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DETECTION OF BACTERIOCIN (STAPHYLOCOCCIN) ACTIVITY OF *STAPHYLOCOCCUS AUREUS* ISOLATES IN CHICKENS.

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Summary

Twenty isolates of *Staphylococcus aureus* were examined, fourteen from them showed staphylococcin activity while six isolates were not producers. The staphylococcin activity of potential producer isolates was increased if $CaCl_2$ was incorporated in the medium and if the cultures were induced with ultra violet radiation.

التحري عن فعالية البكتريوسين (الستافلوكوكسين) لعزلات العنقوديات الذهبية في الدجاج

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الخلاصة

فحصت عشرون عزلة من العنقوديات الذهبية، أربعة عشر منها أظهرت فعالية للستافلوكوكسين بينما ستة عزلات غير منتجة. أظهرت فعالية الستافلوكوكسين للعزلات المنتجة زيادة ملحوظة بوجود كلوريد الكالسيوم في الوسط الزرعي وكذلك عند تعريض المزارع الجرثومية إلى الأشعة الفوق البنفسجية.

Introduction

Bacteriocins (Staphylococcins) are bacterial proteins and highly specific antibiotics produced by many species of bacteria. They are active against members of their own species or closely related species [1]. Bacteriocins have been demonstrated and characterized in many gram-negative and gram-positive bacteria, including species of *Escherichia, Salmonella, Streptococcus* and *Staphylococcus*.Bacteriocins synthesized by Staphylococci are known as staphylo-

coccins. Fredericq, who first studied bacteriocins of *Staphylococcus*, he observed five Staphylococcins each with its own activity spectrum against other *Staphylococcus* strains. They were also active against *Bacillus* strains, although it is difficult to produce and isolate Staphylococcins in large amounts some data on their nature and properties have recently become available [1,2].

With some bacteria, the ability to produce bacteriocins is controlled by a geneticelements existing in the cell as an autonomous replication or in conjunction with the chromosome. They maybe produced spontaneously or may require induction by agents, such as ultra violet (UV) [3].

It was found that no study has been reported on Staphylococcin activity of *S. aureus* isolates obtained from chickens. The purpose of this study is to describing the Staphylococcin activity of several chicken's isolates of *S. aureus*.

Materials and Methods

Twenty isolates of *S. aureus* were collected from various diseases of chicken coming to different veterinary clinics in Baghdad City, in the period between March to September 1998. Each specimen was collected by sterile swab and dipped in brain heart infusion broth, and then transported to the Microbiology lab. of Vet. Med. College, Univ. of Baghdad.

All the bacterial isolates of *S. aureus* were identified according to Sneath *et al.* [4]

Tryptic Soya broth and agar mediums, both richer with 0.3% yeast extract, were used, $CaCl_2$, $MgCl_2$ were each added to individual amounts of tryptic broth at 2 concentrations (0.01M and 0.001M) before autoclaving.

The isolates for Bacteriocin (Staphylococcin) susceptibility (indicators) were grown in 3ml of tryptic Soya broth for 18 hours. A concentration of 10^5 organism/ml was prepared for each of the indicator isolates, and 3ml of each were then poured on to separate tryptic Soya agar plates. Excess fluid was removed and the partially open up –Ended plates were placed in the incubator at 37°C for minutes to dry [5].

The isolates tested for Staphylococcin activity (producers) were grown in 3 ml of Tryptic Soya broth for 18 hours in the same way as the indicator isolates[5,6].

The isolates (3ml) were exposed to UV in sterile petri dishes for 10 seconds. The treated cultures were then spotted on indicator isolates as described previously. Spotting of the potential producers isolates was accomplished with an inoculating loop having a 0.01ml capacity .Six producer isolates could be readily

screened on each indicator by this technique .The plates were then incubated at 37°C for 18 hours, after which inhibition of growth was recorded.
Cultures of the producer isolates (Table 1) in mediums containing $CaCl_2$, $MgCl_2$ respectively, were also treated and spotted in the same way as described in mediums.

Results

Of the twenty isolates tested for staphylococcin activity, fourteen isolates were positive while six isolates are non-producer.

The patterns of their activity are shown in Table 1. The zone of inhibition ranged in size (width) from 2 to 7mm. It was observed that isolates, which exposed to UV, gave good staphylococcin producers compared with that before the expose. Also showed stimulatory for staphylococcin production at the concentrations 0.01M and 0.001M $CaCl_2$, while the addition of $MgCl_2$ gave neither inhibitory nor stimulatory effects.

Table 1: patterns of staphylococcin production and effects of ion on staphylococcin producing isolates if tested by spotting technique.

Producer Isolates	Indicators	Source of ions			
		$CaCl_2$		$MgCl_2$	
		0.01 M	0.001M	0.01M	0.001M
1	A	+	+	N	N
2	A	+	+	-	-
3	A	+	+	N	N
5	A	+	+	+	+
8	A	N	N	N	N
9	B	N	N	N	N
10	A	+	+	N	N
11	B	+	+	-	-
12	B	+	+	N	N
13	B	+	N	+	+
15	B	+	+	N	N
16	A	+	+	N	N
18	A	+	N	N	N
20	A	N	N	N	N

+ = Enhances activity, N= no effect on activity, - =decrease in activity.
A, B= susceptible isolates.

Discussion

The results indicate that Staphylococcin occur widely among chickens isolates of *S. aureus* .In this study approximately 70 % of the cultures produced them. Some isolates acted on several cultures, whereas several isolates were active against single cultures e.g. No.9,11,12,13,15 were bactericidal for isolate B only. This indicates that the Staphylococcin production maybe associated with the virulence of the organism. In the present experiment it was also shown that UV was abutter induce for Staphylococcin production .The Ca^{+2} stimulated the production of Staphylococcin. Whereas Mg^{+2} had no effect, Similar finding was observed by Chengappa and Cater [7].

The function of these ions in the production of Staphylococcin is not well understood. Although it has been suggested that Ca^{+2} induces a lag in initial multiplication of indicator cells, which permits sufficient accumulation of Staphylococcin to inhibit growth.

Conversely the addition of Mg^{+2} might stimulate rapid initial multiplication of the indicator cells resulting in the evental appearance of confluent growth about the producer colony [1,7].

We hope that further studies employing cell-free systems may clarify some of the complex-interrelationships controlling the activity of staphylococcin.

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