Diversity of soil Allelopathic Actinobacteria in Tiruchirappalli district, Tamil Nadu, India

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Received 27 April 2013; accepted 25 July 2013
Available online 3 August 2013

KEYWORDS
Biodiversity; Actinobacteria; Soil; Paddy field; Weeds; Allelopathic activity

Abstract
The present study deals with the diversity of paddy field actinobacterial flora in Tiruchirappalli district. Totally 118 actinobacterial colonies were isolated from 12 different paddy fields, among which 45 isolates were morphologically distinct on the basis of spore color, mycelia formation, pigment production and reverse side color formation. Totally 45 actinobacterial isolates were screened for allelopathic activity against Cyperus rotundus. Among them 6 (13.33%) isolates showed maximum growth inhibition and 17 (37.77%) isolates showed minimum inhibition of weed germination. An attempt was made to correlate the actinobacterial diversity and physico-chemical properties of soils which revealed positive relationship with nitrogen, potassium, copper, and phosphorus. Species diversity indexes (H'), species richness (SR) and species evenness (J') were also studied.

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1. Introduction
Actinobacteria represents a large part of the rhizosphere microbial community (Strobel et al., 2004), which is involved in the turnover of recalcitrant plant organic matter producing a balance in the ecosystem (Miyadoh, 1997). Majority of the actinobacteria are isolated from soil, rhizosphere region and rice fields (Kimura and Asakawa, 2006). In the study of Crawford et al. (1993) the interactions such as nitrogen fixation, plant growth hormone production and protection of plants against infection have been demonstrated. In addition, the production of lytic enzymes by the actinobacteria facilitates the survival of plants by degrading diverse substrates occurring in plant litter and soil, which again has the ability to inhibit the growth of phytopathogens. The degradative ability of actinobacteria is important for carbon cycle and humus formation in the environment which ultimately enhances soil nutrition for plant growth. Actinobacteria, especially Streptomyces are prolific producers of secondary metabolites, used as biocontrol agents to control soil-borne and seed borne diseases of plants.

Paddy field is a unique agro-ecosystem containing flood water, rice root, rice straw and stubble, composted materials and percolating water which makes it as an important habitat for the microorganisms. These habitats are microenvironments...
physicochemically different from each other exhibiting biologically distinct properties. Such heterogeneity of these habitats influences the structure and diversity of microbial communities in the paddy field ecosystem supporting various microbiological processes in paddy fields, most of which are agronomically and biogeochemically important.

The word “weed” means any wild plant growing and interfering the growth of beneficial or cultivated plants in agricultural field. Weeds act as a host plant for pest and reduce the quality and yield of the cultivated plant. Weeds also indirectly affect all living beings. Due to the above problems there is a serious need for controlling the weeds. The major weeds found in Tamilnadu are Cassia occidentalis L., Echinocloa crus-galli L., Amaranthus spinosus L., Amaranthus viridis L., Gynandropsis pentaphylla L., Cyperus rotundus L. and Vicia sativa L. The secondary metabolite (allochemicals) production by plants, viruses and fungi influences the growth and development of agricultural and biological systems (excluding animals). The secondary metabolites of certain microbes have been used as alternative for agrochemicals. The environmental and health hazard of the weeds has led to the use of allochemicals and rising of allelopathic crops. The allelopathy mechanism of the microbes is an eco-friendly approach and helps in promoting the growth of plants, production of phyto-hormones and degradation of complex molecules such as cellulose, lignin, and xylene. The biological weed control agents do not generate any public health diseases. In this regard, the present work aims to study the biodiversity of actinobacteria and their allelopathy potential in the paddy soil samples of 12 different regions in Tiruchirappalli district.

2. Materials and methods

2.1. Description of the sampling sites

Soil samples were collected from 12 different paddy fields in Tiruchirappalli locations, during the period of January 2011–May 2011 which includes Thiruvanaikovil (10°51’N, 78°38’E), Lalgudi (10°72’N, 78.94’E), Kallai (10°63’N, 78°17’E), Manaparai (10°38’N, 78°21’E), Vayampatti (10°31’N, 78°12’E), Marungapuri (10°20’N, 78°20’E), Manachanallur (10°98’N, 78°32’E), Musiri (10°96’N, 78°39’E), Thottiyam (10°98’N, 78°10’E), Thuraiyr (11°15’N, 78°34’E), Uppiliyapuram (11°20’N, 78°29’E) and Yerakudi (11°18’N, 78°26’E).

2.2. Physicochemical analysis of soil samples

Samples were collected randomly from the depth of 15 cm from each location, brought to the laboratory and stored for further study. Physico-chemical parameters such as pH, electrical conductivity, nitrogen, phosphorus, potassium, soil texture, lime status, ferrous, manganese, zinc and copper were analyzed according to Jackson (1973).

2.3. Correlation co-efficient analysis

The correlation co-efficient analysis between physico-chemical parameters of soils and actinobacterial populations was performed using SPSS statistical software (Version 17.0 for Windows, SPSS, Chicago, IL, USA). The diversity indices like Simpson’s index, Shannon index, Shannon evenness, species richness, Menhinick index, Margalef index, brillouin, Equitability and dominant index were performed using PAST statistical software version 2.10 for Windows, PAST, copy right Hammer et al. (2001).

2.4. Soil Samples’ pretreatment

Pretreatment of the soil was performed according to the method of El-Nakeeb and Lechevalier (1963), with slight modifications. The air dried soil (10 g) was mixed in a mortar with 1% of calcium carbonate (CaCO₃) and was incubated for 2 days at 30 °C in a closed inverted sterile Petri dish in which a high relative humidity was maintained by water saturated filter paper. To assess the effect of pretreatment, soils without CaCO₃ served as a control.

2.5. Isolation of actinobacteria

Starch casein agar medium (g/l: Starch 10, Casein 0.3, KNO₃ 2, NaCl 2, KH₂PO₄ 2, MgSO₄ 7H₂O 0.05, CaCO₃ 0.02, FeSO₄ 7H₂O 0.01 and agar 18; supplemented with Griseofulvin and Cycloheximide (Himedia, Mumbai) 25 and 10 mg/ml) was used for the isolation and enumeration of actinobacteria Kuster and Williams (1964). The diluted sediment samples (0.1 ml) were spread over the medium with a sterilized bent (L) rod and plate spinner. The inoculated plates were incubated at 30 °C for seven to 10 days. After incubation, colonies were purified using streak plate technique and maintained for further investigation.

2.6. Identification of isolated cultures

Purified isolates of actinobacteria were identified using morphological and cultural characteristics by the methods described in the International Streptomycetes Project (ISP) Shirling and Gottlieb (1966). The morphology of the spore bearing hyphae with the entire spore chain, the structure and arrangement of the spore chain with the substrate and aerial mycelium of the actinobacteria were examined and identified using slide culture technique Williams et al. (1989). After growth, cultures were examined under light microscope. Color of spore mass was visually estimated by using the color chart (Pridham, 1965).

2.7. Allelopathic activity of soil actinobacteria

All the isolates were screened for allelopathic activity by moist chamber technique and Roll towel assay method Dhanasekaran et al. (2010, 2012). The actinobacterial isolates were grown in starch casein broth and incubated at 28 ± 2 °C for a week. The culture broth was spun at 10,000 rpm for 10 min. The resultant supernatant was collected and used as a test sample to evaluate the allelopathic activity.

3. Results

Tiruchirappalli district lies at the heart of Tamilnadu slope toward east. It has number of detached hills and is punctuated by river Cauvery, which supports irrigation to the agriculture and
serves as lifeblood (Fig. 1). The alluvial plain created by the river is fertile and favors agriculture. In addition, a large amount of soil carrying spores of terrestrial actinobacteria enters into the agricultural fields.

Totally 118 actinobacterial isolates were isolated from twelve different sampling sites. Maximum isolates were recorded in Kallanai paddy field followed by Manachanallur and Thottiyam whereas minimum number of isolates from Yerakudi soil (Fig. 2). Out of 118 isolates, 45 isolates were morphologically distinct including white, gray, pink, brown, grayish white, green and red color colonies. Most of the isolates were morphologically white. The morphological characteristics like colony nature, aerial mycelium color, reverse side color, diffusible pigments, growth rate, and size of the colony were observed.

Out of 45 isolates, 14 were identified as genus *Streptomyces* (spore chain with coiling, spiral and looped), ten isolates as *Streptoverticillium* (spore chain are long, straight filament with regular intervals), nine as *Actinomadura* (spore chains are straight and open hooked), six as *Kitasatosporia* (spore chains are long with more than 20 spores) and two each belonging to *Nocardiopsis* (aerial mycelium totally sporulated), *Pseudonocardia* (spore chains are long, irregularly zig-zagged), and *Kibdelosporangium* (spore chains are long with irregularly curved) (Williams et al., 1989), (Fig. 3). Thus they have worldwide distribution, which indicate their plasticity and adoptability to the various environments.

Frequencies of identified genera of actinobacteria in different sampling sites were fluctuated. The frequency of the genus *Streptomyces* was 31.1% followed by *Streptoverticillium*.
22.2%, Actinomadura 20%, and Kitasatosporia 13.3% whereas other genera such as Nocardiopsis, Kibdelosporangium, and Pseudonocardia recorded low percentage of frequency (4.4%) (Fig. 4). Besides Streptomyces, the genera most frequently appeared on media were Streptoverticillium, Actinomadura, Kitasatosporia, Nocardiopsis, Kibdelosporangium and Pseudonocardia.

In spite of the fact that the actinobacteria have a wide distribution, they also show variation in their population dynamics. The correlation co-efficient analysis of physico-chemical properties of soil samples and total actinobacterial count revealed a significant positive correlation between total actinobacterial count and nitrogen ($r = .688$, $P < 0.01$), TAC and potassium ($r = .606$, $P < 0.01$), TAC and copper ($r = .585$, $P < 0.01$), and TAC and phosphorous ($r = .567$, $P < 0.01$) (Table 1). Similar type of study was reported by Mansour, (2003). The range of biodiversity indexes viz. dominance, simpson’s index, shannon index, and evenness at all stations were 0.14–1.0, 0.73–0.85, 0.6–1.9, and 0.9–1.0 respectively. The range of Brillouin, Margalef, Menhinick index, and Equitability at all stations were 0.3–1.4, 1.4–2.7, 0.7–2.2, and 0.9–1.0 respectively (Fig. 5).

Among the forty-five isolates only six isolates (DDBH001, DDBH010, DDBH012, DDBH016, DDBH019 and DDBH020) showed maximum allelopathic activity against C. rotundus (Fig. 6). Isolates DDBH001, DDBH012, and DDBH019 were identified as Streptomyces sp. belonging to Thiruvanaikovil, Kallanai, and Manaparai paddy soils, whereas DDBH010 was identified as Kitasatosporia sp. from Kallanai paddy soil, DDBH016 as Streptoverticillium sp. from Kallanai paddy soil and DDBH020 as Actinomadura sp. from Manaparai paddy soil. This result clearly revealed that Kallanai paddy soil isolates have stronger allelopathic activity when compared to other paddy soils. The positive six allelopathic isolates belong to high calcium carbonate rich soils that favor the growth of actinobacteria and the isolates are highly depend on nitrogen, copper, phosphorus and potassium sources for their survival. The moderate allelopathic activity was observed in 17 isolates and 22 isolates showed minimum secretion of weed growth inhibitory compounds. C. rotundus is a common weed in paddy; hence it was selected to test the allelopathic activity.

4. Discussion

Microorganisms in the paddy field environment has much role in the growth and development of plants through nutrient regeneration by biological processes either decomposition or by fixing the nitrogen and phosphorous. Assessments of microbial diversity in the paddy field ecosystem are not
attempted so far. Hence, the present study has been undertaken to find out the diversity and function of actinobacteria.

CaCO$_3$ is commonly employed to increase the number of actinobacterial population from air-dried soils (El-Nakeeb and Lechevalier, 1963; Oskay, 2009). However, the precise mechanism of CaCO$_3$ effect is not well-studied. The increase in actinobacteria population due to the addition of CaCO$_3$ might be attributed as increase in pH and stimulation of Ca$^{2+}$ by aerial mycelium formation, which was observed in some actinomycetes viz., *Streptomyces ambofaciens* and *Streptomyces alboniger* (Natsume et al., 1989), Qin et al. (2009).

Vanajakumar et al. (1991) reported that, white color series of actinobacteria were the dominant forms than the gray, pink and red color series. Among the genera recorded in the present study, *Streptomyces* was the most predominant one when compared to other genera. The dominance of *Streptomyces* among the actinobacteria especially in soils has also been reported by many workers (Dhanasekaran et al. (2009), Vijiyakumar et al., (2007), Moncheva et al. (2002), Balagurunathan et al., (1996), Mansour, 2003).

Jiang and Xu (1996), Saadoun and Al-Momani (1996) have studied the pH, moisture, organic matter, nitrogen and phosphorous content of the soils and correlated with actinobacterial population.

The physicochemical parameters clearly indicate that important nutrients such as nitrogen and potassium were found maximum between the soil depths of 0–10 cm. The distribution and abundance of actinobacteria were found maximum between the depths of 10–20 cm. Ghanem et al. (2000) reported that, the counts of actinobacteria were found maximum in the upper sediment layers (0–20 cm).

The correlation between salinity, pH and organic content of marine sediments and actinobacterial population has been reported by several workers (Jensen et al. (1991); Ndonde and Semu (2000); Dhanasekaran et al. (2009). Ghanem et al.

### Table 1

Carl Fisher coefficient analysis of physico-chemical properties of soil and total actinobacterial species.

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* Correlation is significant at the 0.05 level (1-tailed).
** Correlation is significant at the 0.01 level (2-tailed).
Diversity of soil Allelopathic Actinobacteria in Tiruchirappalli district, Tamilnadu, India

Figure 6  Allelopathic activity of actinobacterial isolates.

(2000) reported that the variation in temperature, pH and dissolved phosphate showed insignificant values, but variation in total nitrogen and organic matter was significant in the population in Alexandria.

The allelopathic results revealed that the actinobacterial extract has the potential to reduce the germination of C. rotundus and these results were similar to the observation of earlier workers Dhanasekaran et al., 2010, 2012; Priya Dharsini et al., 2013a, b). In their findings Cyperus germination inhibitory compounds were identified as N-phenylpropanamide and N-(naphthalen-1-yl) propanamide from Streptomyces sp. KA1-3.

The results of the present study showed that the actinobacterial dominance, diversity, richness and evenness are associated with the environmental factors of the soil. In conclusion, actinobacterial communities at the respective habitats in the paddy field ecosystem are different in diversity and stability to each other, which may contribute to the diversity of the whole world of allelopathic actinobacterial communities in the paddy field ecosystem. The present results also show that, actinobacteria are evenly distributed according to the microenvironments governed by intermediate factors. The present investigation proved that, the paddy field ecosystem in the South west coast of India is eminently a suitable ecosystem for the diversity of actinobacteria which could thus enhance crop yield and quality.

Acknowledgement

The first author is financially supported by the Ministry of Social Justice and Empowerment and Ministry of Tribal Affairs (University Grants Commission approval No and Date: F.14-2 (SC)/2009 (SA-III) dated 18.12.2010.

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