٧-٩ على التوالي، وثبط نمو السلالتين في الوسط الحامضي والقاعدي العالي.