**Actinobacteria from Cow Feces: Isolation, Identification and Screening for Industrially Important Secondary Metabolites**

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**Introduction**

Increasing incidences of fungal infections across the world among plants and animals have driven the search and development of new antifungal compounds. In this context, Actinobacteria represents a prolific source of bioactive secondary metabolites. Under phylum Actinobacteria, genus Streptomyces contribute significantly to the production of secondary metabolites with different properties such as antibacterial, antifungal, anticancerous, immunosuppressants etc. [1, 2]. Sixty percent of antibiotics developed for agricultural purpose was isolated from the same genus [3]. Due to declining rate of discovery of new lead compounds from common sources, the cultivation of rare taxa of Actinobacteria from under-explored habitats has become a focus in the search for the next generation of antimicrobial agents [4]. It is clear from the evidence that researchers have been focussing on the screening of unusual and unexplored niche for new Actinobacterial strains and also to overcome the increasing demand of the bioactive molecules.

Cow feces is a cheap and easily available bioresource on our planet. It is used as manure, biofertilizer, biopesticides, pest-repellent and as a source of energy [5]. Antimicrobial activity of Cow feces has been reported by many researchers [6–8]. Ayurveda suggests the use of panchgavya (Panchgavya in Sanskrit means a mixture of ghee, feces, urine, curd, milk) alone or in combination
with the drug [9]. Cow urine (a part of panchgavya) increases phagocytic activity of macrophage and is helpful in prevention and control of bacterial infection [10]. According to Dhama et al. [11], Cow milk is considered to be effective in curing fever and pain; tumors; diabetes; kidney disorders and weaknesses and also act as a medium to administer medicine while curd (dahi) is a blood purifier, and found useful in blood related problems; piles and gastro-intestinal disorders; Cow ghee has immunostimulatory properties. The application of Cow feces is helpful in killing the germs of malaria and tuberculosis [11]. Being wealthy in nutrients like nitrogen, potassium, along with trace amount of sulfur, iron, magnesium, copper, cobalt, and manganese; Cow feces supports a rich microbial diversity including different species of bacteria (*Bacillus* spp., *Corynebacterium* spp. and *Lactobacillus* spp.), protozoa and yeast (*Saccharomyces* and *Candida*) [9]. Cow feces associated *Bacillus* strains were found active in plant growth promoting traits with biocontrol activity against *F. oxysporum* and *B. thobromae* [9]. Radha and Rao [12] prepared three Cow feces based biodynamic preparations viz., Panchagavya (PG), BD500 and ‘Cowpat pit’ (CPP) and found these to be dominated by *Bacillus* species. The isolated bacterial strains exhibited plant growth promoting attributes like indole acetic acid production, phosphate solubilization, antagonism to *R. bataticola* and improved growth of maize plants. Hence, it is necessary to identify promising Actinobacteria in the Cow feces to find out bioactive molecules. The present study was conducted with decisive objectives to explore the potential of the Actinobacteria having industrial importance in the discovery of bioactive molecules.

**Materials and Methods**

**Sample collection**

In the current study Ghurdauri region of Pauri, Uttarakhand, India was selected. The latitude and longitude of Ghurdauri are 30.18°N, 78.69°E respectively. It is about 1800 meters above from the sea level. Ten Cow (*Bos taurus*) feces samples were selected from nearby locations of Ghurdauri. Approximate 500 g of fresh Cow feces sample was collected in sterile poly bags, transferred to the laboratory and stored at 4°C for further use.

**Isolation of Actinobacteria**

The sample was pretreated by drying at 60°C in hot air oven for 3 h. Actinobacteria were isolated from non-selective method [13]. In brief, ten grams of pre-treated sample was suspended in 90 ml of sterile water, stirred well and one ml of suspension was plated on four selective media (starch casein agar, Bennet’s agar, Gause no. 2 agar and Water agar) supplemented with nystatin (50 µg/ml) and nalidixic acid (25 µg/ml) (HiMedia, India) to minimize fungal and bacterial contamination respectively. The plates were incubated at 27°C for 14–21 days. The colonies were selected on the basis of morphological appearance (dry, tough and leathery colonies). Pure cultures were maintained on yeast extract malt extract (ISP-2) agar plates.

**Identification**

**Morphological and chemotaxonomic characterization.** The isolated Actinobacterial strains were identified on the basis of morphological and physiological characteristics described in the ISP (International Streptomyces Project) [14] and Bergey’s Manual of Systematic Bacteriology [15]. Morphological characteristics including an arrangement of spore (i.e. retinaculiperti, rectiflexibles), substrate and aerial mycelia were studied by cover slip technique. The color of aerial mycelium, substrate mycelium, and pigmentation of *Actinobacteria* were monitored on different ISP media (ISP-2, ISP-3, ISP-4, ISP-5, ISP-6 and ISP-7). The diaminopimelic acid isomers of the cell wall were examined according to Staneck and Roberts [16].

**Molecular characterization.** Promising strains (GBTCF-09, GBTCF-21, GBTCF-26) were identified through 16S rDNA gene sequence analysis. The genomic sequence was determined by the Gujarat State Biotechnology Mission (DST, Gujarat Genomics Initiative) (Gujarat, India). Genomic DNA was extracted by using the Prepman method. The 16S rDNA (ribosomal DNA) gene was amplified by using thermal cycler of Applied Biosystems Veriti (9800 Fast Thermal Cycler) with universal bacterial primer, 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492 (5'-GGTTACCTTGTTACGACTT-3'). PCR amplification was performed and various stages were mentioned as: stage-1 initially denaturation of template
for 3 min at 95°C followed by 15 cycles of denaturation at 95°C for 40 sec, annealing at 64°C for 1 min, extension at 72°C for 2 min (stage-2), stage -3 was followed by 10 cycles of denaturation at 95°C for 40 sec, annealing at 64°C for 1 min, extension at 72°C for 2 min and final extension (stage-4) at 72°C for 10 min and stored at 4°C for further use. The PCR products were purified using the ExoSAP-IT PCR product clean-up reagent (ThermoFisher Scientific, USA) and sequenced using automatic Applied Biosystems 3500 genetic analyzer (Applied Biosystems, USA). The 16S rRNA sequences were manually aligned with the published sequences of validly described species available from the GenBank databases. The evolutionary history was inferred using a neighbor-joining method based on the Kimura 2-parameter model [17, 18]. The topography of the constructed tree was evaluated by bootstrap analysis with 1000 replicates [19]. The evolutionary analyses including phylogenetic tree construction were performed in MEGA 5.05 [20].

Effect of salt, temperature and pH on growth of strains. The effect of salt concentration, variable temperature, and variable pH was analyzed to determine the optimum growth conditions of strains on ISP-2 media. The strains were streaked and incubated at different temperature (20°C, 27°C, 37°C and 45°C) for 14 days to determine the optimum temperature. The strains were streaked over different salt concentrations (1% to 10%) and incubated at 27°C for 14 days to determine the salt tolerance capability of the strains. The optimum pH was determined by streaking the media plates having different pH (5–10) and incubated at 27°C for 14 days.

Preliminary screening for antifungal activity

All the strains were preliminarily screened for their antagonistic activity against fungal phytopathogens including Aspergillus niger, Fusarium solani, Fusarium oxysporum, Macrophomina phaseolina and Rhizoctonia solani by agar plug method [21]. In this method, the strains were grown over ISP-2 media and 6 mm plug were made using sterile cork borer. The test fungal pathogens were seeded on sabouraud dextrose agar (SDA) and plugs of different strains were transferred. The plates were kept under incubation at 27°C for 72 h. A zone of inhibition around the plug was observed as an antifungal compound produced by the active strains. For the selection of the most effective antagonistic strains, percent inhibition against test phytopathogens was evaluated by dual culture technique. Visual observation of the inhibition of pathogenic fungal growth was recorded after 72–96 hours of incubation in comparison with the SDA plate simultaneously inoculated with fungal pathogen only as control. The radial growth of fungal mycelium was measured and afterwards, percent inhibition of radial growth (PIRG) was recorded using the following formula:

$$ \text{PIRG} = \left( \frac{R1 - R2}{R1} \right) \times 100$$

$R1$ = Radial growth of fungus in control plate
$R2$ = Radial growth of fungus interacting with antagonistic bacteria

Screening for extracellular enzymes production

Chitinolytic activity was explored using the method defined by Saima and Roohi [22]. Colloidal chitin was prepared from the chitin powder (Hi-Media, India), in which chitin powder (40 g) was slowly added with 600 ml of concentrated HCl and kept for 60 min at 30°C with vigorous stirring. Chitin was precipitated as a colloidal suspension by adding it slowly to 2 liter of water at 4–10°C. The suspension was collected by filtration with suction on a coarse filter paper and washed by suspending in distilled water. pH of the suspension was neutralized with 10 N NaOH. The suspension was centrifuged at 5,009 × g for 10 min and the precipitate was ready to be used as a medium substrate. Strains were screened for the chitinase activity on the colloidal chitin agar (CCA) ($\text{Na}_2\text{HPO}_4$ -6.0 g/l ; $\text{KH}_2\text{PO}_4$ -3.0 g/l ; $\text{NH}_4\text{Cl}$ -1.0 g/l ; $\text{NaCl}$ -0.5 g/l ; Yeast extract -0.05 g/l ; agar, 15.0 g/l and colloidal chitin -10.0 g/l). The six mm agar plugs of strains were placed over CCA plates and incubated at 27°C. The plates were observed for clear zone around plugs after 96 h of incubation, which showed the competency of strains to produce the enzyme chitinase and zone was measured. The production of different extracellular enzyme including cellulase, amylase, gelatinase, and caseinase was analyzed according to the consultative procedure [23]. The lipase production was detected through the method defined by Hou and Johnston [24].
Statistical analysis

One way analysis of variance (ANOVA) test of the different zone of inhibition (in triplicates) of different strains was performed and the results with \( p < 0.05 \) were considered to be statistically significant.

Results

Isolation

This screening was carried out with an intend to explore the presence of Actinobacteria in the Cow feces with their potential to produce antifungal molecules against different phytopathogens and enzymes. After the incubation period, Actinobacteria having distinct morphology on the isolating media were monitored and thirty strains were isolated from ten samples of Cow feces. The maximum number (90%) of strains were recovered from traditionally used isolating media i.e. starch casein agar while rest of 10% of strains were obtained on Bennet’s agar. No isolate was recovered from Gause no.2 and water agar. Strains were maintained on ISP-2 media and preserved in 20% glycerol.

Morphological and chemotaxonomic identification

Streptomyces is a dominant genus of phylum Actinobacteria and widely recognized from various habitats including soil, marine, plants etc. The strains showed velvety or powdery appearance along with extensively branched aerial and substrate mycelia on different ISP media and some of them had potential to produce pigments (Fig. 1). At maturity, the aerial mycelium bears 14–20 numbers of spores which were arranged in straight to recti flexibles spore chains as observed under light microscope. 56.7% (17) of strains produced gray spores, 36.7% (11) strains produced white spore and 6.7% (2) strains produced yellow color spores on their mycelia. Melanin pigment was shown by three strains on ISP-6 (peptone yeast extract iron agar) and on ISP-7 (tyrosine agar) media, but no diffusible pigmentation was observed on ISP-5 media. All the strains were having LL-DAP isomeric form in their cell wall, which is an indicator chemical for a genus of Streptomyces. On the basis of morphological and chemotaxonomic results observation, it was concluded that all strains belong to the genus Streptomyces.

Molecular identification

Three strains, having highest antifungal activity, were investigated for 16S rRNA gene sequencing. The 16s rDNA region of strains, GBTCF-09, GBTCF-21 and GBTCF-26, were amplified, sequenced and deposited in GenBank with accession no.; MF136812, MF136813, and MF136814 respectively. The obtained 16S rRNA gene sequences were compared with the sequences in 16S rRNA gene database (Bacteria and Archea) available at GenBank database of NCBI by using BLAST.
Table 2. Inhibition zone (mm) of active strains against fungal pathogens.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>A. niger</th>
<th>F. oxysporum</th>
<th>F. solani</th>
<th>M. phaseolina</th>
<th>R. solani</th>
</tr>
</thead>
<tbody>
<tr>
<td>GBTCF-01</td>
<td>0.0 ± 0.0</td>
<td>11.3 ± 0.47</td>
<td>12.3 ± 0.94</td>
<td>11.7 ± 0.47</td>
<td>10.3 ± 0.47</td>
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<tr>
<td>GBTCF-02</td>
<td>7.7 ± 0.47</td>
<td>10.3 ± 0.47</td>
<td>11.3 ± 0.47</td>
<td>8.3 ± 0.47</td>
<td>9.3 ± 0.47</td>
</tr>
<tr>
<td>GBTCF-05</td>
<td>11.3 ± 0.47</td>
<td>10.7 ± 0.47</td>
<td>11.7 ± 0.47</td>
<td>15.7 ± 0.47</td>
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<tr>
<td>GBTCF-06</td>
<td>8.7 ± 0.82</td>
<td>11.7 ± 0.47</td>
<td>14.3 ± 0.94</td>
<td>16.3 ± 0.47</td>
<td>10.0 ± 0.82</td>
</tr>
<tr>
<td>GBTCF-07</td>
<td>9.3 ± 0.47</td>
<td>14.3 ± 0.47</td>
<td>15.3 ± 0.47</td>
<td>15.3 ± 0.94</td>
<td>10.7 ± 0.47</td>
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<tr>
<td>GBTCF-08</td>
<td>9.7 ± 0.47</td>
<td>13.7 ± 0.47</td>
<td>15.7 ± 0.47</td>
<td>14.3 ± 0.47</td>
<td>15.7 ± 0.47</td>
</tr>
<tr>
<td>GBTCF-09</td>
<td>10.3 ± 0.47</td>
<td>13.7 ± 0.47</td>
<td>15.3 ± 0.47</td>
<td>15.3 ± 0.94</td>
<td>14.3 ± 0.47</td>
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<td>GBTCF-10</td>
<td>7.7 ± 0.47</td>
<td>9.7 ± 0.47</td>
<td>11.3 ± 0.47</td>
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<td>8.7 ± 0.47</td>
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<tr>
<td>GBTCF-11</td>
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<td>11.3 ± 0.47</td>
<td>11.7 ± 0.47</td>
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<td>8.7 ± 0.47</td>
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<tr>
<td>GBTCF-12</td>
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<td>11.7 ± 0.47</td>
<td>11.0 ± 0.0</td>
<td>11.3 ± 0.47</td>
<td>11.7 ± 0.47</td>
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<tr>
<td>GBTCF-13</td>
<td>9.7 ± 0.47</td>
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<td>13.3 ± 0.47</td>
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<td>12.3 ± 0.94</td>
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<td>GBTCF-17</td>
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<td>10.0 ± 0.0</td>
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<tr>
<td>GBTCF-18</td>
<td>0.0 ± 0.0</td>
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<td>9.7 ± 0.47</td>
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<tr>
<td>GBTCF-19</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>8.7 ± 0.47</td>
<td>9.3 ± 0.47</td>
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<tr>
<td>GBTCF-20</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>10.7 ± 0.47</td>
<td>10.3 ± 0.47</td>
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<tr>
<td>GBTCF-21</td>
<td>11.0 ± 0.82</td>
<td>12.3 ± 0.47</td>
<td>15.0 ± 0.82</td>
<td>15.7 ± 0.47</td>
<td>15.0 ± 0.82</td>
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<tr>
<td>GBTCF-22</td>
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<td>11.3 ± 0.47</td>
<td>11.3 ± 0.47</td>
<td>11.0 ± 0.82</td>
<td>11.3 ± 0.47</td>
</tr>
<tr>
<td>GBTCF-23</td>
<td>8.3 ± 0.47</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>10.3 ± 0.47</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>GBTCF-24</td>
<td>7.7 ± 0.47</td>
<td>12.0 ± 0.82</td>
<td>10.0 ± 0.0</td>
<td>11.3 ± 0.47</td>
<td>9.3 ± 0.47</td>
</tr>
<tr>
<td>GBTCF-25</td>
<td>10.3 ± 0.47</td>
<td>11.7 ± 0.47</td>
<td>12.3 ± 0.47</td>
<td>13.7 ± 0.47</td>
<td>13.3 ± 0.47</td>
</tr>
<tr>
<td>GBTCF-26</td>
<td>10.3 ± 0.47</td>
<td>14.0 ± 0.0</td>
<td>16.3 ± 0.47</td>
<td>16.7 ± 0.47</td>
<td>12.3 ± 0.47</td>
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</table>

Value are means ± Standard deviation.

(Basic Local Alignment Search Tool) algorithm and the result reflects that sequence had a significant resemblance to a number of species of genus Streptomyces (family Streptomycetaceae). 16S rRNA gene sequences of the strains were having 99–100% similarities to their promising neighboring strain sequence in GenBank as shown in Table 1. The phylogenetic tree was constructed by the neighbor-joining method with Kimura 2-p model along with 1000 times of bootstrapping of 16s rRNA gene sequence (Fig. 2).

Fig. 2. Evolutionary relationship among Taxa: Neighbor-joining tree based on 16S rRNA gene sequences showing relationships between the representative Actinobacteria and nearest strains. A number of nodes indicates bootstrap values based on 1000 replicates.
Growth of strains on variable salt, temperature and pH conditions

All the strains were tolerant up to 10% salt concentration but no growth and sporulation were observed on increasing the concentration of salt. All the strains were well grown and sporulated at variable pH range from 5 to 10. Optimum growth and sporulation of all strains were observed at temperature 27°C and 37°C. At 20°C, slight sporulation was observed for isolate GBTCF-26 and no sporulation was observed for rest of the strains while no growth was observed at 45°C. Interestingly, isolate GBTCF-05 produced blue color soluble pigment at basic pH and at 37°C on ISP-2 media.

Antifungal activity

Antifungal activity of all the strains was assessed against five fungal phytopathogens (A. niger, F. oxysporum, F. solani, M. phaseolina and R. solani) during screening using agar plug method (Fig. 3). From the current study, 66.7% of strains were active against M. phaseolina and R. solani while 63.3% of strains were active against A. niger, F. oxysporum, and F. solani. However, 15 strains (50%) inhibited the growth of all of the test phytopathogens. Antifungal activities of the strains with a zone of inhibition against test pathogens are represented in Table 2. There was a significant difference ($p < 0.05$) among antifungal activity of strains against test pathogens with an inhibition zone of $11.3 \pm 0.47$ (GBTCF-05), $14.3 \pm 0.47$ (GBTCF-07), $16.3 \pm 0.47$ (GBTCF-26), $16.7 \pm 0.47$ (GBTCF-26) and $15.7 \pm 0.47$ (GBTCF-08) against A. niger, F. oxysporum, F. solani, M. phaseolina and R. solani respectively. Percent inhibition of the selected strains (GBTCF-09, GBTCF-21, GBTCF-26) was also calculated against test phytopathogens (Fig. 4). Percent inhibition was ranged from 35.7 to 62.2% (Table 3). GBTCF-26 was found to be most active against R. solani with percent inhibition of 62.2% while GBTCF-09 was prominent against F. solani and F. oxysporum with percent inhibition of 61.1% and 58.8% respectively.

Enzymatic activity

All the strains were screened for their potential to produce extracellular enzymes (Fig. 5). It was observed that out of 30 strains, 19 strains (63.3%) produced amylase, 16 strains (53.3%) produced caseinase, 11 strains (36.7%) produced gelatinase, 10 strains (33.3%) produced lipase, 4 strains (13.3%) produced chitinase and 8 strains (26.7%) produced cellulase, while three strains produced all these enzymes. On the basis of colloidal chitin degradation and zone of clearance on CCA plate, isolate GBTCF-13, GBTCF-18, GBTCF-25 and GBTCF-28 were found to exhibit chitinolytic activity with the clearance zone of 9 mm, 12 mm, 18 mm and 15 mm respectively. Further, strains (GBTCF-26, GBTCF-21, GBTCF-09) exhibited good antifungal activity but did not show chitinase activity. However, isolate GBTCF-25 was able to show high antifungal activity along with

![Fig. 3. Preliminary screening of strains against (A, B, C) Rhizoctonia solani and (D) Macrophomina phaseolina.](image)

<table>
<thead>
<tr>
<th>Isolate</th>
<th>% inhibition against</th>
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<tbody>
<tr>
<td></td>
<td>A. niger</td>
</tr>
<tr>
<td>GBTCF-09</td>
<td>35.71</td>
</tr>
<tr>
<td>GBTCF-21</td>
<td>41.17</td>
</tr>
<tr>
<td>GBTCF-26</td>
<td>35.89</td>
</tr>
</tbody>
</table>
high chitinase activity. A larger number of Actinobacteria representing genera *Streptomyces* from cow feces origin produced amylase followed by caseinase, gelatinase, lipase, cellulase, and chitinase activity.

**Discussion**

As per literature studies, new bioactive molecule originate from natural sources [25] and this is the output of the vigorous screening for microorganism from different niches and extract of different plants [26]. Among microorganism, *Actinobacteria* have important role in production of variable bioactive metabolites and many industrially significant compounds have been used in the current scenario. Cow feces is an important biomat that is extensively used as manure for the enhancement of diverse crops. Microbes that inhabit Cow feces, possess plant growth promoting traits along with antagonistic activity against phytopathogens. Hence, this study had been done to explore the potential of actinobacteria from Cow feces for their industrially important secondary metabolites including enzymes and antagonist activity against phytopathogens. The Cow feces samples were screened for successful isolation of *Actinobacteria* and were demonstrated as an important natural source of secondary metabolites. In a similar study, Tan et al. [27], recovered and characterized actinomycetes isolated from goat faeces; of which, *Oerskovia* was most frequently observed genus. *B. subtilis* strains, isolated from Cow feces inhibited the in-vitro growth of fungi, *F. oxysporum* (25–34%) and *B. theobromae* (100%) [9]. The strains were recognized on the basis of their morphological, biochemical and DAP in whole cell hydrolysate study recommended as per International *Strepto*
myces Project (ISP) criteria and Bergey’s Manual of Systematic Bacteriology [14]. The dominant genera were Streptomyces which also have different color appearance of aerial mycelium (Grey, white, yellow) on growing medium [28]. Some of the strains were further characterized on the basis of 16S rRNA sequencing. The obtained sequences were analyzed for chimera check by using online tool Decipher’s [29], as obtaining satisfactory result means the absence of chimeric form in the sequence. Sequences were aligned by using MUSCLE program and the phylogenetic tree was constructed with Mega 5.0 software with bootstrap method [17–20]. The phylogenetic relationship among the strains were clearly represented by molecular marker i.e. gene for 16S rDNA that has vital role in explaining the diversity of the strains [30].

Fungal phytopathogens pose a serious threat all over the world in the cultivation of economically important crops and people are using chemical fungicides to neutralize these microorganisms. Further, the excessive use of these chemicals in agronomy has enhanced the resistant fungal pathogens, waning human health and creating a polluted environment. To overcome this problem, interest is inclining toward the search for an alternate method to protect crops from fungal infections. As per results of this study, the test phytopathogens were sensitive in order as follow: F. solani, F. oxysporum, R. solani, M. phaseolina and A. niger. Besides, strain GTBCF-26 displayed inhibition against all test fungal pathogens. It is well established in the literature that a large number of Actinobacteria are antagonistic to phytopathogens. Kaur [31] reported 35 endophytic actinomycetes from Azadirachta indica. Strain AZR-12, which is identified as Micromonospora, was showing a wide range of antifungal activity against four phytopathogens (F. oxysporum; Aspergillus brassicicola; R. solani; Phytophthora dreselea) and the maximum percentage inhibition was observed against F. oxysporum (75%). Similarly as per the report of Andreoli et al. [32], 74 and 122 endophytic bacteria were isolated from 3 years and 15-year-old Corvina vines respectively including Actinobacteria. One of the most dominant Actinobacterial strain was having antagonistic activity against fungal pathogen Botrytis cinerea. In addition, these beneficial Actinobacteria were able to colonize in the tissue of the plant and showed biocontrol competence against the fungal phytopathogens.

In the current scenario, antifungal metabolites and enzymes of biological origin should attract the industries to replace chemical compounds or catalyst in various food, paper and pulp, pharmaceuticals and textile industries. Several researchers have reported different enzymes of actinomycetes origin from various habitats [33, 34]. In a similar study, Kumar et al. [28] reported various enzymes including xylanase, lipase, amylase, cellulase, gelatinase and caseinase from earthworm casting. Chitinolytic activity, in which enzyme chitinase play an antagonistic role by attacking the cell wall of the fungus, of Actinobacteria provides importance in control of diseases that are caused by fungal phytopathogens [35]. In the present study, chitinase activity was examined by degradation of chitin in CCA plate method and only four strains were having chitinolytic activity. Similarly in a study conducted by Singh and Gaur [36], 68 endophytic actinomycetes were isolated from healthy medicinal plants; 12 strains having good chitinolytic activity with more than 25 mm clear zone and these 12 selected strains were having antagonistic nature against S. rolfsii, R. solani, F. oxysporum and A. solani. Maximum inhibition was observed against S. rolfsii and more than 60% inhibition was recorded against R. solani and F. oxysporum [36].

The results of the study revealed that the Actinobacteria associated with Cow feces have an immense potential against fungal phytopathogens and enzymes important to industries.

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