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Short Communication

Identification of Bacitracin Produced by Local Isolate of *Bacillus licheniformis*

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Bacillus licheniformis **was isolated from soil of different house gardens. Diagnosis was performed according to Gram stain, motility, shape forming, aerobic condition and other tests. Bacitracin was primary identified after its activity was tested against some species of Gram positive and Gram negative bacteria. Identification was completed by using thin layer chromatographic technique.**

Key wards: Bacitracin, *Bacillus licheniformis*, soil.

INTRODUCTION

A compilation of the microbial sources of antibiotics in the soil discovered in the United States and Japan between 1953 and 1970 revealed that approximately 85% are produced by actinomycetes, 11% by fungi and 4% by bacteria (Tyler et al., 1988). *Bacillus* species occur mainly in the soil, and because of spore forming the bacteria have the ability for survival in soil environment. Thirty one strains of *Bacillus sp*. Have been isolated from soil sample in different region of Iraq Kurdistan, including 7 isolates of *Bacillus stearothermophilus* with one showing high production of alkaline phosphatase (Hamza and Hassan 2005). In another study, 30 of 40 strains were identified as *Bacillus* species in six different soil samples taken from many places in Turkey. These include two species (*B. thuringiensis, B. megatwerian*) showing an inhibitory effect against *Escherichia coli, Staphylococcus aureus, Yersinia enterocolitica* and *micrococcus* (Aslim et al., 2002).

Bacillus species produce many kinds of antibiotics which share a full range of antimicrobial activity such as bacitracin, pumulin and gramicidin. (Todar, 2005). Bacitracin is produced by *Bacillus licheniformis* which is a mixture of at least 5 polypeptides. This antibiotic consists of 3 separate compounds, bacitracin A, B and C. Bacitracin A is the chief constituent. It is active against many Gram positive organisms, such as *Staphylococcui, Streptococci, anaerobic cocci, Corynebacter* and *Clostridia*, but not against most other Gram negative organisms (McEvoy, 1993).

In this study, a *Bacillus* species (*B. licheniformis*) was isolated from the soil, and its ability for antibiotics production was investigated.

MATERIALS AND METHODS

Isolation and identification of bacteria

Bacillus licheniformis was isolated after different samples of soil were collected from the upper layers of four house gardens in Karbala Govern event in December 2005. Each sample was distributed randomly on the surface of nutrient agar medium in a Petri dish. The plates were incubated under anaerobic condition at 37°C for 24 h. The isolated bacteria were identified according to Gram characteristics, spore morphology and motility. In addition, the following identified tests were carried out: utilization of citrate, VP test, and gelatin liquefaction, and starch hydrolysis, production of acid from D-glucose, L-arabinose and D-xylose. The production of gas from glucose, catalase production and nitrate reduction were also tested (Claus and Berkeley, 1986).

Inhibitory effect

The determination of the inhibitory effect of isolate on test bacteria was carried out according to the agar diffusion method. The diagnosed isolate was cultured under anaerobic condition in nutrient broth and after 72 h of incubation at 37°C, media was filtrated through Millipore filter (0.4 µm) to obtain media containing antibiotic.

In this study, *Staphylococcus aureus*, non hemolytic *Streptococci*, beta- hemolytic *Streptococci*, *Bacillus cereus* and *E. coli* were used as test bacteria which were isolated from male volunteer (35 year) by culturing of skin swap, throat swap and stool in Muller Hinton agar and blood agar. The cultures were incubated at 37°C for 24 h and identified (Claus and Berkeley, 1986). 0.1 ml of filtrated media was dropped in each well (four wells in a Petri) in nutrient agar see-

ded with tested bacteria and the diameter of inhibition zones were measured after 24 h of incubation at 37°C.

Detection of bacitracin

According to the result obtained, bacitracin or polymxin was suspected to be the antibiotic produce by isolated bacteria. The antibiotic was extracted with n-butanol from cell grown, at 37°C for 72 h under anaerobic condition, in nutrient broth medium. A butanol extract was then subjected to thin layer chromatography (TLC; Tamehiro et al., 2002) with n-butanol: acetic acid: water (4: 1: 2) as a solvent (Gasparic et al., 1998). Pure standard bacitracin or polymxin B and butanol extract were loaded on the plate of thin layer chromatography. As soon as the solvent reached the top of the plate, dry TLC plate was sprayeded with iodine solution and the distance traveled by each spot was measured.

RESULTS AND DISCUSSION

B. licheniformis was successfully isolated from the soil samples. Identification was done using various parameters (Table 1). Antibacterial activity of the antibiotic showed a clear inhibition zone in the media seeded with *S. aureus* (20 mm), beta-hemolytic *Streptococci* (22 mm) non hemolytic *Streptococci* (21 mm) and *B. cereus* (22 mm), whereas no inhibition zone was noted in the culture of *E. coli* (Table 2). Antibiotic production by *B. licheniformis* was comparable with that of pure standard bacitracin, each having an R_f value of 0.34.

Table 1. Identification tests of *Bacillus licheniformis*.

Test type	Result
Citrate	+
VP test	+
Gelatine liquefaction	+
Starch hydrolysis	+
Acid production	
D- glucose	+
L- arabinose	+
D- Xylose	+
Gas from glucose	Few
Catalase	+
Nitrate reduction	+

In searching for new antibiotics, relatively simple and rapid methods have been developed for screening microorganisms for antibiotic producing ability. Soil samples are commonly employed in the screening because they are rich sources of antibiotic producing organisms. *Bacillus* species are ubiquitous in nature.

Bacitracin may be bactericidal or bacteriostatic in action. It inhibits bacterial cell wall synthesis by preventing the incorporation of amino acids and nucleotides into the cell wall (McEvoy, 1993). Production of bacitracin by *B. licheniformis* is affected by many factors including nitrogen and carbon source (Hanlon and Hodges, 1981). Temperature variation also affects the synthesis of the bacitracin (Egorov, et al., 1983).

Table 2. Inhibition zones (mm) of bacitracin produced by *Bacillus licheniformis* against test bacteria.

Test bacteria	Inhibition zones (mm)
Staphylococcus aureus	20
Non hemolytic *Streptococci*	21
Beta-hemolytic *Streptococci*	22
Bacillus cereus	22
E. coli	zero

Bacitracin is synthesized during the trophophase stage of *B. licheniformis* growth (Egorov et al., 1986) and there is no correlation between the bacterial sporulation and bacitracin production (Lukin et al., 1986) (Lukin et al., 1983). In conclusion, this study has achieved the isolation of *B. licheniformis* which produced the antibiotic, bacitracin.

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