

EFFECT OF TEMPERATURE AND SODIUM CHLORIDE CONCENTRATION ON GROWTH OF *Staphylococcus aureus* MUTANTS

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SUMMARY

The study included the identification of *Staphylococcus aureus* (wild type and mutant) isolates. All these isolates were identified as coagulase and catalase positive and able to ferment mannitol.

Strain number 24 was chosen & undergone mutation with NTG to isolate temperature sensitive osmotically fragile (TOF) mutants. TOF mutants were successfully isolated by exposing the cell suspension to 200 Mg/ml of NTG for 75 minutes. The results of characterization of TOF mutants, showed that these mutants were identical to their mother strain morphologically and biochemically. The result of this mutation is to make the lysozyme able to lyse the cell wall of the TOF mutants in pattern similar to lysostaphin .

Antibiotic sensitivity pattern was demonstrated for all the isolates and multiple resistance of some isolates were detected. The obtained results showed that the two mutant isolates were resistant to Methicillin, Benzathin, Penicillin, Cloxacillin Sodium and Lincomycin, whereas these isolates were sensitive to Amoxicillin, Cefotaxine Sodium, Cephalexin Sodium and Tetracyclin but intermediate in resistance to Clindamycin.

The effect of different temperature showed that the growth of the mother strain (strain No.24) increased with the increase of temperature. The mutant strains (3M,10M) are not stable in the different temperature and their growth is very low at 40C.

The effect of different NaCl concentration showed that the growth of the mother strain (strain No.24) and the 2 mutant strain (3M,10M) would increase with the increase of the NaCl concentration but decrease at NaCl concentration = 1.5 M .

Keywords: *Staphylococcus aureus*, temperature sensitive osmotically fragile mutants and TOF mutants.

Introduction

The Staphylococci are Gram-Positive, spherical cell usually arranged in grape like irregular clusters. They grow readily on many types of media and are active metabolically. Fermenting carbohydrates and producing pigments that vary from white to deep yellow(Cynthia M.*et.al*,2002) .Some are members of normal flora of the skin and mucous membranes of humans. Other cause, abscess

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formation a variety of pyogenic infections and even fetal septicemia. *Staphylococci* produce a variety of extracellular enzymes and toxins.

Temperature-Sensitive (ts) mutants : The phenotype of conditional mutant is expressed only when the organism is grown under a particular set of conditions. Ts stains grow normally at the permissive temperature (30-33C), but exhibit the mutant phenotype at the elevated, restrictive (or non permissive) temperature (39-43C). The Ts phenotype is thought to arise from unfolding and consequent inactivation of the mutant polypeptide chain at the restrictive temperature "Glass, 1982" . An amino acid replacement that alters the gene product's conformational stability is, presumably, responsible for this conditional response (Alžbeta *et.al.*,2009).

Conditional mutants have proved extremely useful in the analysis of essential genes. Lesions at these loci are normally lethal (unless back-up mechanism or second, Functional gene copy is also present), where as the viability of Ts conditional-lethal mutant is maintained at the permissive temperature; the existence of a lethal mutation in an indispensable gene is essentially cryptic under these non-restrictive conditions. Identification of the mutant protein may lead to the isolation of the essential gene product, itself Nonsense mutants are particularly amenable to this type of analysis because of the difference in size between the wild type and foreshortened polypeptides "Darke, 1970" .

Material and methods

Antibiotics were used for antibiotic sensitivity test for *S.aureus* strains as:

- 1- Methicillin
- 2- Benzathin Pencillin
- 3- Amoxicillin
- 4- Cloxacillin Sodium
- 5- Clindamycin
- 6- Cefotaxime Sodium
- 7- Lincomycin Hcl
- 8- Choramphenicol
- 9- Cephalexin Sodium
- 10- Tetracyclin

Bacterial Strain

Three strains of *S.aureus* were used :

The one type strain is *Staphylococcus aureus* ATCC 25923 (no. 24) and two are mutants (3M and 10M) taken from Biotechnology laboratory of Baghdad unversity.

Culture media

The following culture media were used through the study :

Broth media:

- 1) Casipeptone 2.5 gm
- 2) Thiopeoptone 2.5 gm

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3) Beef extract (Bactigrade) 3.0 gm

25 gm were dissolved in 1 L distill water and sterilized in the autoclave for 15 min. at 121 °C pH was adjusted to 7.0-7.2 at 15 pounds/In². (Baron *et al.*, 1996)

Agar media :

- 1) Beef extract, Bactigrade 1.0 mg
- 2) Yeast extract, Bactigrade 2.0 mg
- 3) Peptone, Bacteriological 5.0 gm
- 4) Sodium chloride 5.0 gm
- 5) Agar, Bactigrade 12.5 gm

25 gm were dissolve in 1000 ml distill water was sterilized by autoclave for 15 min at 121° C pH was adjusted to 7.2. . (Baron *et al.*, 1996)

Brain heart infusion broth :

- 1) Calf brains 200 gm
- 2) Beef heart 250 gm
- 3) Proteose peptone 10 gm
- 4) Bacto Dextrose 2 gm
- 5) Sodium chloride 5 gm
- 6) Disodium phosphate 2.5 gm

37 gm were dissolve in 1000 ml distill water was sterilize in the autoclave for 15 min at 121 °C the PH was adjust to 7.4. add 2 gm agar to get BHI agar . (Baron *et al.*, 1996)

Sterilization methods

The methods were used to sterilize glassware by oven at 180 °C for 2hr

Preservation of *S.aureus* isolates :

The isolates were preserved on slant of nutrient media or brain heart infusion agar under 4° C in refrigerator and these slants subcultured every 3 weeks .

Antibiotic Sensitivity Test :

(Bauer - Kirby *et al.*, 1966) : A few colonies of the organism to be tested were picked with a wire loop from the original culture plate and introduced into a test tube containing 5 ml Brain-Heart-Infusion broth. These tubes were then incubated overnight at 37 °C. The bacterial broth suspension was streaked evenly onto the surface of BHI agar plates with a spreader. After the inoculum had dried (3 to 5 minutes), the disks are placed on the agar with flamed forceps and gently pressed down to ensure contact then plates were incubated at 37 C.

Growth curve (Miller, 1972) :

Bacterial strain number 24,3M & 10 M was grown in BHI broth overnight. Bacterial cells were collected from the overnight culture by centrifugation & inoculated into new flask containing BHI broth. Every 1 hr the O.D was measured at (540 nm) and serial dilutions were made to establish the total viable count on a BHI agar plates.

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Effect of temperature shift on the growth of mutants (Nieuwlandt & pattee, 1989) :-

An overnight culture of the wild type (24) and the mutant (3M and 10M) were prepared each of these cells were inoculated into 3 flasks containing media without NaCl. The Flasks of each isolate were incubated at 36, 40, 43 C and the O.D (540 nm) was measured every 1 hour for 5 hours.

Effect of NaCl on the growth of mutant&wild type at 36° C, 40 °C and 43 °C (Good and pattee, 1970) : The wild type bacterial strain and the mutant (3M and 10M) were grown in nutrient broth containing 0.5, 1 and 1.5 M NaCl at 36° C over night. The bacterial cells were collected by centrifugation & inoculated into BHI broth plus 0.5, 1 and 1.5M NaCl. The growth was monitored during subsequent incubation at 36, 40 and 43° C. The O.D was measure at (540 nm) every 1 hr for 5 hours.

Results & Discussion

Antibiotic Sensitivity of mutants :-

Level of resistance was measured as resistant (R), intermediate (int) or sensitive (S)"David, Ian, J.David, Richard (1978) " .

Antibiotic sensitivity test was performed for the mutants and the type strain to see whether the mutagen (NTG) has made changes in the pattern of antibiotic sensitivity. As shown in tables (1, 2, 3) the antibiotic sensitivity pattern did not change for methicillin, Benzathin pencillin, Amoxicillin, Cefotaxime Sodium, Lincomycin Hcl, Cephalexin Sodium and Tetracyclin. (Mulla S *et.al*,2007) In this regard, the type strain was intermediate in resistance to choramphenicol, whereas the mutant strain 3M showed an intermediate level of resistance but the other mutant 10M was sensitive. The type strain was cloxacillin sodium and clindamycin sensitive but the two mutants (3M and 10M) were resistant to cloxacillin sodium and intermediat in resistant to the clindamycin. (Omololu-Aso, J. 2011) The differences in pattern of resistance between wild type and mutants might be due to mutational changes that occurred as a result of NTG treatment (Drake, 1970).

Table 1: the sensitivity of *S. aureus* no. 24 toward the antibiotics

No.	Antibiotic disk	Inhibition zone (mm)	sensitivity
1	Methicillin	8	R
2	Benzathin pencillin	15	R
3	Amoxicillin	32	S
4	Cloxacillin sodium	27	S
5	Clindamycin	22	S
6	Cefataxime sodium	27	S
7	Lincomycin HCl	8	R
8	Choramphenicol	15	Int.
9	Cephalexin sodium	25	S
10	Tetracyclin HCl	25	S

R: Resistance, S: sensitive, int.: intermediate

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Table 2: the sensitivity of *S. aureus* no. 3 M toward the antibiotics

No.	Antibiotic disk	Inhibition zone (mm)	sensitivity
1	Methicillin	8	R
2	Benzathin pencillin	16	R
3	Amoxicillin	27	S
4	Cloxacillin sodium	18	R
5	Clindamycin	14	Int.
6	Cefataxime sodium	24	S
7	Lincomycin HCl	8	R
8	Choramphenicol	15	Int.
9	Cephalexin sodium	27	S
10	Tetracyclin HCl	26	S

R: Resistance, S: sensitive, int.: intermediate

Table 3: the sensitivity of *S. aureus* no. 10 M toward the antibiotics

No.	Antibiotic disk	Inhibition zone (mm)	sensitivity
1	Methicillin	8.2	R
2	Benzathin pencillin	18	R
3	Amoxicillin	38	S
4	Cloxacillin sodium	20	R
5	Clindamycin	14	Int.
6	Cefataxime sodium	25	S
7	Lincomycin HCl	7.8	R
8	Choramphenicol	18	S
9	Cephalexin sodium	26	S
10	Tetracyclin HCl	25	S

Growth curve of *S.aureus* number 24, 3M and 10M :-

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The growth curve of *S.aureus* isolates (24,3M and 10M) was determined the lag phase lasted two hours when the bacterial cells were grown in Brain hear infusion broth. After two hours a gradual and steady increase was observed as the cells entered the log phase which lasted for 16 hours after which the cells entered the stationary phase. The results showed that the generation time of isolated was 27 minutes .

Effect of temperature on growth :-

To determine the response of the bacterial strains (The type and the two mutants strains) to the temperature, a temperature shift experiment was performed. The results obtained with mother and mutants .

Growth was measured for five hours by means of optical density. The shift in temperature was applied after two hours of growth. The results indicated that the growth of the mother and the mutant strains was stopped and remained at a constant O.D (540 nm) value after temperature shift viable count showed a similar result in which the number of cells remained constant after the temperature shift .

The result showed that the growth of the mother strain increased with the increase of the temperature (at 36, 40, 43 °C) in figuar 1,2 and3 but the 3M and 10M mutants is affected by 40C this temperature slow down the growth .

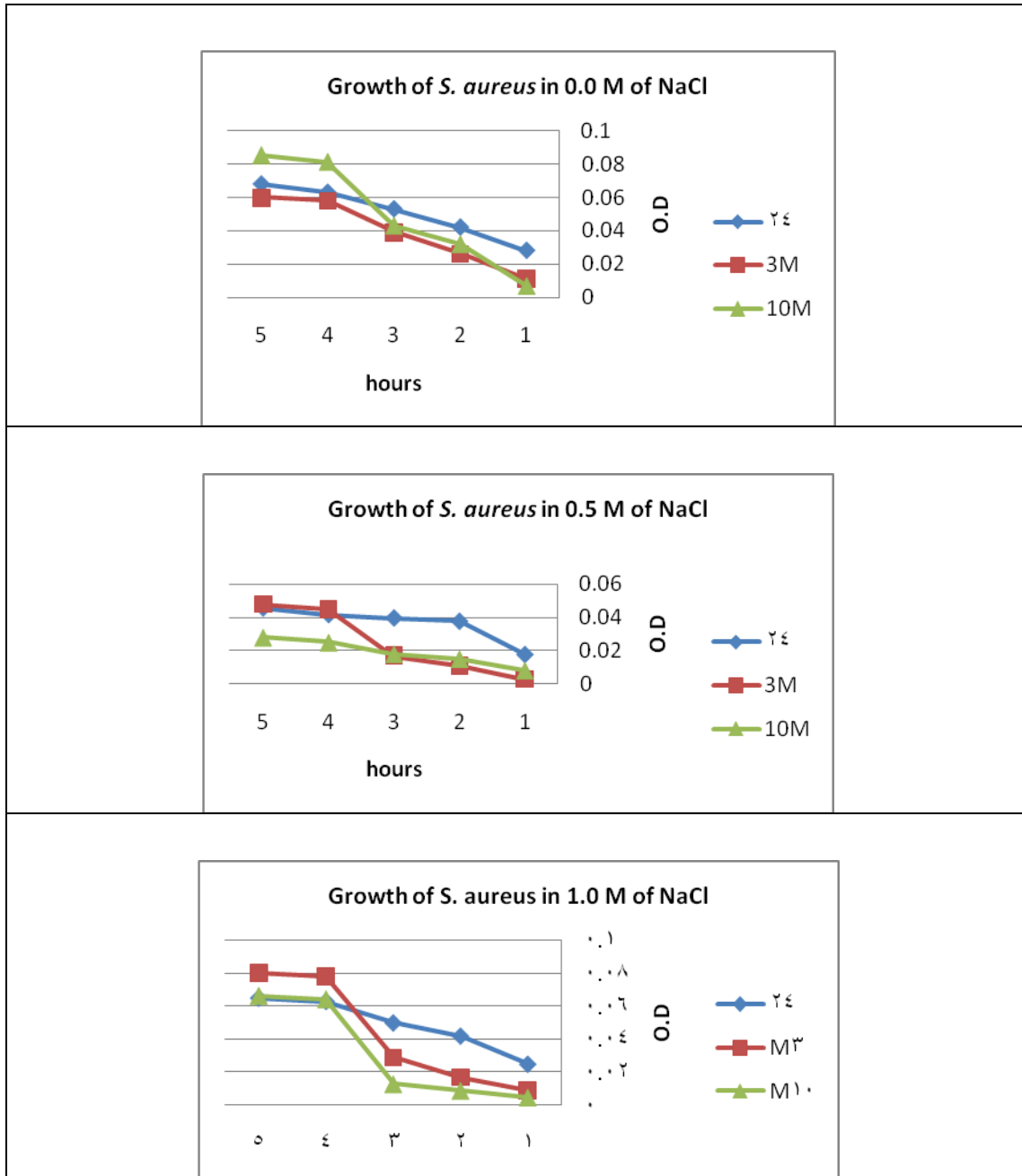
When mutants cells were exposed to lysozyme protoplasts were formed as examined under microscope.

The result of this experiment indicated that the mutants (3M and 10M) were conditionally temperature sensitive and the defect might in cell wall . This defect could be due to change in peptidoglycan biosynthesis or to the action of autolytic enzymes, or other factors required for the maintenance of the cell wall integrity “Nieuwlandt and pattee, (1959) , Mitchll and Moyle, (1975) “.

Effect of NaCl on growth :-

The effect of NaCl on growth of the wild type strain number (24) and the mutant strains (3M and10M) at 36, 40, 43 °C was ascertained. The strains are also grown in Brain-Heart-Infusion broth at 36, 40, 43 °C in the presence and absence of NaCl at concentration of (0.5M, 1M, 1.5M). As shown in the figures (1,2and3), the absence of NaCl from the media, do not effect the growth of wild type *S.aureus* at different temperatures. These results revealed that NaCl supported the growth of the mutant strain at different temperatures, which comes in agreement with the observation of (Mitchell and Moyle, 1975) who demonstrated that sugar or NaCl could stabilize the mutants of *S.aureus*.

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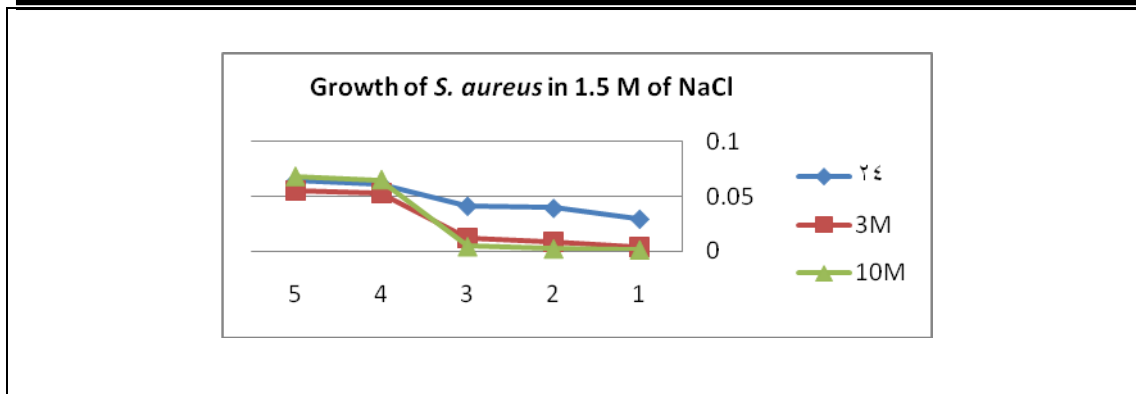
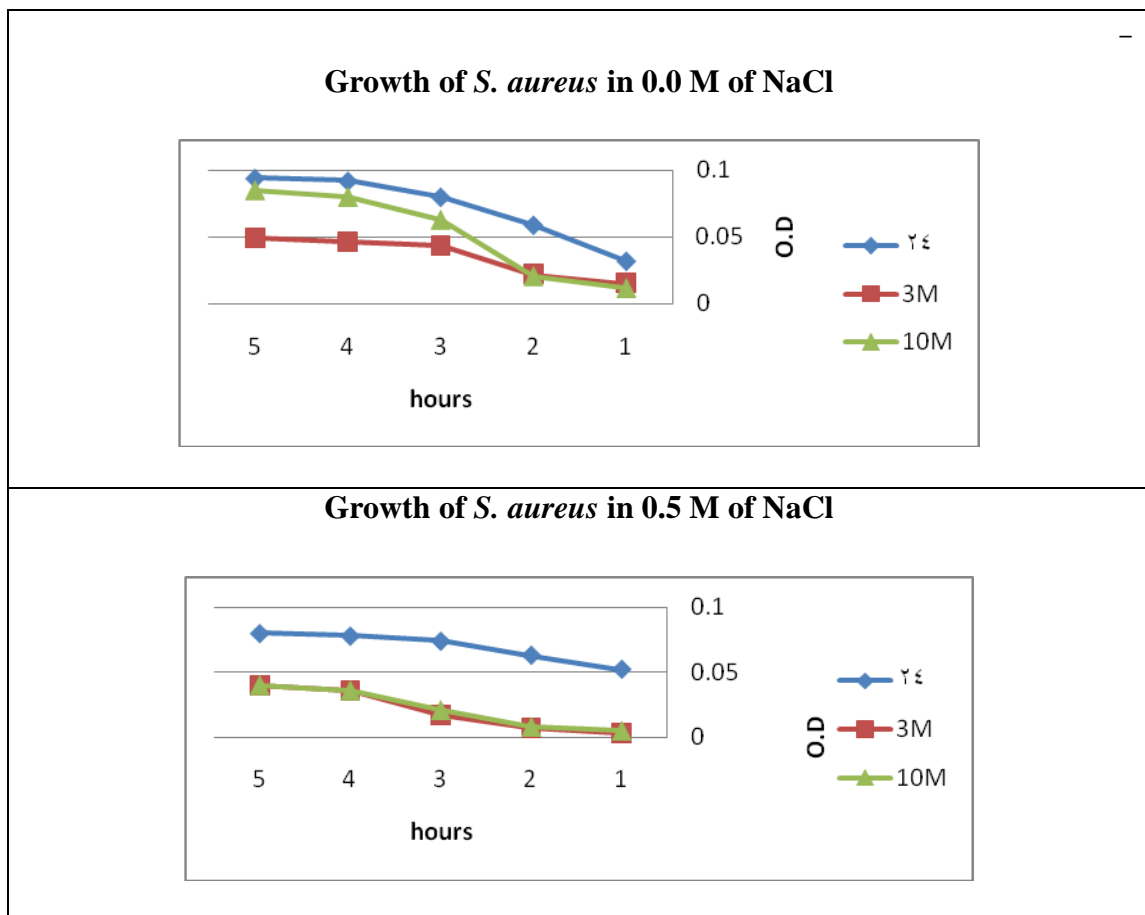


Fig 1: Effect of NaCl concentration on the strains of *S.aures* at 36° C



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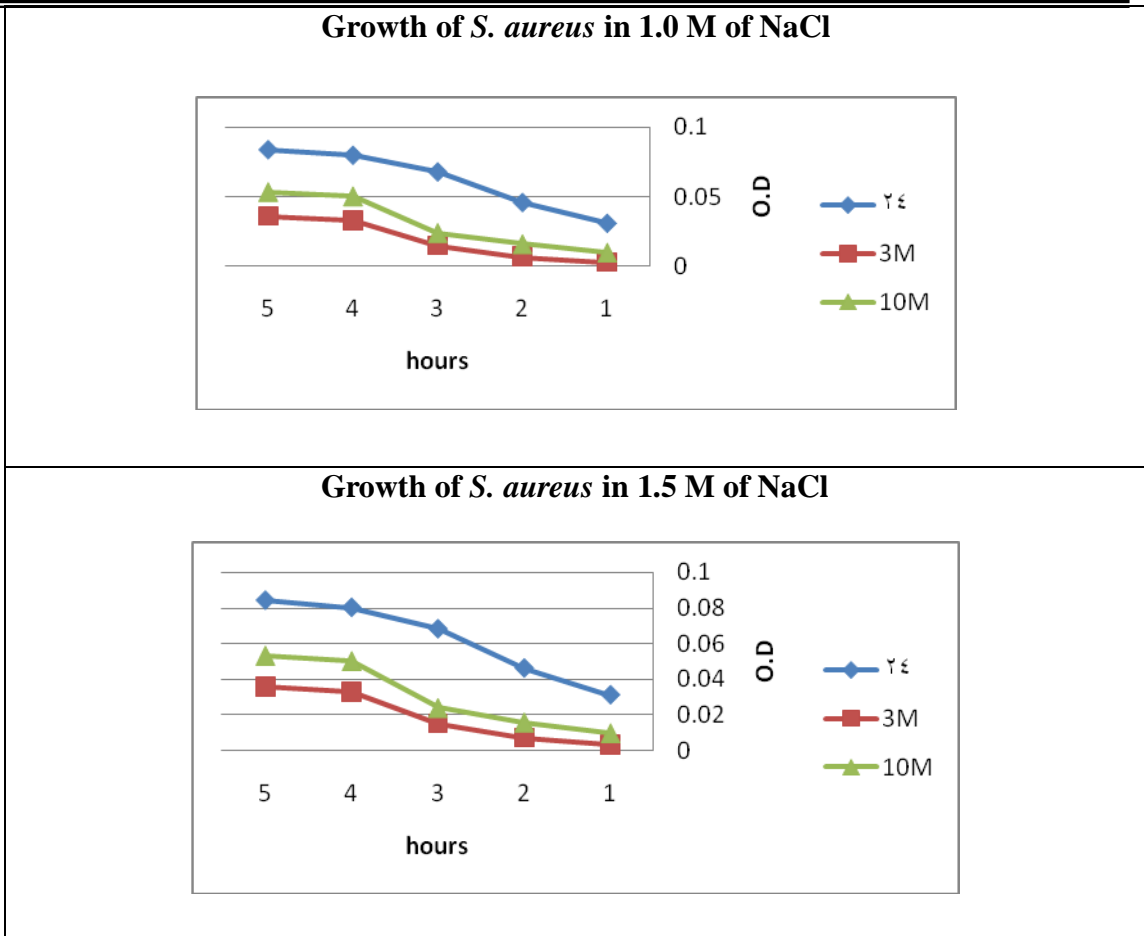
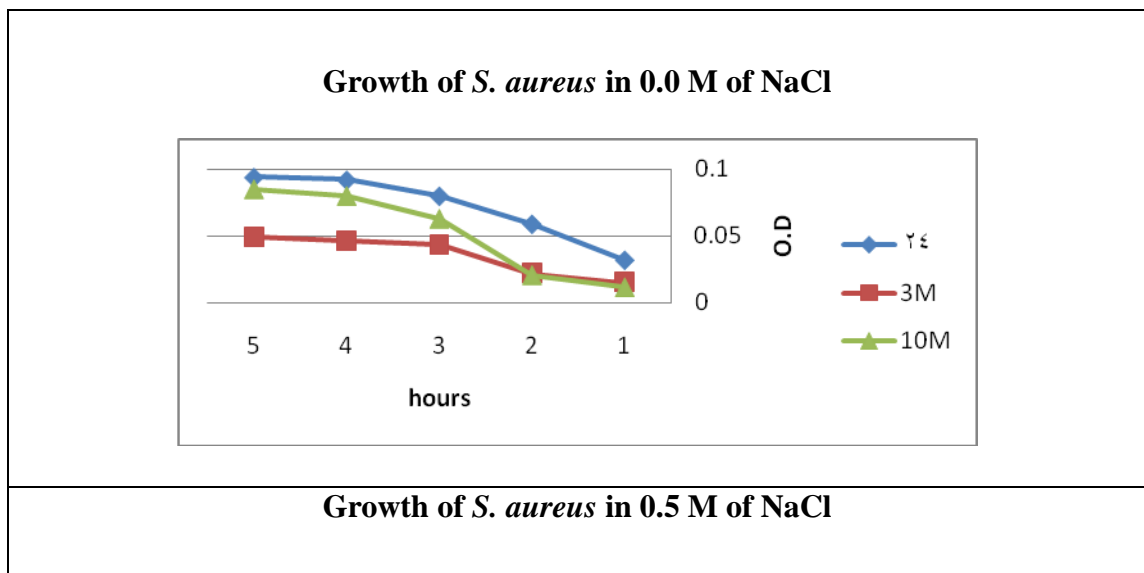


Fig 2: Effect of NaCl concentration on the strains of *S. aureus* at 40° C



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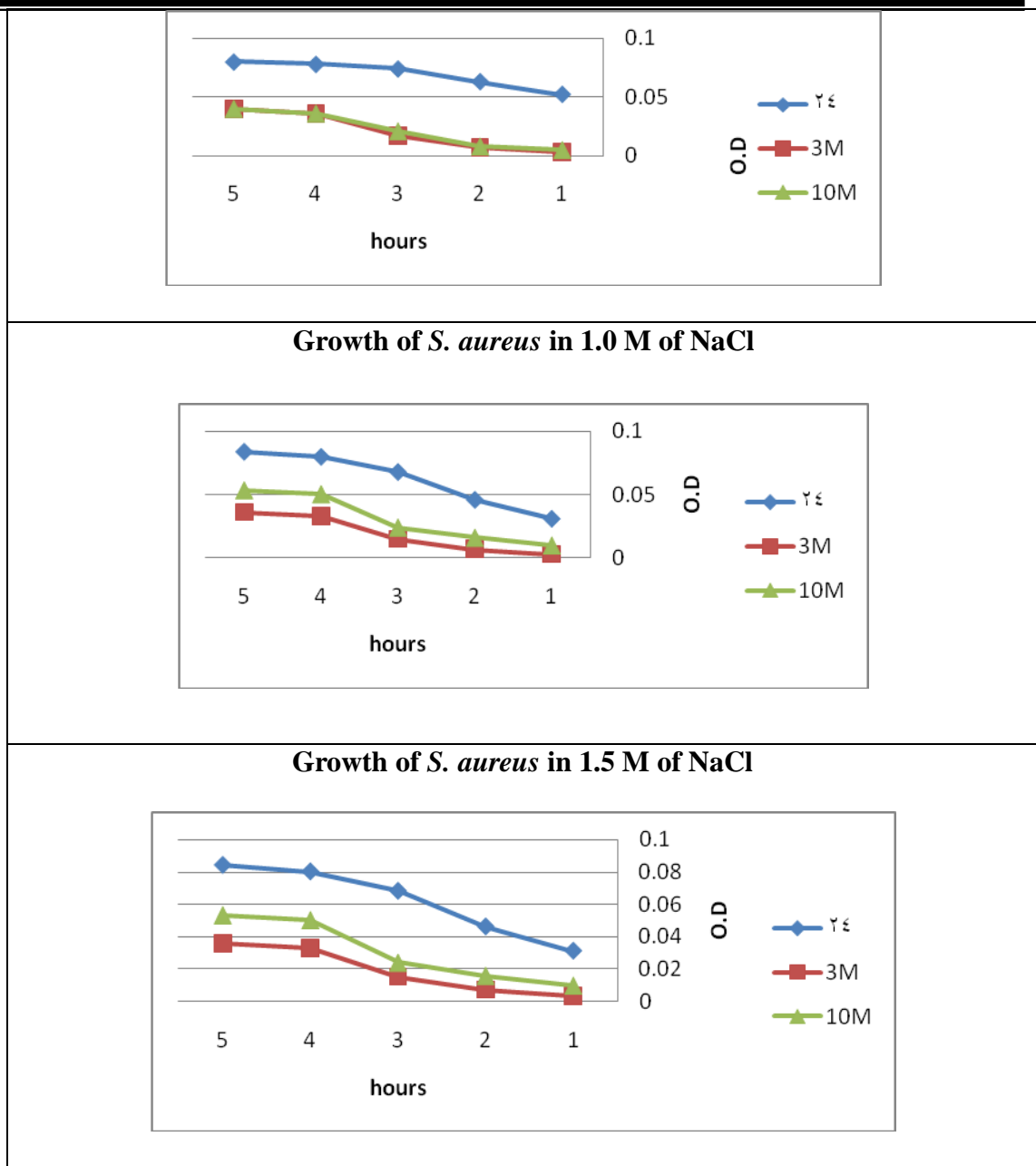


Fig 3: Effect of NaCl concentration on the strains of *S. aureus* at 43 C

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تأثير درجة الحرارة وتركيز كلوريد الصوديوم على نمو بكتريا

staphylococcus aureus

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فرع التقنيات الإحيائية، قسم العلوم التطبيقية، الجامعة التكنولوجية

الخلاصة:

تضمنت هذه الدراسة تشخيص عزلات المكورات العنقودية (النوع العادي والمطفرة). وشخصت كل العزلات على اساس تخميرها لسكر المانيتول و افرازها لكل من coagulase و الكاتلايز.

وقد تم اختيار سلالة (24) للدراسة وعرضت لطفرة بواسطة NTG للعزلات الحساسة للحرارة (TOF). وقد تم عزل الخلايا المطفرة TOF بنجاح من خلال تعريض معلق الخلايا إلى 200 ملغ / مل من NTG لمدة 75 دقيقة. نتائج التطهير TOF أظهرت أن هذه الخلايا المطفرة كانت مطابقة لسلالة الأم مظهرها. نتيجة التطهير جعل الليزوزيم قادرة على تحليل جدار الخلية TOF مماثل لـlysostaphin.

وقد اظهر نمط الحساسية للمضادات الحيوية لجميع العزلات المقاومة ومتعددة لبعض العزلات تم الكشف عنها. أظهرت النتائج أن عزلتين مطفرة كانت مقاومة للميثيسيلين، بنزائين، البنسلين، كلوكساسيلين الصوديوم ولينكوميسين وأيضا هذه العزلات كانت حساسة للأموكسيسيلين، Cefotaxine الصوديوم ، الصوديوم وسيفاليكسين وتيترايسايكلين ومتوسطة في مقاومة كلينداميسين. وأظهرت تأثير درجات الحرارة المختلفة إلى أن نمو السلالة الأم (سلالة رقم 24) تزداد مع زيادة درجة الحرارة والسلالات الطافرة (M3 ، M10) ليست مستقرة في درجات الحرارة المختلفة ونموها منخفضة جدا في 40 م.

وأظهرت ان تأثير تراكيز كلوريد الصوديوم المختلفة على النمو من سلالة الأم (سلالة رقم 24) والسلالتين الطافرة (M3 ، M10) تزيد مع زيادة تركيز كلوريد الصوديوم ولكن تنخفض في تركيز كلوريد الصوديوم = 1.5 مولاري.