The Isolation of Halotolerant Pigmented Actinomycetes from Jeddah Sea Shore

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ABSTRACT. Seventy-four actinomycete isolates were obtained from mud of Jeddah Sea shore. 15 isolates were designated as the pink spore series, 7 of yellow, 4 of grey and one isolate to a violet spore series. The remaining isolates were designated as the white spore series. Green and blue spore series were not found in these isolates. Eight isolates produced soluble pigments. The maximum specific growth rate are found in isolates M15, was 0.1 h^{-1} at a concentration of 9.0% NaCl. An inhibition zone of *Staphylococcus aureus* growth for the same isolate was 34 mm at a concentration of 3.0% NaCl, the specific growth rate was also, 0.1 h^{-1}.

Introduction

Microorganisms have an ability to survive high concentration of sodium chloride, and halophilic bacteria can tolerate up to 20 to 30% of NaCl[1]. Since Waksman[2] reviewed the tolerance of *Streptomyces* to NaCl in 1959, no complete work has been carried out in this area. Tresner, *et al.*[1] used the tolerance of streptomycetes to NaCl as a taxonomic aid while Okazaki and Okami[3] studied marine actinomycetes and their tolerance to NaCl concentrations. The latter authors suggested that these organisms could be deemed to be one of the most important sources for a screening program of biological active substances. Kitahara *et al.*[4] produced a new antibiotic from actinomycetes isolated from seawater that has inhibitory activity against tumors. Okami, *et al.*[5] produced aplasmomycin that effected both bacteria and plasmodia from marine actinomycete isolates. In this report the author has investigated the flora of the Jeddah seashore as part of the Red Sea and to study the activity and growth characteristics of producing actinomycete strains isolated from sea mud.

Materials and Methods

Mud samples were collected from five different sites on the Jeddah seashore mud and store in polyethylene bags. Each sample contained a quantity of seawater, which was

used for dilution procedures. Mud was also spread directly onto the surface of agar media. All samples were incubated for 2-5 weeks at 30ºC.

***Medi*a**

Two types of agar and broth media were used for actinomycete isolation, modification of media used by Okazaki and Okami[3] being used. Glycerine medium contains glycerol 20 ml.L^{-1}; K_2HPO_4 1G.L^{-1}; KNO_3 1G.L^{-1}; $MgSO_2.7H_2O$ G.L^{-1}. Water used was an equal mix of distilled and seawater. Starch medium contained the following components: soluble starch 20 g.L^{-1}; yeast extract 5 g.L^{-1} and the same mixture of water was added to one liter. 17 g.L^{-1} agar was added for solid media. The pH of both media was 7.4. Cultures maintenance, normal starch medium recommended by ISP[6] was used. For investigation of salinity tolerance a starch medium of ISP[6] was used with addition of NaCl according to the concentration of 1-10%.

For shaking batch culture, 250 ml capacity conical flask containing 100 ml of starch medium with different NaCl concentrations was used.

Test Organisms

Antibiotic production was tested against methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) as Gram-positive and for Gram-negative bacteria *E. coli* (25922) β-lactamase negative and *E. coli* (11954) β-lactamase positive obtained lyophilized from King Khaled Military Hospital were used.

Determination of Antibacterial Activity

Two methods of determining actibacterial activity were used. An agar disk of a grown actinomycete culture was removed aseptically and placed on a seeded plate of test organisms. An alternate method using culture filtrate was also used. A sterilized disk of multilayer filter paper of 8 mm diameter was soaked in culture filtrate aseptically to a seeded plate of test organism[7]. All plates were incubated at 35ºC for 24 hours. The diameter of the growth inhibition zones was recorded. In salinity tolerance experiments, the determination of antagonistic activity was investigated using crude extracted compound. Extraction of antibiotic substances was described by Mansour[7]. Some modifications to the published method was used. Liquid cultures with acidic pH (4) were extracted with equal volumes of ethyl acetate, and evaporated under vacuum. Solid residues were dissolved in 10 ml sterilized distilled water and the culture filtrate method was used.

Dry Weight

Growth parameters were determined using dry weight measurements. Quadruplicate flasks of different NaCl concentrations were carried out. For the first 48 hours each flask was used for dry weight determination. The remaining flasks were incubated for seven more days. A sample of 10 ml was collected from each culture every 24 hours, and filtered through a 0.45 μm filter membrane. The collected biomass was dried at 70ºC for 48 hours. Specific growth rate was estimated accordingly.

Mathematical Consideration

Pirt[8,9] described the basic law of growth as follow

$$\mu x = \frac{dx}{dt}$$

where x is the biomass, t is time and m is the specific growth rate. This equation can be converted to natural logarithms with a known exponential phase time (t) as follow

$$\mu = \frac{\ln x - \ln x_o}{t}$$

Results and Discussion

The sea is not the original environment for actinomycetes and they present in marine environment due to wash out from land. The tolerance of actinomycetes and other microorganisms to salt concentrations is the main factor in their survival. Therefore, all strains studied showed a high ability to tolerate salinity. A preliminary investigation of antagonistic activity (Table 1), showed that all isolates investigated gave a noticeable results against MRSA strains except isolate P09. Isolates G03, K06, M15 and p15 recorded the highest zone of growth inhibition of MRSA using culture filtrate method, where it was 18, 26, 25 and 21 mm respectively. Isolates K06, M15 and S11 gave the highest zone of growth inhibition for *E. coli* (11954 β-lactamase positive) where it was 22, 22 and 17 mm respectively. The detection of antagonistic activity for NaCl containing cultures was investigated by ethyl acetate extraction. Plotting the antagonistic activity obtained against NaCl concentration revealed that all strains produced an inhibition zone within 2% of NaCl concentration, except isolate M15 where the zone of inhibition gradually increased to reached 34 mm at 3% NaCl and then began to decreased gradually (Fig. 1). Data presented in Figure 2 show the specific growth rate (μ) under different concentrations of NaCl. Obviously, all tested isolates show a tolerance 2% NaCl. An inconsistent result was obtained by isolate M15, in which specific growth rate decreased gradually down to the 6% of NaCl then increased up to 9% NaCl. What is more surprising is that isolate M15 seemed to be halotolerant. This isolate showed contrary results in both antibiotic and specific growth rate among all tested strains. Rezanka and Votruba[10], reported that when *Streptomyces avermitilis* was grown under different concentration of NaCl, avermectin was produced constantly up to 0.5% NaCl, then strongly dropped. In contrast Okami, *et al.*[5] reported that supplementation of NaCl to the Kobu-Cha medium produced encouraging aplasmomycin production by a marine actinomycete.

The preparatory identification of the tested actinomycete isolates depending on the morphological and some of physiological characteristics recommended by Buchanan and Gibbons[11] and Goodfellow[12] revealed that these isolates belong to the genus *Streptomyces.*

These preliminary results revealed that the investigated isolates can tolerate a high salinity, and could probably be adaptive to the high concentration of salts as reported be-

fore by Kuster and Neumeier[13]. Also, sea mud and marine organisms particularly actinomycetes could be useful screening sources for bioactive metabolites[3].

TABLE 1. Antagonistic activities of pigment producer actinomycete grown in starch broth and agar media against tested organisms.

Isolate no.	Inhibition zone (mm)							
	Staphyloccocus aureus				*Escherichia coli*			
	MSSA		MRSA		Lactamase – ve		Lactamase + ve	
	Agar disk	Culture filtrate	Agar disk	Culture filtrate	Agar disk	Culture filtrate	Agar disk	Culture filtrate
G03	–	16	12	18	12	–	–	–
H12	18	20	16	19	–	–	–	–
J22	24	24	22	21	12	–	10	
K06	22	24	20	26	16	14	20	22
M15	28	26	26	25	27	25	26	22
P09	17	16	13	–	–	–	8	9
P15	15	14	20	21	17	19	15	14
S11	22	24	12	16	15	17	17	17

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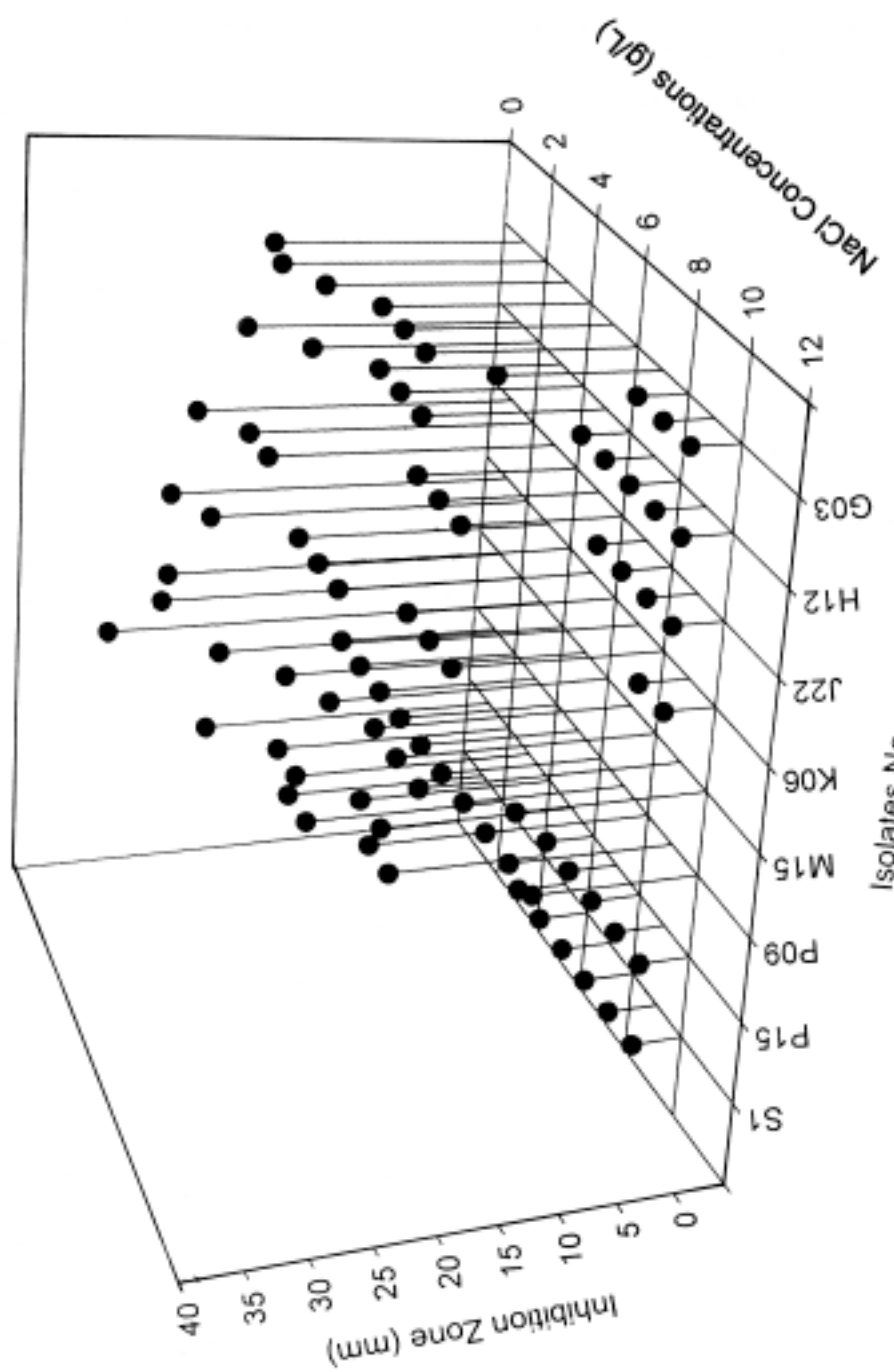

FIG. 1. Antagonistic activity of investigated actinomycete isolates on the growth of *Staphylococcus aureus* under different NaCl concentrations.

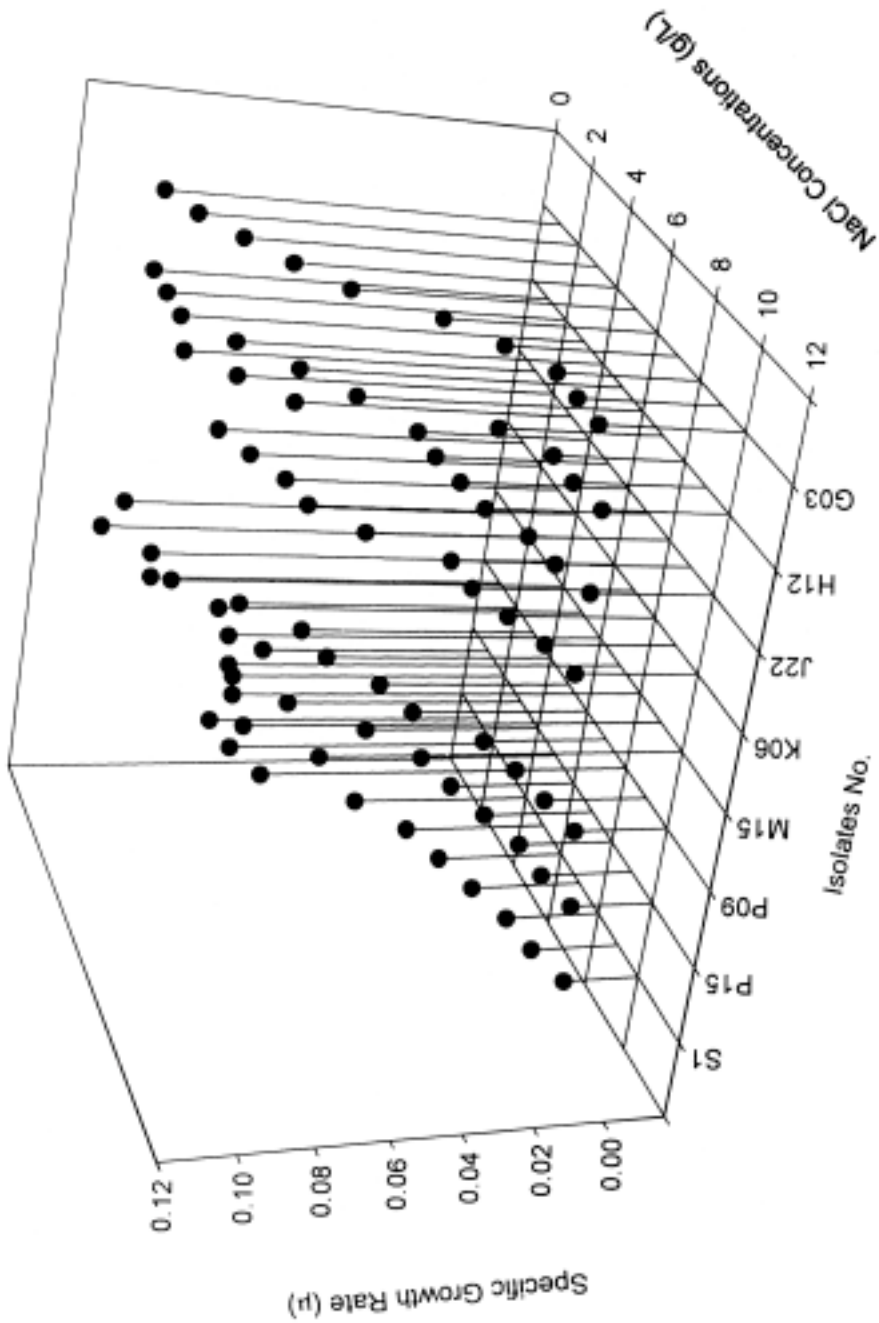

FIG. 2. Specific growth rate of investigated actinomycete isolates grown under different NaCl concentrations.

عزل الاكتينوميسيتا الملونـة والمتحملة للملوحـة في شاطيء مدينـة جـدة ، المملكة العربية السعودية

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المستخلص . تم عزل أربعة وسبعين عزلة من الاكتينوميسيتا من طين عدة مناطق من شـواطيء مدينة جـدة . تتكون هذه المجموعة من العزلا من ١٥ عزلة تقع ضمن السلاسل البوغية الوردية ، ٧ عزلا من السلاسل البوغية البنفسجية . أما باقي العزلا فكانت تقع ضمن مجموعة السلاسل البوغية البيضاء . ولم يتم عزل أي عزلا من فئة السلاسل البوغية الخضراء أو الزرقاء . ثمان عزلا من مجموع العزلا كانت منتجة للصبغا الذائبة والتي كانت موضع الدراسة . تم الحصول على أعلى معدل نمو تخصصي للعزلة M15 حيث بلغت ١ , ٠/ الساعة . أما أعلى منطقة التثبيط لنمو ميكروب *Staphylococcus aureus* فقد بلغت ٣٤مم لنفس العزلة السابقة عند تنميتها في مزارع محتوية على ٣٪ كلوريد صوديوم ، وبلغ عندها معدل النمو التخصصي ١ , ٠/ الساعة أيضًا .