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# Screening and Evaluation of Antimicrobial Activity of Tannery Fleshing Utilizing Fish Gut Bacteria

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**Abstract:** The focal theme of the present investigation is to explore the antimicrobial activity of Tannery Fleshing (TF) utilizing *Labeo rohita* gut bacteria against common microbial pathogens. The study involves isolation of the bacteria through culture technique, biochemical characterization, Scanning Electron Microscopic and phylogenetic cluster analysis. Antimicrobial activities were determined through agar diffusion technique. Partial 16S-rRNA gene sequencing analysis shows that the isolate exhibited homology with *Bacillus megaterium*. Among the bacterial pathogens maximum inhibition was observed against *Salmonella typhi* and *Bacillus subtilis* when compared to standard antibiotic followed by *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus vulgaris*. Minimum inhibitory effect was observed with *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Most susceptible fungal pathogens include *Microsporum canis* and *Aspergillus fumigatus* whereas *Trichophyton mentagrophytes* and *Penicillium chrysogenum* were least susceptible. Antifungal activity of *Bacillus megaterium* remained almost similar to standard drug in *Fusarium moniliforme*, *Aspergillus niger*, *Candida albicans*. The *in vitro* studies indicate that *Bacillus megaterium* ANFLR1 isolated from *Labeo rohita* can inactivate pathogenic Gram-positive and Gram-negative bacteria as well as fungi and can be of potential use as an antibiotic and probiotic.

**Key words:** *Bacillus megaterium*, tannery fleshing, *Labeo rohita*, antibacterial, antifungal

## INTRODUCTION

Disposal of non-biodegradable solid wastes from leather processing units and unused leather in the dumpsite makes solid waste management ineffective (Dhayalan *et al*., 2007). Utilization of TF for the production of enzymes using bacterial or fungal sources has been well documented (Katsifas *et al*., 2004; Kumar *et al*., 2008). Tannery waste hydrolyzing strains are necessary for efficient conversion of this organic waste into valuable products (Chen *et al*., 2001; Taylor *et al*., 2002). The use of microorganisms that grows in tannery solid waste fleshing and transforms the waste into an antimicrobial substance is a promising approach for identifying novel source of antibiotic production.

Compounds that eliminate or inhibit the development of bacteria, viruses and fungi are called antimicrobials (Betina, 1983). Resistance of microorganism to existing antimicrobials is a serious problem and necessitates the discovery of novel unconventional sources of antibiotics (Cowan, 1999; Gaynes and Edwards, 2005). The use of microorganisms for the biological purpose has become an effective alternative to control pathogens as most of them produce broad spectrum classical antibiotics (Motta *et al*., 2004). Recent researchers have revealed the presence of indigenous microflora in the gut of fish (Sugita *et al*., 1997), earthworm (Aruna *et al*., 2008) and termites (Ramin *et al*., 2008; Kucharoenphaisan and Sinma, 2012) with antimicrobial activity providing natural defense mechanism against invading pathogens (Gibson and Roberfroid, 1995) and physiologically active materials like enzymes, amino acids and vitamins through decomposition of nutrients (Sugita *et al*., 1997).

Currently most of the antibiotics are derived from a relatively small group of microorganisms that includes *Penicillium* (Gharaei-Fathabad *et al*., 2009), *Streptomyces* (Bairagi *et al*., 2002; Arasu *et al*., 2009; Kumar *et al*., 2010; Deepika and Kannabiran, 2010; Raja and Prabakaran, 2011; Reddy *et al*., 2011), *Cephalosporium*, *Micromonospora* (Zinsser, 1988). *Bacillus,* a genus of rod-shaped bacteria belonging to Firmicutes (Turnbull, 1996) are the preferred hosts for the production of many new and improved

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products (antibiotics, amino acids and enzymes) as we move through the genomic and proteomic era (Klein *et al*., 1993; Gutowski-Eckel *et al*., 1994; Stover and Driks, 1999; Kim *et al*., 2003; Motta *et al*., 2004). Several strains of Bacillus have been isolated from traditional fermented foods including various cereal, legume and vegetable based fermented foods (Gadaga *et al*., 1999; Dhayalan *et al*., 2007; Valero *et al*., 2007) that displays antimicrobial activity against *Pseudomonas aeruginosa*, *Pseudomonas. fluorescens* RSKK 380, *Bacillus thuringiensis* RSKK 380, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *Micrococcus luteus*, *M. flavus*, *Yersinia enterocolitica*, *Bacillus subtilis* (Perez *et al*., 1992, 1993; Aslim *et al*., 2002). The *Bacillus* species that produces antibiotics *are B. subtilis*, *B. polymyxa*, *B. brevis, B. licheniformis*, *B. circulans* and *B. cereus* (Morikawa and Imanaka, 1992; Drabløs *et al*., 1999) and have a wide range of applications as antiviral (Gadaga *et al*., 1999), anti-fungal (Milner *et al*., 1995), anti-mycoplasma (Peypoux *et al*., 1999) and anti-ameobocytic agents (Galvez *et al*., 1994).

*Bacillus megaterium* is a spore-forming bacterium found in soil, seawater, sediments, rice paddies, dried food, honey and milk (Vary, 1994) that produces plasmid-borne oxetanocin, a potential antiviral agent (Morita *et al*., 1999) and penicillin G acylase (Pinotti *et al*., 2000). So far, the information regarding isolation of Tannery fleshing utilizing *Bacillus megaterium* from freshwater fish *Labeo rohita* is perhaps nil. This is the first report on isolation and identification of tannery fleshing utilizing fish gut bacteria *Bacillus megaterium* as an antimicrobial agent.

Hence, the focal theme of the present investigation deals with the isolation of *Bacillus megaterium* from fresh water fish *Labeo rohita* gut fed Tannery fleshing diets and evaluation of its antimicrobial activity against common pathogenic bacteria and fungi.

## MATERIALS AND METHODS

**Sample collection and isolation of bacteria:** The Indian major Carp, rohu (*Labeo rohita*) were obtained from a local fish farm, Chennai (India) and maintained in tanks designed in laboratory for a period of 30 days and fed with tannery fleshing based diets (3%, data not shown). The fish was sacrificed and surface sterilized using 1% iodine solution. The intestine was dissected on ice slab in sterile conditions and used for microbiological examination. The intestine samples were homogenized in a surface sterilized mortar and pestle and was serially diluted to ten folds with sterile saline solution. Diluted samples (0.1 mL) were spread aseptically within a laminar airflow on sterilized Schaedler HiVeg TM agar to determine the total cultured gut heterotrophic bacterial population. The chemicals and bacteriological media used for analysis were procured from Merck and Hi Media, India.

**Cultivation media:** The optimized medium for the growth of *Bacillus megaterium* ANFLR1 consists of (g $L^{-1}$) sodium chloride: 1.4, ammonium chloride: 0.005, dipotassium hydrogen phosphate: 1.25, potassium dihydrogen phosphate: 0.9 with 60 g tannery fleshing (Sumathi and Sekaran, 2011).

**Biochemical characterization and 16S rRNA Phylogenetic sequence analysis:** The morphological properties of the isolate were examined using light microscope as well as scanning electron microscope. The biochemical tests like gram staining, endospore staining, motility test, indole production, methyl red and Voges Proskauer, citrate utilization, oxidase, catalase, Nitrate reduction, urease production, $H_2S$ production, gelatin liquefaction, casein and starch hydrolysis and carbohydrate fermentation tests were performed for the selected strains.

DNA was extracted from the isolated strain following the modified method of Hykin *et al*. (Therese *et al*., 1998). Universal eubacterial primers which have a broad specificity for gram positive and gram negative bacteria were custom synthesized by Bangalore Genie Pvt. Ltd., India. The sequence of primers used for the first round of eubacterial nested PCR were U1: 5' TTGGAGAGTTTGATCCTGGCTC 3' and rU4: 5' GGACTACCAGGGTATCTAA 3' which generated a 766 bp product. The Primers U2: 5' GGCGTGCTTAACACATGCAAGTCG 3' and rU3: 5' GCGGCTGGCACGTAGTTAG 3' for the second round used the PCR products of first round as templates generated a 470 bp product after amplification by PCR. The 16S rRNA sequence was analyzed for the similarity and homology with the existing sequences available in the data bank of National Center for Biotechnology Information (NCBI) using BLAST search. The isolate was sub cultured and maintained in slant culture at 4°C as well as at 20% (v/v) glycerol stock at -80°C.

**Preparation of bacterial cell free supernatant:** Isolated bacteria was grown in 250 mL Erlenmeyer flasks containing 100 mL of Tannery Fleshing broth incubated for 48 h at 35±1°C in a shaker at 125 cycles/min. After growth, culture media were centrifuged at 10,000 g for 15 min and the supernatants were filtered through 0.22 μm. The resulting filtrates were used to evaluate antimicrobial activity.

**Bacterial and fungal pathogens:** The bacterial and fungal pathogenic strains were obtained from Microbial Type Culture Collection (MTCC, Chandigarh, India) and ATCC. Antimicrobial activity were evaluated against pathogenic bacterial strains viz., *Escherichia coli* (MTCC 2939), *Salmonella typhi* (MTCC 98), *Proteus vulgaris* (MTCC 1771), *Pseudomonas aeruginosa* (MTCC 1688), *Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 441), *Klebsiella pneumoniae* (ATCC 10273) and *Serratia marcescens* (MTCC 97) and against various fungal pathogens viz., *Aspergillus niger* (MTCC 281), *Trichoderma viride* (MTCC 167), *Penicillium chrysogenum* (MTCC 160), *Microsporum canis* (MTCC 2820), *Candida albicans* (MTCC 183), *Fusarium moniliforme* (MTCC 156), *Aspergillus niger* (ATCC No. 16404), *Trichophyton rubrum* (MTCC 296), *Trichophyton mentagrophytes* (ATCC 9533).

***In vitro* antimicrobial assay:** Antimicrobial assays were conducted using the agar well diffusion assay method (Vaseeharan and Ramasamy, 2003). Briefly, 1 cm diameter wells were punched in each plate before adding 200 μL aliquots of 24 h bacteria wherein fourth well on each plate served as a negative control and was inoculated with 200 μL sterile growth medium. Antibacterial activity is defined as the clear inhibitory zone formed around the wells. Each test was repeated three times and the antibacterial activity was expressed as the mean of diameter of the inhibition zones (mm). Chloramphenicol at 1 $g^{-1}$ final concentration was used as positive control.

***In vitro* antifungal assay:** Antifungal activity of the crude extract was determined using the standard method CLSI M38-A (formerly NCCLS) (NCCLS, 2002). The fungal cultures were maintained in 0.2% dextrose medium. Each fungal inoculums were applied on plate and evenly spread on Sabouraud's Dextrose agar (HiMedia, India) using a sterile swab. Agar diffusion assay was followed to evaluate the antimicrobial activity along with Amphotericin B. The petri plates were incubated at 30°C for 2 days. At the end of the 48 h, inhibition zones formed in the medium were measured in millimeters (mm). All experiments were done in triplicates.

**Minimum inhibitory concentration (MIC):** MIC was determined by the broth 2-fold macro dilution method (Andrews, 2001). Briefly, 1 mL of cell free supernatant bacteria (containing $10^6$ or $10^8$ CFU $mL^{-1}$) was added in Mueller Hinton broth for identifying bacterial growth and in Sabouraud's dextrose broth for fungal growth inhibition. The tubes were incubated aerobically at 37°C for 24 h for bacteria and 30°C for 48 h for fungi. Positive controls were prepared separately for both bacteria and fungi with respective organisms in the same culture media without the extract. The OD at a wavelength of 600 nm was taken every 3 h for 33 h. After incubation, the tube which shows no growth was taken as the MIC value for the respective organism. Triplicate samples were maintained in each experiment.

**Statistical analysis:** The assays were conducted in triplicates wells in three independent experiments and are expressed as Mean±SD. Results were analyzed statistically by using SPSS software.

## RESULTS

**Screening and identification of antimicrobial bacteria:** About 25 bacterial strains were isolated from the gut of freshwater fish *Labeo rohita* and screened for antimicrobial activity. 11 isolates produced maximum inhibitory zone on plates. Those bacteria producing inhibition zones higher than 10 mm against both bacteria and fungi were selected for further characterization of their antimicrobial activity. Tannery fleshing media had high productivity value. Commercial media often include relatively expensive components which support suboptimal growth, or undesirable for use on a large scale production (Sharp *et al*., 1989). Therefore, tannery fleshing media being economical may probably be replaced in commercial production. The enhancement of growth of *Bacillus megaterium* utilizing Tannery fleshing will have dual beneficial effect wherein solid waste is converted into antimicrobial agent.

P5 strain was found to produce maximum activity on both bacterial and fungal strains. The isolated P5 was found to be gram positive rod, in chain and motile. The strain showed negative response to indole, VP, citrate utilization and nitrate reduction tests and produced acid slant in the triple sugar ion agar and also fermented glucose, lactose and maltose. The bacterial exhibited positive response to methyl red, catalase and weakly positive for oxidase test (Table 1). SEM analysis revealed

Table 1: Biochemical tests of *Bacillus megaterium* ANFLR1

| Biochemical tests | *Bacillus megaterium* |
|---|---|
| Indole | −ve |
| MR/VP | +ve/−ve |
| Citrate utilization | −ve |
| Nitrate reduction | −ve |
| Urease production | −ve |
| Catalase | +ve |
| Oxidase | +ve |
| Triple sugar ion agar | H2S −ve, acid slant |

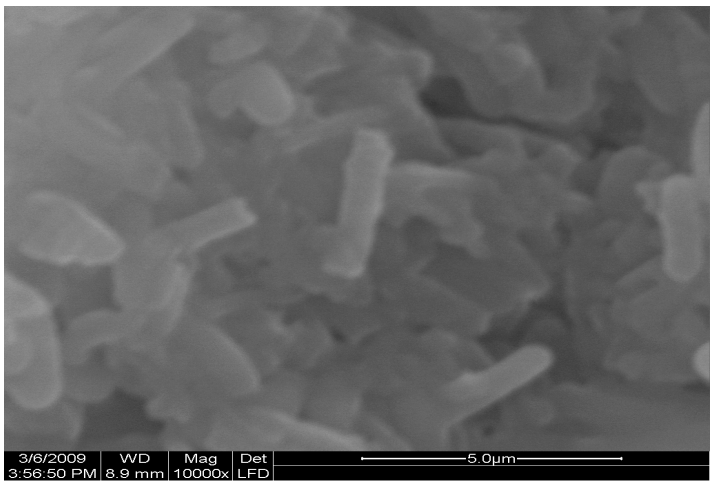


Fig. 1: SEM image of *Bacillus megaterium* isolated from *Labeo rohita* fish gut

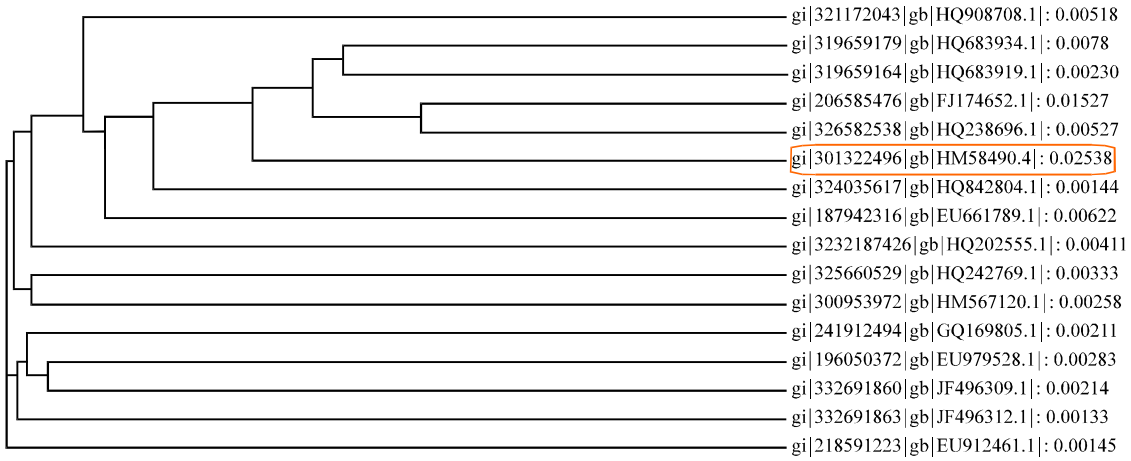


Fig. 2: Phylogenetic analysis of *Bacillus megaterium* ANFLR1

the presence of *Bacillus megaterium* in fish gut (Fig. 1). The 16S rRNA genes sequences are deposited in NCBI GenBank database with the following accession number HM584904 and have 98% phylogenetic similarity to *Bacillus megaterium*.

**Phylogenetic analysis of the isolated bacteria:** *Bacillus megaterium* strain ANFLR1 16S ribosomal RNA gene, partial sequence (HM584904.1) was analyzed using similarity search tool BLAST. This indicated high degree of similarity (98%) with annotated sequences of around 50. Phylogenetic tree based on 16S rDNA sequences was constructed using Neighbor joining method over a panel of 55 strains which exhibited 98% similarity. Fourteen isolates were clustered in 2 different groups and further divided into 7-8 species which include *Bacillus aryabhatti* and *megaterium* strains and closest type strain *Bacillus aryabhatti* strain F77063. Additionally pairwise alignment reveals complete 756 sequences identity and similarity with the 1487 sequences (Fig. 2).

**Antibacterial activity:** Based on MIC values the susceptibilities of pathogenic bacterial and fungal species as measured by the NCCLS method are summarized in Table 3. As the concentration of the antagonist bacteria increased, the antimicrobial activity increased. The highest diameter of inhibitory zone was observed against *Salmonella typhi* followed by *Bacillus subtilis*

Table 2: Antimicrobial activity (zone of inhibition) of *Bacillus megaterium* on pathogenic microorganisms

| Pathogenic strains (bacteria) | $1\times10^{6}$ CFU $mL^{-1}$ | $1\times10^{8}$ CFU $mL^{-1}$ | Positive control chloramphenicol |
|---|---|---|---|
| *Escherichia coli* (MTCC 2939) | 16.2±0.7 | 19.5±0.77 | 18.5±0.62 |
| *Salmonella typhi* (MTCC 98) | 22.4±0.34 | 26.1±0.49 | 16..2±0.78 |
| *Proteus vulgaris* (MTCC 1771) | 16.8±0.54 | 17.4±0.39 | 21.8±0.65 |
| *Pseudomonas aeruginosa* (MTCC 1688) | 14.3±0.33 | 15.8±0.58 | 19.6±0.87 |
| *Staphylococcus aureus* (MTCC 96) | 12.6±0.55 | 13.3±0.58 | 17.8±0.68 |
| *Bacillus subtilis* (MTCC 441) | 21.8±0.32 | 22.6±0.26 | 20.5±0.55 |
| *Klebsiella pneumoniae* (ATCC 10273) | 16.7±0.32 | 17.6±0.36 | 15.5±0.48 |
| *Serratia marcescens* (MTCC 97) | 16.7±0.24 | 15.4±0.49 | 18.2±0.56 |
| Pathogenic strains (fungi) | $1\times10^{6}$ CFU $mL^{-1}$ | $1\times10^{8}$ CFU $mL^{-1}$ | Positive control amphotericin B |
| *Aspergillus fumigatus* (MTCC 1811) | 18.2±0.64 | 20.4±0.55 | 19.8±0.52 |
| *Trichoderma viride* (MTCC 167) | 15.8±0.47 | 16.6±0.37 | 17.5 ±0.66 |
| *Penicillium chrysogenum* (MTCC 160) | 13.5±0.52 | 14.3±0.61 | 18.6 ±0.53 |
| *Microsporum canis* (MTCC 2820) | 19.2±0.44 | 21.4±0.75 | 20.5 ±0.65 |
| *Candida albicans* (MTCC 183) | 15.1±0.39 | 17.8±0.69 | 22.5 ±0.72 |
| *Fusarium moniliforme* (MTCC 156) | 18.2±0.58 | 19.6±0.70 | 19.7 ±0.59 |
| *Aspergillus niger* (ATCC 16404) | 16.5±0.63 | 18.2±0.51 | 17.5 ±0.41 |
| *Trichophyton rubrum* (MTCC 296) | 16.4±0.45 | 17.2±0.54 | 18.2 ±0.39 |
| *Trichophyton mentagrophytes* (ATCC 9533) | 11.2±0.53 | 12.6±0.78 | 20.8 ±0.52 |

Data is expressed as Mean±standard deviation (n = 3)

Table 3: Minimum inhibitory concentration of isolated bacteria against various microbial pathogens

| Pathogenic strains | Optical density with $1\times10^{6}$ CFU $mL^{-1}$ | Optical density with $1\times10^{8}$ CFU $mL^{-1}$ | +ve control (with antibiotic) | −ve control (without antibiotic) |
|---|---|---|---|---|
| **Bacteria** | | | | |
| *Escherichia coli* (MTCC 2939) | 0.53 | 0.46 | 0.45 | 0.80 |
| *Salmonella typhi* (MTCC 98) | 0.43 | 0.37 | 0.69 | 1.00 |
| *Klebsiella pneumoniae* (ATCC 10273) | 0.34 | 0.29 | 0.48 | 0.85 |
| *Pseudomonas aeruginosa* (MTCC 1688) | 0.32 | 0.29 | 0.38 | 1.02 |
| *Staphylococcus aureus* (MTCC 96) | 0.43 | 0.39 | 0.46 | 1.19 |
| *Bacillus subtilis* (MTCC 441) | 0.38 | 0.32 | 0.53 | 1.26 |
| *Klebsiella pneumoniae* (ATCC 10273) | 0.37 | 0.31 | 0.65 | 1.13 |
| *Serratia marcescens* (MTCC 97) | 0.45 | 0.36 | 0.33 | 0.92 |
| **Fungi** | | | | |
| *Aspergillus fumigatus* (MTCC 1811) | 0.42 | 0.36 | 0.58 | 0.98 |
| *Aspergillus niger* (ATCC 16404) | 0.55 | 0.43 | 0.64 | 1.12 |
| *Trichoderma viride* (MTCC 167) | 0.52 | 0.39 | 0.54 | 1.23 |
| *Penicillium chrysogenum* (MTCC 160) | 0.68 | 0.63 | 0.69 | 1.20 |
| *Microsporum canis* (MTCC 2820) | 0.55 | 0.49 | 0.53 | 1.50 |
| *Candida albicans* (MTCC 183) | 0.80 | 0.68 | 0.78 | 0.99 |
| *Fusarium moniliforme* (MTCC 156) | 0.52 | 0.38 | 0.50 | 0.87 |
| *Trichophyton rubrum* (MTCC 296) | 0.66 | 0.58 | 0.71 | 1.04 |
| *Trichophyton mentagrophytes* (ATCC 9533) | 0.58 | 0.47 | 0.66 | 1.15 |

at $10^{8}$ CFU $mL^{-1}$. On the other hand lowest activity was seen against *Staphylococcus aureus* at $10^{8}$ CFU $mL^{-1}$ in comparison with standard drug. Antibacterial activity displayed by these isolates encouraged us to consider them for further investigations to screen antifungal activity. Among the fungal pathogens most prominent activity was shown against *Microsporum canis* and *Aspergillus fumigatus* and minimum activity against *T. mentagrophytes*. MIC Value was less when pathogenic strains were incubated with *Bacillus megaterium* at different levels (Table 2).

## DISCUSSION

Screening of fish gut bacterial antimicrobial activity has not yet been described. In the present investigation, presence of a considerable population of bacterial flora has been found in the gastrointestinal tracts of the fish species and certain strains exhibit antimicrobial property. The 16S rRNA genes of fifty five representative bacteria were sequenced and the closest type strains of isolates were retrieved from NCBI. A Phylogenetic tree based on 16S rRNA gene sequences of antagonistic bacteria and type strains were established.

It was exciting to discover that *Bacillus megaterium* was strongly active against common pathogenic bacteria which includes *Escherichia coli*, *Salmonella typhi*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Serratia marcescens* and against fungi viz., *Aspergillus niger*, *Trichoderma viride*, *Penicillium chrysogenum*, *Microsporum canis, Candida albicans*, *Fusarium moniliforme*, *Aspergillus niger*. The reason for different sensitivity between Gram-positive and Gram negative bacteria could be ascribed to the morphological differences between these microorganisms. Gram-negative bacteria have an outer phospholipidic membrane carrying the structural lipopolysaccharide components which

makes the cell wall impermeable to lipophilic solutes while porins constitute a selective barrier to the hydrophilic solutes with an exclusion limit of about 600 Da (Nikaido and Vaara, 1985). The Gram-positive bacteria are more susceptible since they have only an outer peptidoglycan layer which is not an effective permeability barrier (Trust and Sparrow, 1974).

Furthermore, *Bacillus* can act as a friendly bacteria by strengthening natural host defenses that acts as a guardian against invading pathogens by creating a barrier against microbial infection and produces antagonistic activity against both Gram-negative and Gram positive bacteria (Pinotti *et al*., 2002). *Bacillus* sp. are considered safe biological agents (Kim *et al*., 2003) based on different antagonists studies carried out with *Bacillus subtilis*, *B. cereus*, *B. pumilus* and *B. polymyxa* (Utkhede, 1984; Silo-suh *et al*., 1994), *B. thuringiensis* (Sarker *et al*., 2010) and *B. subtilis* (Nakayama *et al*., 2009). *Bacillus species* (*B. subtilis*) are reported to produce antibiotics peptides, lipopeptide and proteins substances (Klein *et al*., 1993; Paik *et al* 1998; Motta *et al*., 2004) which are mostly active against Gram-positive bacteria (Kuipers *et al*., 1992), Gram-negative bacteria (Katz and Demain, 1987; Bechard *et al*., 1998; Stover and Driks, 1999) and fungi (Tosato *et al*., 1997). Furthermore, D'Arienzo *et al*. (2006) established a new model of reduction in *C. rodentium* infection with *Bacillus subtilis*.

Similarly, *Bacillus megaterium* ANFLR1 possess wide range of antimicrobial spectrum and results further corroborates with *Bacillus* sp. NM12 isolated from fish intestine (Sugita *et al*., 1998) and brittlestar *Amphipholis gracillima* (Ahmad *et al*., 2002). The ability of the *Bacillus megaterium* to inhibit fungal indicators also varied and these findings also coincide with the previous reports (Tosato *et al*., 1997). The variation of the susceptibility of the tested microorganisms could be attributed to their intrinsic properties.

Patterson and Burkholder (2003) have anticipated that the competitive exclusion of pathogens by *Bacillus* probiotics will result from one or more modes of action, including immune exclusion, competition for adhesion sites and production of antimicrobial agents, such as bacteriocins (Servin, 2004; Kim *et al*., 2003; Pinchuk *et al*., 2001; Premalatha and Dhasarathan, 2011). Lactic Acid Bacteria possess antagonistic activity against common pathogens (Ahmad *et al*., 2002; Pishva *et al*., 2009; Raja *et al*., 2009; Bali *et al*., 2011) and exerts probiotic properties (Abdelhamid *et al*., 2009). Since, *Bacillus* isolate showed interesting antimicrobial properties, it would be also useful to investigate their probiotic properties as it have been comparatively less studied as a probiotic (Patterson and Burkholder, 2003). The obtained results are considered to be sufficient for the further studies and ongoing studies deals with the process of isolation and identification of the active principles and probiotic characterization.

## CONCLUSION

It is interesting to note that cell free supernatant of *Bacillus megaterium* showed prominent activity against various fungal and bacterial pathogens. Results of this study indicate that the potential of this microorganism to produce antimicrobial activity and application value is great and must be better explored. Future studies will be based on the identification of the active metabolite which has inhibitory effects against various microbial pathogens.

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