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Bacillus Amyloliquefaciens MS-3: An Antagonistic Bacterium Against Clinical Isolates

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ABSTRACT

In the present study, six isolates of bacteria were isolated from the rhizosphere soil and assayed for its capability to show antagonism characteristics against the bacterial isolates *Salmonella typhimurium*, *Pseudomonas aeruginosa* and fungal isolates *Aspergillus flavus* and *Aspergillus niger*. Among the isolates, *Bacillus amyloliquefaciens* MS-3 was found to have maximum antagonism against the host organisms. The nature of the protein was found to be extracellular product. This investigation shows the importance of antimicrobial protein against the pathogens.

Keywords: Antagonism, *Bacillus amyloliquefaciens*, Antimicrobial protein.

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INTRODUCTION

Microbial antagonism is a common phenomenon in nature [1] which is facilitated through release of antimicrobial proteins (AMP) which seems to be a general phenomenon for most bacteria. The microbial defence system includes broad spectrum antibiotics, metabolic products such as organic acid and lysozyme; a lytic agent. Additionally, biologically active protein moieties like exotoxin and bacteriocins with bactericidal mode of action, were already well reported in literature [2,3]. Since, the cosmopolitan distribution of microbes are not free from the theory; survival of fittest, thus their defence mechanism i.e., toxins to kill the contender species in the locality is included and termed as antagonism.

With the report of current genetic evolution at microbial community and its plasticity toward environment; resulted in existence of multiple drug resistant strains (MDR) and superbugs, whose number is increasing at alarming rate worldwide [4] among pathogens, search for new and effective antimicrobial agents against key pathogens is a field of utmost priority due to this reason. Most of the antimicrobial compounds available today is associated to the members of bacillus genus, associated strains are generally found in soil and most of them have proteolytic activity i.e. ability to kill other microbes through production of proteins which bring lysis to cells, with their ecological role while playing defensive action helped them to upcome as wide phylogenetic branches and established community among microbial world [5].

In this study, different bacterial strains were isolated and assayed for their ability to have antagonistic property against some key pathogens. Identification and characterisation was done only for isolates having strongest antagonism property against host organisms.

MATERIALS AND METHODS

Sample collection

Rhizosphere soil sample was aseptically collected from locality around Tambaram, Chennai, India, using sterile pharmacol (Hi-Media, PW1148).

Isolation of antagonistic bacterial strain

One gm of soil sample was suspended in 9 ml of sterile distilled water and incubated for 10 min with shaking. Then the suspension was serially diluted and 10^{-7} dilution was used for plating on nutrient agar (NA) plates. The streaked plates were incubated at 37°C for 18-24h. The pure colonies were subcultured and maintained on NA slant at 4°C.

Test microorganisms

To find out the antagonistic effect, two bacteria, *Salmonella typhimurium* and *Pseudomonas aeruginosa*, and two fungi, *Aspergillus niger*, *Aspergillus flavus* were used.

Antimicrobial activity assay

For the antimicrobial screening, all six strains were grown in nutrient broth (NB) at 37 °C in a rotary shaker (150 rpm) overnight. The culture broth was centrifuged at 10 000 rpm for 10 min and the cell-free supernatant was tested. Well diffusion inhibition assay was conducted against the indicator strains [6]. The indicator strains were grown in broth culture overnight according to their specific growth requirements. The culture of pathogen strains was diluted in sterile distilled water until turbidity equal to 0.5 McFarland ($1x10^8$ CFU/ml).). The final inoculum was spread with sterile cotton swab on Muller Hilton Agar; MHA (Hi-Media, India). Bacterial crude extract concentration was 20 mg/ml was added (50µl) into wells (6 mm) formed by cork borer on the MHA layer. The plates were incubated in suitable temperatures for 24-48 h; the zone of inhibition was measured and recorded [7]. Each experiment was performed in triplicate.

Identification of antagonistic bacteria

The bacterial strain MS-3 was characterized by morphological and biochemical tests based on microscopic appearance, Gram staining and according to Bergey's Manual of Systematic Bacteriology [8]. The isolate that showed clear antagonism against the tested pathogens was subjected to further identification on the basis of phenotypic and physiological characteristics by analysis of the 16S rDNA sequence. To obtain 16S rDNA sequences, polymerase chain reaction (PCR) was carried out using universal primers fP1 (5'-GAGTTTGATCCTGGCTCA-3') and rP2 (5'-ACGGCTACCTTGTTACGACTT-3') and PCR protocol as follows: 94 °C for 2 min, 30 cycles of 94 °C for 30 s, 50 °C for 30 s, 68 °C for 30 s and 72 °C for 10 min. Purified PCR fragments were sequenced with both primers and compared with 16S rDNA gene sequences in the public database using BLAST.

RESULTS AND DISCUSSION

It is of interest to determine the in vitro antagonistic potential of natural isolate against several pathogenic microorganisms. This study revealed that the presence of high spectrum of antimicrobial protein in the isolated bacterial strain.

Determination of antimicrobial activity

Screening of the antimicrobial activity of six bacterial isolates was carried out against four test organisms such as two bacteria and two fungi. Among these, bacterial isolate MS-3 exhibited highest inhibitory potential against the clinical isolates. The isolate MS-3 showed more significant antimicrobial activity against bacteria than fungi. The antimicrobial property against the pathogenic bacteria for all six isolates was shown in Figure 1. The result indicates that isolate MS-3 produced maximum zone of inhibition against the indicator strain. This isolate exhibited strong antagonistic property against *Salmonella typhimurium* of 20 mm than *Pseudomonas aeruginosa* of 16 mm. Similarly, isolate MS-3 showed efficient fungicidal effect among the all. Isolate MS-3 produced highest zone of inhibition of 13 mm against *Aspergillus niger* than *Aspergillus flavus* of 11 mm.

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Identification of antagonistic strain

Morphological characterization of the strain MS-3 was gram positive, rod-shape, and endospore forming bacteria. This strain showed catalase, oxidase, hydrolysis of casein and acid produced from glucose, sucrose, starch and glycerol positive and indole production negative. Based on the results of morphological, physicochemical and biochemical test (Table 1), the strain was categorized as *Bacillus* according to Bergey's Manual of Systematic Bacteriology. Comparison of 16S rDNA amplified gene to sequence of GenBank was shown 99% similar to the *Bacillus amyloliquefaciens* and the isolate was identified as *Bacillus amyloliquefaciens* MS-3.

Table 1. Morphological and biochemical characteristics of the isolated strain MS-3

Characters	Results
Morphology	Rod shaped, gram +ve
Motility	+ve
Indole production	-ve
Urease, oxidase	+ve
Citrate utilization	+ve
Acid production from glucose, fructose, glycerol, sucrose, ribose, starch, lactose, mannitol Acid production from galactose, sorbitol	+ve -ve
Catalase	+ve
Hydrolysis of starch	+ve
Hydrolysis of casein	+ve
Acetoin production	-ve
H_2S production	+ve
Nitrate reduction	+ve
Urease	-ve
Gelatinase	-ve

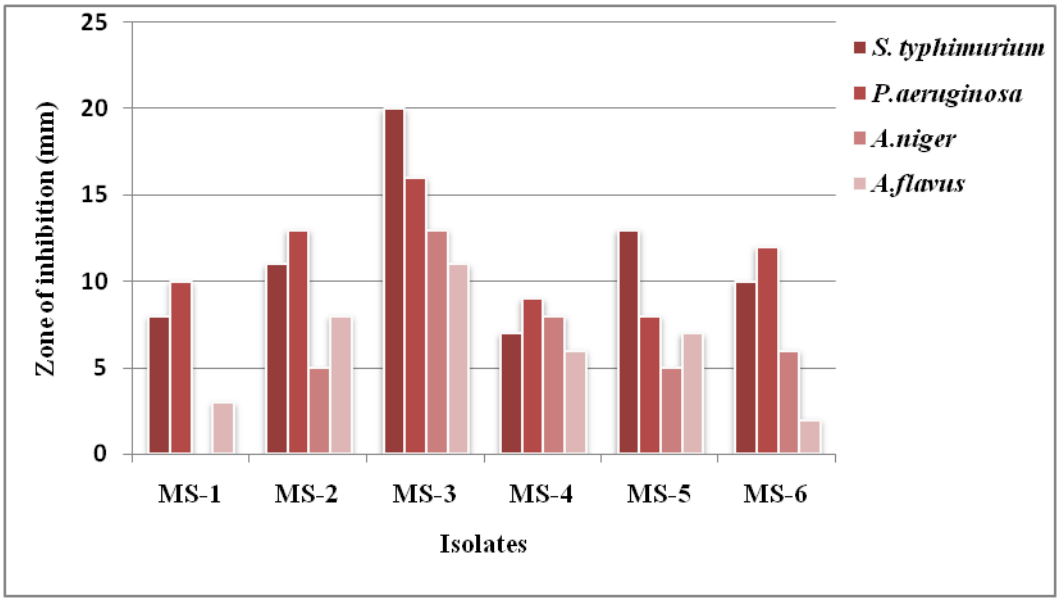

Figure 1. Antimicrobial activity of isolate MS-3 against clinical isolates

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CONCLUSION

The results of this study indicate that the crude extract of isolate MS-3 (later identified as *Bacillus amyloliquefaciens* MS-3) was effective against the growth of bacteria and fungi. The crude extract had high potential antimicrobial activity; this implies that the presence of antimicrobial protein as extracellular product can be used to treat infections against host organisms.

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