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# Antagonistic activities of local actinomycete isolates against rice fungal pathogens

**Debananda S. Ningthoujam*, Suchitra Sanasam, K. Tamreihao and Salam Nimaichand**

Microbial Biotechnology Research Laboratory, Department of Biochemistry, Manipur University, Imphal, India.

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**Several actinomycete isolates obtained from various habitats in Manipur were screened for activity against some major rice fungal pathogens such as *Curvularia oryzae* MTCC 2605, *Pyricularia oryzae* MTCC 1477, *Bipolaris oryzae* LSMU1, and *Fusarium oxysporum* MTCC 287. LSCH-10C, NRP1-14, NRP1-18 and NRP1-26 showed potent antagonistic activities in dual culture assay. Among 33 indigenous actinomycete isolates, LSCH-10C isolated from Loktak lake sediment on chitin agar, was found most promising to be developed as biocontrol agent (BCA) for rice. The nature of the activity in terms of fungitoxic or fungistatic nature was also determined. This report presents the preliminary results of these bio-control actinomycetes. Some of the strains have been selected for further studies towards application as rice BCAs. This paper also deals with characterization of the most promising agroactive strain.**

**Key words:** Biocontrol, antagonism, rice, fungal pathogens, actinomycetes, *Streptomyces vinaceusdrappus*, Manipur.

## INTRODUCTION

Actinomycetes are unparalleled sources of bio-active metabolites including antibiotics, plant growth factors, and other substances (Keiser et al., 2000; Omura, 1986; Shahidi et al., 2004). *Streptomyces* and other action-mycetes are major contributors to biological buffering of soils and have roles in organic matter decomposition conducive to crop production (Dhingra and Sinclair, 1995). Biological control is slow but can be long lasting, inexpensive, and harmless to living organisms and the ecosystem; it neither eliminates the pathogen nor the disease, but brings them into natural balance (Ramanathan et al., 2002). Intensive research on plant growth promoting bacteria(PGPB) is underway worldwide for developing biofertilizers and biocontrol agents (BCAs) as better alternatives to agrochemicals, as the latter harm the environment and human health besides demanding high costs. As most PGPBs show inconsistent perfor-mance in the field conditions, there is urgent need for survey of indigenous strains suited to local conditions.

Actinomycetes are known to produce bioactive sub-stances, especially antibiotics that are effective against phytopathogenic fungi (Crawford et al., 1993; Bressan, 2003). Biocontrol with beneficial bacteria is one promising alternative to fungicides (Li et al., 2008). Hydrolases such as chitinase contribute to degradation of fungal cell walls (Korsten et al., 1994) Paulitz and Belanger, 2001; Helisto et al., 2001). Chitin is the second most abundant polysac-charide in nature and a major component of fungal walls, insect exoskeletons and crustacean shells. Chitinase secreted by a BCA is likely to be effective against pathogenic fungi, the cell walls of which are mainly chitin.

The present study aims to isolate and screen efficient native biocontrol strains for disease control (and growth promotion) in rice (which feeds more than 70% of global population and whose yield needs to be enhanced by 100% by 2020) suited to local conditions in Manipur (Indo-Burma Biodiversity Hotspot), holding special significance as meagre studies have been done so far here.

*Corresponding author. E-mail: n.debananda@gmail.com.

## MATERIALS AND METHODS

Actinomycetes were isolated from various biotopes of Manipur such as agricultural soils, forest soils, caves, salt springs, limestone quarries, lake, and river sediments and plants (for endophytic actinomycetes), following standard protocols (Kuster and Williams, 1964; Hsu and Lockwood, 1975) on Starch Casein Nitrate agar (SCNA) and Chitin agar media. Biocontrol assay was done using dual culture method (Hamdali et al., 2008) against the rice patho-

(a) (b) (c)


**Figure 1.** Growth inhibition of *Fusarium oxysporum* MTCC 287 by the bioactive actinomycete isolates. (a) Control plate (b) NRP1-14 and LSCH-10C (c) NRP1-26 and NRP1-18

(a) (b) (c)


**Figure 2.** Growth inhibition of *Pyricularia oryzae* MTCC1477 by the bioactive actinomycete isolates (a) Control plate (b) NRP1-14 and LSCH-10C (c) NRP1-26 and NRP1-18.

gen, *Curvularia oryzae* MTCC 2605 (leaf spot disease) . The study is being extended against other pathogens such as *Pyricularia oryzae* MTCC 1477(rice blast disease), *Bipolaris oryzae* LSMU1 (brown spot disease), *Fusarium oxysporum* MTCC 287 (root rot disease). These isolates were tested for phosphate solubilization, IAA production and chitinase production (Hamdali et al., 2008; Pikovskaya, 1948; Hsu and Lockwood, 1975; Ahmad et al., 2008). The best strain was characterized by biochemical, morphological and physiological tests, and 16S rDNA sequencing using primers 8-27F, 357F, 1100R and 1492R.

## RESULTS AND DISCUSSION

Of 33 isolates screened 4(LSCH-10C, NRP1-14, NRP1-18 and NRP1-26) showed bioactivity (Figures 1-4) of which LHCH -10C (Figure 6) showed maximal antagonistic activity against almost all the test pathogens (Table 1). It showed inhibition to *C. oryzae* and *P. oryzae,* even after 14 days of incubation (Figure 5). (16S rDNA sequencing) showed that the strain, LSCH-10C is closely related to *S. vinaceusdrappus* (data not shown). All the strains have phosphate solubilizing and chitinase active-ties, showing their potential for growth promotion and biocontrol. However, none of them showed any IAA producing ability (Table 2).

The most potent biocontrol strain, LHCH-10C was identified by phenotypic (Table 3) and genotypic (16S rDNA sequence analysis) (data not shown here) as *S. vinaceusdrappus*.

Chitinase is a good candidate as a BCA. *Streptomyces plicatus* has been shown to inhibit spore germination and spore tube growth in *F. oxysporum*, *Alternaria alternata*, *Verticillum albo-atrum*, and other pathogenic fungi (Abd-Allah, 2001).

Phosphorous deficiency is limiting crop production worldwide (Hamdali et al., 2008) as most soil phosphates in soil are found bound as insoluble phosphates which can be released by microorganisms (Arcand and Schneider, 2006). P-solubilizing microbes (PSMs) belong to *Bacillus, PSEUDOMONAS, MICROCOCCUS* and ACTINOMYCETES etc. (Mba, 1997; Rudresh et al., 2005). Among these PSMs, actinomycetes are of special interest as they can survive in a variety of soils and produce a plethora of bioactive compounds that may benefit the plant (Hamdali et al., 2008; Pathom-Aree et al., 2006; Ikeda, 2003; Jain and Jain, 2007). However, little work on characterization of PSM actinomycetes and thier mechanism for solubilization has been done (Hamdali et al., 2008). Hamdali et al. (2008) reported actinomycetes like

(a) (b) (c)

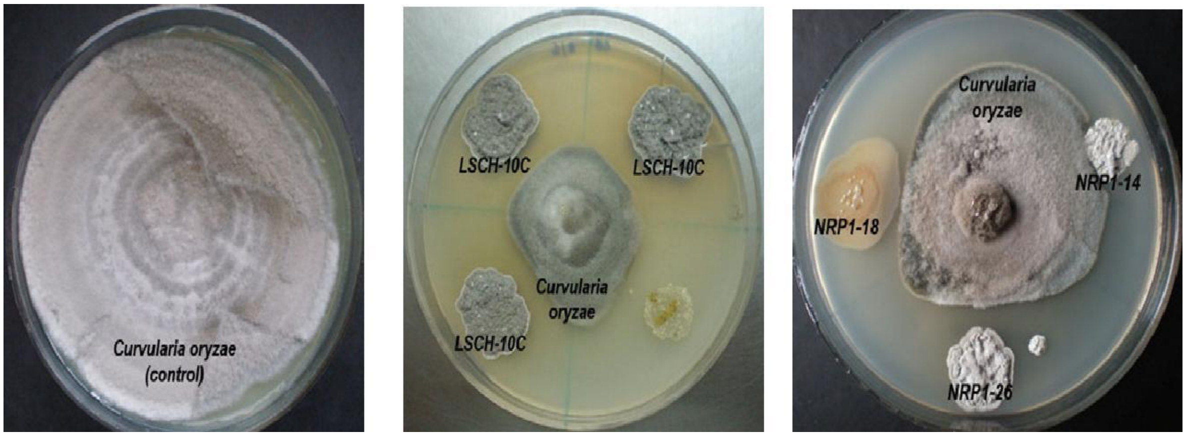


**Figure 3.** Growth inhibition of *Curvularia oryzae* MTCC 2605 by the bioactive actinomycete isolates. (a) Control plate (b) LSCH-10C (c) NRP1-18, NRP1-14 and NRP1-26.

(a) (b)

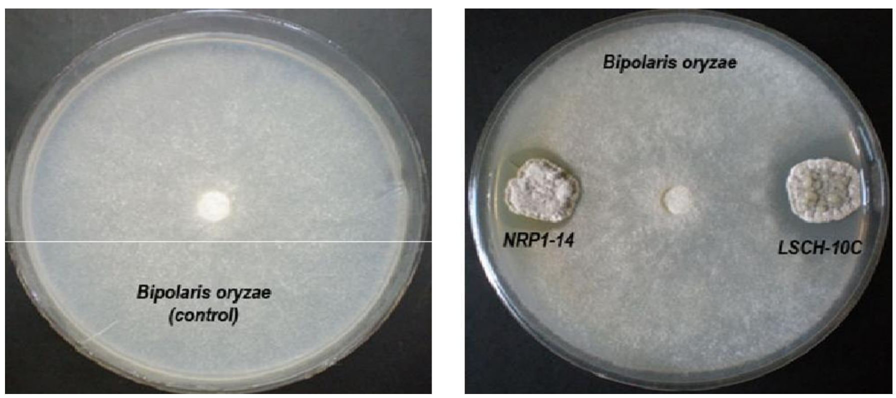


**Figure 4.** Growth inhibition of *Bipolaris oryzae* LSMU1 by the bioactive actinomycete isolates (a) Control plate (b) NRP1-14 and LSCH-10C.

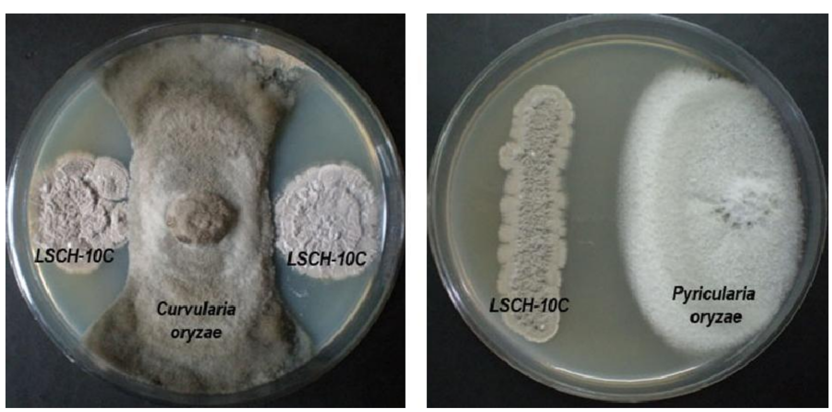


**Figure 5.** Growth inhibition by LSCH-10C even after 14 days of incubation (a) *Curvularia oryzae* MTCC 2605 and (b) *Pyricularia oryzae* MTCC1477.

**Table 1.** Percentage growth inhibition of the rice phytopathogens by the indigenous bioactive actinomycete isolates.

| | % of mycelial growth inhibition | | | |
|---|---|---|---|---|
| **Test isolates** | **MTCC 2605** | **LSMU1** | **MTCC 287** | **MTCC 1477** |
| Control | 0.00 | 0.0 | 0.00 | 0.0 |
| LSCH-10C | 65.00 | 53.5 | 48.84 | 53.5 |
| NRP1-14 | 42.00 | 51.2 | 46.60 | 60.5 |
| NRP1-18 | 43.75 | ND | 39.60 | 44.2 |
| NRP1-26 | 60.00 | 67.5 | 46.50 | 44.2 |
| Days of incubation | 8 | 7 | 8 | 11 |

MTCC 2605, *Curvularia oryzae;* LSMU1, *Bipolaris oryzae;* MTCC 287, *Fusarium oxysporium;* MTCC 1477, *Pyricularia oryzae;* ND, Not determined.


**Figure 6.** LSCH-10C.

**Table 2.** Results for phosphate solubilization (PS), IAA production (IP) and chitinase production (CP) by the bioactive actinomycete isolates.

| Isolates | PS | IP | CP |
|---|---|---|---|
| LSCH-10C | + | - | + |
| NRP1-14 | + | - | + |
| NRP1-18 | + | - | + |
| NRP1-26 | ++ | - | + |

*Streptomyces grisea* and *Streptomyces cavourensis* as powerful solubilizers in SMM broth and found them to act through chelators.

Although there are some reports of actinomycete species with biocontrol activity (Trejo-Estrada et al., 1998; Lee et al., 2002; Loqman et al., 2009; Sharifi et al., 2007; Prapagdee et al., 2008; El-Mehalawi et al., 2004), there are limited reports of *Streptomyces* species that are antagonistic to rice fungal pathogens (Lee et al., 2002) *P. oryzae* (Magnaporthe grisea) causes blast disease in rice worldwide, leading up to 50% yield losses. Similarly, the other phytopathogens studied in this work cause yield losses in rice of varying extents. So studies of such antagonistic strains suited to native conditions are of prime importance. And actinomycetes are especially significant for the following reasons: they can survive in soils of various types (Pathom-Aree et al., 2006), have long survival due to their spore forming abilities, are prolific producers of various bioactive compounds such as antibiotics, siderophores, chitinases, and phytohormones and have phosphate solubilizing abilities (Hamdali et al., 2008; Ikeda, 2003; Jain and Jain, 2007).

**Table 3.** Biochemical, morphological and physiological characteristics of LSCH-10C.

| Test | Characteristics | Test | Characteristics |
|---|---|---|---|
| Gram staining | + | **Effect of temperature on growth** | |
| Pigment production | - | 4°C | - |
| Starch hydrolysis | - | 15°C | + |
| Casein hydrolysis | + | 25°C | + |
| Catalase activity | - | 30°C | + |
| Oxidase activity | - | 37°C | + |
| Methyl Red (M-R) test | | 42°C | + |
| Voges Proskauer (VP) test | - | 60°C | - |
| Citrate utilization | - | **Effect of pH on growth** | |
| Indole production | - | 5.2 | + |
| Urease activity | - | 7.0 | + |
| Nitrate reduction | - | 8.0 | + |
| Gelatin liquefaction | - | 9.0 | + |
| $H_2S$ production | - | 10.0 | + |
| | | | |
| **Degradation of** | | **Effect of salt on growth (NaCl conc.)** | |
| Adenine 0.5% | + | 0% | + |
| Tyrosine 0.5% | + | 2% | + |
| Xanthine 0.4% | - | 5% | + |
| Guanine 0.05% | - | 7% | + |
| | | 10% | w |
| | | | |
| **Utilization of nitrogen sources** | | **Utilization of carbon sources** | |
| Phenylalanine | + | L-Arabinose | + |
| Cysteine | - | D-Fructose | + |
| Valine | + | D-Galactose | + |
| Hydroxyproline | + | meso-inositol | + |
| Methionine | + | Mannitol | + |
| Arginine | + | L-Rhamnose | + |
| Serine | + | Salicin | + |
| Threonine | - | Sucrose | - |
| Histidine | + | D-Xylose | + |
| $KNO_3$ | + | D-Mannose | + |

The most potent biocontrol strain, LHCH-10C was identified by phenotypic (Table 3) and genotypic tests (16S rDNA sequence) analysis (data not shown here)) as *S. vinaceusdrappus.* Next to LHCH-10C, NRP1-14, NRP1-26 and NRP1-18 are other promising isolates were antagonistic against rice phytopathogens. To our knowledge, this is the first report of *S. vinaceusdrappus* that is antagonistic against rice fungal pathogens. Further work on optimization of this strain's antagonistic activity and characterization of the mechanism of action is currently underway.

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