
ANTIBACTERIAL ACTIVITY OF *BACILLUS SUBTILIS* EXTRACT ON PATHOGENIC ORGANISMS

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INTRODUCTION

Most bacteria produce antimicrobial compounds such as broad spectrum classical antibiotics, metabolic products viz. organic acids and lytic agents such as lysozyme. In addition, several types of protein exotoxin and bacteriocins, which are biologically active peptide moieties with bactericidal action (Amanda *et al.* 2004) are also released. Members of the *Bacillus* genus are generally found in soil and most of these bacteria have the ability to disintegrate protein (proteolytic activity). The high proportion of antimicrobial compound producing strains may be associated with ecological role, playing a defensive action to strains into an established microbial community as per Strahl *et al.*(2002)

The subtilin protein has the bactericidal effect on many Gram positive and certain Gram negative bacteria (Beima Aslim *et al.*, 2002). This study is taken with the objective of isolation of *Bacillus subtilis* from the soil and to assess the antibacterial effect of “subtilin”.

Isolation of *Bacillus subtilis*

Soil isolates of *Bacillus subtilis* were obtained by screening soil samples, collected from

the Palar river bed at vellore district, Tamil Nadu. The soil samples were processed as per the method of Beima Aslim *et al.*, (2002). Each gram of soil samples was suspended in 99ml of sterile distilled water and shaken vigorously for 2 minutes. The samples were heated at 60°C for 60 minutes in a water bath. Then the soil suspensions were serially diluted and then streaked onto BHI Agar (Hi Media, Mumbai). The suspected colonies were Gram stained and confirmed by biochemical tests as per Garrity *et al.* (2005). A total of 4 numbers of soil samples were subjected to isolation of organism.

Production of Subtilin

The method of Amanda, *et al.* 2004 was followed for the production of subtilin. *Bacillus subtilis* was grown in BHI broth and incubated for 48 hrs at 32°C at 125cycles/min in an orbital shaker. The culture was centrifuged at 10,000rpm for 15min. The supernatant was then filtered using 0.22/μm filter. The resulting filtrate, is the “subtilin designate” and subjected to evaluation for its antimicrobial activity.

Assessment of antimicrobial activity

Disc diffusion method of Bauer *et al.*(1964) was used for the assessment of antibacterial activity.

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Escherichia coli of calf scour origin and *Staphylococcus aureus* of mastitis origin were used in this study. *Escherichia coli* and *Staphylococcus aureus* culture (0.5 of Mc Farland turbidity standard) were streaked onto Tryptone Soya agar(TSA) plates. 20µl of Subtilin(100mg/ml or 2mg/20µl) was applied on sterile disc and placed on TSA agar and incubated at 37°C for 24hrs.

Experimental Animal inoculation test

The subtilin(100µl) was mixed with equal volume of *S.aureus* and *E.coli* culture separately and incubated at 37°C for 30 minutes for neutralization. For each strain of organism, two mice were inoculated with 0.2ml of the neutralized culture subcutaneously along with two control mice which received 0.2ml of the respective culture alone.

Three soil samples revealed the presence of *B.subtilis* and three isolates were obtained. *B.subtilis* was confirmed based on biochemical characters .

Gram's staining revealed Gram positive bacilli with centrally located spores. Biochemical characterization of the three isolates is presented in Table 1.

The protein concentration of subtilin was assessed by 'Protein estimation kit- BCA method' procured from Bangalore GeNei. The inhibitory concentration of subtilin was found to be 100mg/ml for *S.aureus* and *E.coli* .

A bactericidal protein subtilin was isolated from all the three isolates of *B.subtilis*. The extracted subtilin protein from *B.subtilis* was preserved at 4°C.

In antibiotic sensitivity test, the subtilin protein was found to be most effective against *E.coli* followed by *S.aureus* (Fig1&2).

Riley *et al.*, (2002) have reported that the subtilin protein was found to have inhibitory action against Gram positive bacteria viz *S.aureus*. The findings of this study correlate with the findings of Riley *et al.*,(2002) and Bizani *et.al* (2002)

In experimental animal inoculation test, control mice which were infected with *E.coli* culture, when sacrificed, were found to contain accumulation of fluid in the intestine characteristic of Sirini test for *E.coli* but with mice inoculated with neutralized culture no such reaction could be observed . Control mice infected with *S.aureus* evinced pyogenic infection on sacrifice, whereas no pyogenic infection could be witnessed in mice inoculated with neutralized culture.

In conclusion, the results of this study indicate that the antimicrobial compound "subtilin" produced by *B.subtilis* was found to be effective against the commonly occurring Gram positive *S.aureus* and Gram negative *E.coli*. It also paves the way of exploring the possibilities of large scale production of subtilin to be used as an antibiotic against the infections caused by *S.aureus* and *E.coli*

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Antibacterial activity

Isolates	Citrate	V.P	MR	Indole	Oxidase	Catalase
<i>B.subtilis</i> - 1	-	-	+	+	+	+
<i>B.subtilis</i> - 2	-	-	+	+	+	+
<i>B.subtilis</i> - 3	-	-	+	+	+	+

Maltose	Salicin	Dextrose	Lactose	Sucrose	Lactose	Xylose	Raffinose
+	-	+	-	+	-	-	-
+	-	+	-	+	-	-	-
+	-	+	-	+	-	-	-

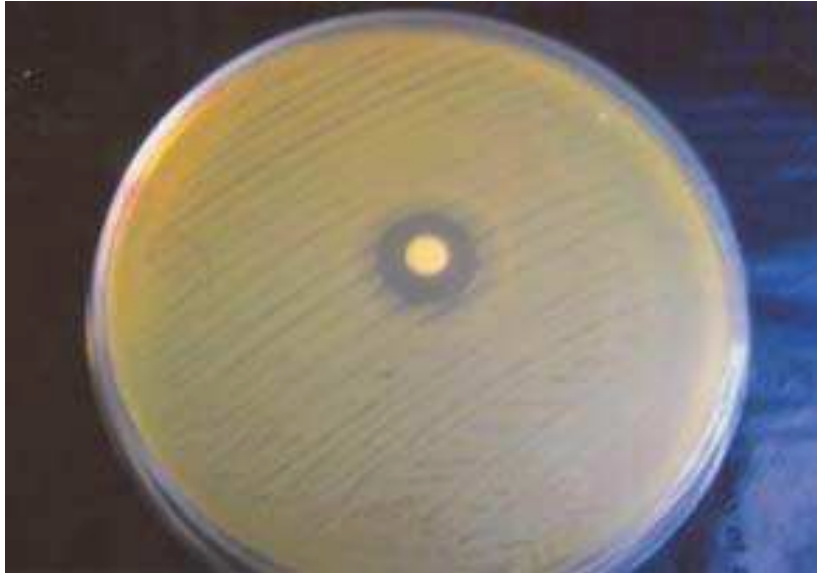
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Sensitivity test of Subtilin Protein against *E.coli*



Sensitivity test of Subtilin Protein against *S. aureus*



Fig-1