ANTIMICROBIAL ACTIVITY OF LIPOPEPTIDE BIOSURFACTANT-
SURFACTIN

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ABSTRACT

Lipopeptides form the most widely reported class of biosurfactants having antimicrobial action. Among the lipopeptides, surfactin, produced by *Bacillus subtilis* is the first and the most well-known member. Other antimicrobial lipopeptides include fengycin, iturin, bacillomycins and mycosubtilins produced by *B. subtilis*. The production of these antimicrobials by Bacillus probiotics is one of major mechanisms by which they inhibit the growth of pathogenic microorganisms in the gastrointestinal tract. In the present study, the crude lipopeptide characterized in the previous studies was tested for its antimicrobial activity against 7 different pathogenic micro-organisms viz; *Salmonella paratyphi A*; *Enterobacter sp.*; *Enterococcus sp*; *Streptococcus sp*; *Staphylococcus sp*; *E.coli* & *Pseudomonas sp*. The maximum zone of inhibition was found to be 6cm at the concentration of 1500µg/ml in *Salmonella paratyphi A*; *Staphylococcus sp* and *E.coli*.

KEYWORDS: *Salmonella paratyphi A*; *Enterobacter spp*; *Enterococcus spp*; *Streptococcus spp*; *Staphylococcus spp*; *E.coli* & *Pseudomonas spp*; surfactin.

INTRODUCTION

Most of the species from the genus *Bacillus* are considered as safe microorganisms and they possess remarkable abilities to synthesize many substances that have been successfully used in agriculture and for industrial purposes. The secondary metabolites produced by several species and strains of the genus *Bacillus* have been found to show antibacterial or antifungal activity against different phytopathogens (Debois D, Ongen M et.al) (1). Among many
structurally diverse antimicrobial compounds that are attributed to *Bacillus subtilis*, cyclic lipopeptides (CLPs) of the surfactin, iturin and fengycin families have well-recognized potential for use in biotechnology and biopharmaceutical applications because of their surfactant properties. In the present study, antimicrobial activity of the crude lipopeptide containing surfactin was tested for its antimicrobial activity against 7 different pathogenic micro-organisms.

**MATERIALS AND METHODS**

The crude biosurfactant containing Bacillus surfactin was tested against 7 different micro-organisms viz; *Salmonella paratyphi A*; *Enterobacter spp*; *Enterococcus spp*; *Streptococcus spp*; *Staphylococcus spp*; *E.coli* & *Pseudomonas spp*. which was maintained in Muller-Hinton agar slants at refrigerated temperature.

**Nutrient Broth culture**

100ml of nutrient broth was prepared and 10mL broth was dispensed into the boiling tubes. They were sterilized by autoclaving at 121°C under 15 lbs pressure for 15 mins. The sterilized broth in the tubes was inoculated with *Salmonella paratyphi A*; *Enterobacter sp.; Enterococcus sp*; *Streptococcus sp*; *Staphylococcus sp*; *E.coli* & *Pseudomonas sp*. Separately and incubated for 24 hours at 35°C (Paulo Andre Vincente et.al) (3).

Muller Hinton agar (Hi-Media) was dissolved in 100 ml distilled water and autoclaved at 121°C under 15 lbs pressure for 15 mins. The sterilized media was poured into the sterile petriplates and allowed to solidify. 100 µL of the 24 hour broth cultures of the respective micro-organisms were spread onto the agar plates separately and 6 wells were created in each of the plates containing the respective pathogens. Wells were numbered from 1-6 for inoculation of different concentrations of crude biosurfactant of the isolate S5. Ethyl acetate was used as control in well 6.

100mg of the crude surfactin sample was dissolved in 10ml distilled water and used as stock. From the stock different concentrations of standard were taken in the order as follows: 25µl (250µg/ml); 50µl (500µg/ml); 75µl (750µg/ml); 100µl (1000µg/ml); 150µl (1500µg/ml) and Control (100µl). The plates were incubated for 24 hours at 37°C and the results were recorded in Table 1.
RESULTS AND DISCUSSION

Antimicrobial Activity of the Lipopeptide.

Table: 1

<table>
<thead>
<tr>
<th>S. No</th>
<th>Organism</th>
<th>25µl (250µg/ml)</th>
<th>50µl (500µg/ml)</th>
<th>75µl (750µg/mL)</th>
<th>100µl (1000µg/ml)</th>
<th>150µl (1500µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Staphylococcus</em> sp.</td>
<td>0.1cm</td>
<td>0.2cm</td>
<td>0.3cm</td>
<td>0.5cm</td>
<td>0.6cm</td>
</tr>
<tr>
<td>2</td>
<td><em>Streptococcus</em> sp.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td><em>Enterobacter</em> sp.</td>
<td>0.2cm</td>
<td>0.3cm</td>
<td>0.4cm</td>
<td>0.45cm</td>
<td>0.5cm</td>
</tr>
<tr>
<td>4</td>
<td><em>Enterococcus</em> sp.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td><em>Salmonella paratyphi A</em></td>
<td>0.2cm</td>
<td>0.3cm</td>
<td>0.4cm</td>
<td>0.5cm</td>
<td>0.6cm</td>
</tr>
<tr>
<td>6</td>
<td><em>E.coli</em></td>
<td>0.1cm</td>
<td>0.2cm</td>
<td>0.4cm</td>
<td>0.5cm</td>
<td>0.6cm</td>
</tr>
<tr>
<td>7</td>
<td><em>Pseudomonas</em> sp.</td>
<td>0.1cm</td>
<td>0.2cm</td>
<td>0.3cm</td>
<td>0.35cm</td>
<td>0.4cm</td>
</tr>
</tbody>
</table>

From Table 1, the antimicrobial activity of the crude lipoprotein was found to be maximum for *Staphylococcus* (Fig.1), *Salmonella paratyphi A* (Fig.5) and *E.coli* (Fig.6) at 1500µg/ml.
concentration exhibiting the zone of inhibition for about 0.6cm diameter. *Streptococcus* (Fig.2) and *Enterococcus* (Fig.4) were found to be resistant against the lipoprotein extract and hence did not show any zone of inhibition. In 250µg/ml concentration of the lipopeptide extract, *Enterobacter* (Fig.3) and *Salmonella paratyphi A* (Fig.5) had shown 0.2cm dm inhibition and rest of the organisms had shown 0.1cm inhibition zone. 500µg/ml concentration 0.3cm dm inhibition zone for *Enterobacter* (Fig.3) and *Salmonella paratyphi A*(Fig.5) and 0.2cm dm inhibition for *Staphylococcus* (Fig.1), *E.coli* (Fig.6) and *Pseudomonas* (Fig.7). In 750µg/ml concentration of the lipopeptide 0.4cm of inhibition zone formed in *Enterobacter* (Fig.3), *Salmonella paratyphi A* (Fig.5) and *E.coli*(Fig.6).1000µg/ml concentration of the lipopeptide showed 0.5cm of maximum inhibition zone for *Staphylococcus* (Fig.1), *Salmonella paratyphi A*(Fig.5) and *E.coli*. (Fig.6). Similar results were shown in the studies by *P.Das et.al* (2).

**REFERENCE**

