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# Actinomycetes from Shifting Cultivation (*Jhum*) of Mizoram, Northeast India

**Marcy D. Momin, Shri Kant Tripathi**

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**Abstract** The main objectives of the present study were to isolate actinomycetes from shifting cultivation of Mizoram, Northeast India and screening for their salt-tolerance activities. This study was carried out in two areas of shifting cultivation (Reiek and Tanhril) of Mizoram. Actinomycetes were isolated by standard method of scrial dilution technique. Inoculated on selected medias like SCA, AIA, ISP5 and incubated at $28^0$C for 3-30 days. Total 30 actinomycetes strains were isolated and isolates were screened for salt-tolerant activity. Out of 30 isolates, 10 actinomycetes can able to tolerate salts. Further, positive isolates were morphological studied.

**Keywords** Actinomycetes, Shifting cultivation, Salt- tolerance activity.

## Introduction

Actinomycetes is a special group of prokaryotes, Gram-positive bacteria, high guanine and cytosine content, branching growth pattern in their growth habit. They are ubiquitous in nature. They play an important role in the ecosystem because many soil actinomycetes can transform different forms of insoluble organic and inorganic substances into a soluble form suitable for plant uptake. These organisms are recognized for their important bioactive compounds perform certain specialized roles including ability to tolerate high temperature, prevent attack of phytopathogens, protect soil borne diseases and capabilities to promote plant growth and development by producing plant growth promoting traits (siderophore, indole acetic acid, nitrogen fixation, ammonia production and phosphate solubilization). Thus, investigation on actinomycetes are of prime attention.

Actinomycetes are widely distributed in different habitats and different soil ecosystems such as forest, pasture, rain-fed and irrigated cultivated land (Ghorbani-Nasrabadi et al. 2013), soils at altitudes (Bull et al. 2018), located in various climatic zones. They can inhabit various ecological niches including the most extreme conditions. Actinomycetes occurrence in mountainous environment have been produce novel compounds. Northeast India Himalayan region gift bioresource of microflora and great variation, particularly in respect of topographic, geographic and the climatic conditions (Idris et al. 2017). Shifting cultivation basically refers to slash and burn is a traditional agricultural practice of Northeast India. It a cyclic process that includes clearing land through the burning of natural vegetation. Shifting cultivation leads to mountain ecosystem instability, soil erosion, loss of beneficial microorganisms, that, in turn, affects fertility of soil. Mizoram topography is conspicuous with the presence of hills and mountain ranges. Mizoram is extended between $21^058'$-$24^035'$ N and $92^015'$ - $93^029'$ E. It covers an area of 21087 $km^2$ (Sati and Rinawma 2014). The average height

Marcy D. Momin*, Shri Kant Tripathi
Department of Forestry, Mizoram University, Aizawl, Mizoram 796004, India
e-mail : mominmarcy@gmail.com
*Corresponding author

of the hills to the west of the state are about 1,000 meters (3,300 feet). These gradually rise to 1,300 meters (4,300 feet) to the east. Some areas, however, have higher ranges which go up to a height of over 2,000 meters (6,600 feet). Information of the microbiology of high-altitude mountain soils universal are scant (Bull et al. 2018). Therefore, present study is to isolate actinomycetes from mountain areas of shifting cultivation in Mizoram, Northeast India and screening for their salt-tolerant activity.

**Materials and Methods**

Study sites

The present study was carried out from two shifting cultivation areas of Mizoram i.e., Reiek shifting cultivation located between $23^0$67′.77′′N and $092^0$60′.37′′ E. Reiek is a district of Mamit situated in the western part of Mizoram about 29 km from the Aizawl city. The topography has 1,465 m. The temperature of the area is subtropical (20-30 $^0$C in summer, 12-22 $^0$C in winter) with considerable seasonal variation and their soil temperature was 22 $^0$C. Average annual rainfall is between 15 and 167.7 mm and relative humidity is over 70% throughout the year (Anonymous 2010).

Tanhril is situated in the central part of the Aizawl district and located at 15 km from Aizawl, Mizoram, located between $23^0$44′55.25′′ N and $092^0$38′36.68′′ E. Its elevation has 535 m. The climate of the area is a humid subtropical. The annual average rainfall is amounting to 5.6-477 mm and their soil temperature was $27^0$C. The ambient air temperature ranges from 20-$30^0$C in summer and 11-$21^0$ C in winter. It is situated with Tlawng river valley to its west and the Tuirial river valley to its east (Anonymous 2010). Both the study sites had limited access compared with the capital city, Aizawl. The area is a part of the Indo-Burma hotspot region with high biodiversity and the terrains are steep.

Isolation of actinomycetes

Actinomycetes was isolated from collected soil samples by soil dilution plate method. One gram of dried soil was suspended in 99 ml sterile water and serially diluted in sterile water up to $10^{-7}$. An aliquot of 0.1 ml of each dilution was taken and spread evenly over the surface of Actinomycete Isolation Agar (AIA) medium, SCA (Starch Casein Agar), International Streptomyces Project (ISP5) will be used as selective media for isolation of actinomycetes. All media were supplemented with 100 lgnystatin/ml, 100 lgcycloheximide/ml and 50 lgnalidixic acid/ml. The plates were incubated at $28^0$C for 4 weeks. Individual colonies were re-grown at $28^0$C on ISP-2 agar for purification.

Characterization of actinomycetes

The isolates based on micro-morphological observation and biochemical characterization was identified. Visual observation of both morphological and microscopic characteristics like aerial mycelia, spore distinctive reverse colony color, color diffusible pigments, spore chain morphology will be studied by Thampayak et al. (2008). Purified isolates were identified to genus level according to Bergey's Manual of Determinative Bacteriology.

Determination of actinomycetes salt-tolerance

Purified actinomycetes were inoculated on medium, in which NaCl concentrations were 0, 50, 70, 100, 150, 180, 200, 250, 300 g/L respectively, and observed their growth conditions. The highest NaCl concentration that the strain could tolerant expressed the strain salt-tolerance (Cai et al. 2009).

**Results and Discussion**

Salt -tolerance actinomycetes are less explored from Northeast, India region. Northeast region is well-known for its high biodiversity. There is rare exploration of microbial bioactive secondary metabolites particularly salt-tolerance activities from a unique habitat of Mizoram, Northeast hilly region. Previous study, occurrence of actinomycetes at high temperature were isolated (Malviya et al. 2013). Although traditional practices of slash and burn have been reported to effect on soil microbes, however, interestingly potential actinomycetes can be isolated. Survival of actinomycetes under stress conditions may have novel bioactive compounds. Salt-tolerance in actinomycetes have been studied by many researchers from various

**Table 1.** Morphological characterization of 10 isolated actinomycetes strains of Mizoram. AM and SM-representatives of Aerial Mycelium and Substrate Mycelium.

| Sl. No. | Isolate code | Nature of the colony | | | Color of the colony | | Days of growth | Media | Identified |
|---|---|---|---|---|---|---|---|---|---|
| | | Form | Elevation | Margian | AM | SM | | | |
| 1. | SKT8 | Entire | Convex | Undulate | Light yellow, sticky, colony size with 0.8 mm in dia. | Light yellow | 21 days | SCA | *Actinoplanes* sp. |
| 2. | SKT9 | Entire | Createriform | Undulate | Light grey, smooth, sticky, colony with 2 mm in dia. | Light cream | 26 days | SCA | *Streptomyces* sp. |
| 3. | SKT12 | Filamentous | Pulvinate | Undulate | Off-white, sticky, colony with 1 mm in dia. | Dark cream | 7 days | SCA | *Streptomycetes* sp. |
| 4. | SKT24 | Entire | Flat | Undulate | Grey-white, colony with 1 mm in dia. | Cream | 3 days | AIA | *Streptomyces* sp. |
| 5. | SKT27 | Irregular | Convex | Curled | Off-white, thick, sticky, hard, colony with 1 mm in dia. | Cream | 5 days | AIA | *Streptomyces* sp. |
| 6. | SKT29 | Circular | Convex | Entire | Off-white, soft sticky, dry, colony with 0.5 mm in dia. | Off-white | 5 days | AIA | *Streptomycetes* sp. |
| 7. | SKT33 | Filame | Flat | Lobate | Off-white, light pink pigment, thick, colony with ring, 1 mm in dia | Dark brown | 3 days | SCA | *Nocardia* sp. |
| 8. | SKT36 | Wrinkle | Raised | Entire | Dark cream, sticky, thick, colony with 2 mm in dia. | Dark cream | 3 days | AIA | *Streptomyces* sp. |
| 9. | SKT40 | Circular | Flat | Undulate | Grey with pink pigment, sticky, colony with 1.5 mm in dia. | Off-white | 6-7 days | SCA | *Streptomycetes* sp. |
| 10. | SKT48 | Circular | Flat | Undulate | Grey with pink pigment, sticky, colony with 1.5 mm in dia. | Off-white | 6-7 days | SCA | *Streptomyces* sp. |

part of the countries and the world (Jose et al. 2011, Hamedi et al. 2013, Cai et al. 2009). Manna et al. (2010) reported that salinity is a key factor to regulate the ecosystems in Sundarbans mangrove. Ballav et al. (2015) suggested that salt- tolerant actinomycetes are a potential source of anti-bacterial compounds. Highest enzyme activity (200 IU/ml) were obtained from salt-tolerance actinomycetes (Krishnakumar et al. 2015). Actinomycetes from unexplored environments have prolonged prime attention in recent years to produce bioactive metabolites (Singh et al. 2014). Total 30 actinomycetes were isolated from mountainous shifting cultivation soil of Mizoram. According to their morphological characteristics, the representative 30 actinomycetes strains were identified as up to the genus level (Table 1), which were observed aerial and substrate mycelium of the strains; growing within 5-21 days; they vary from off-white, creamy, light-yellow, dark-brown in color, entire, raised and filamentous in nature of the colony (Fig. 1). Based on media employed, isolates were well growth in Starch Casein Agar (SCA) medium followed by Actinomycetes Isolation Agar (AIA) and International Streptomyces Project 5 (ISP5) (Table 1). Out of 30 isolates, 10 *Streptomyces* were showed positive for salt-tolerance activity (Table 2). Among 10 salt tolerance *Streptomycetes* were the maximum (80%), where best strains belong to *Streptomyces* sp. (SKT12) followed by *Nocardia* sp. (SKT33) and *Streptomyces* sp. (SKT27) (Table 2). Strain SKT12 can able to grow at 50, 70, 100, 150, 180, 200, 300 concentrations. Strain SKT33 has capacity to grow at 150, 180, 200, 250 concentrations. Strain SKT27 has showed growth at 150, 180, 200 concentrations (Table 2). In the present study, most of the actinomycetes grow at 150 concentrations of NaCl g/L (Table 2). *Streptomycetes* are well known as a rich source of novel bioactive compounds have been exposed to

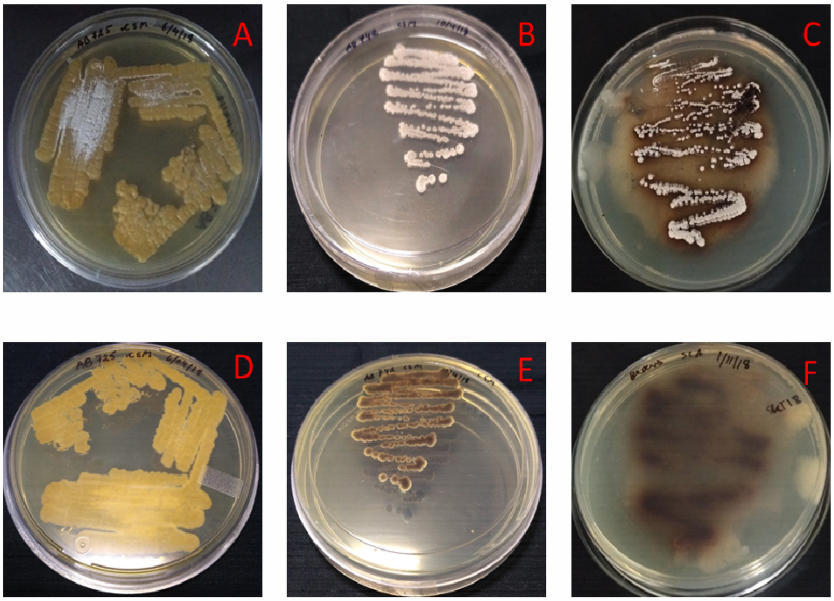


**Fig. 1.** A, B and C -representatives of actinomycetes *(Aerial mycelium)* on agar plates, D, E and F- representatives of actinomycetes *(Substrate mycelium)* on agar plates.

thorough isolation and screening which has directed to over speciation and taxonomic chaos within the genous (Guo et al. 2008). The study on isolation and characterization of actinomycetes from West Coast of India showed that majority (47%) of the solates belonged to the genera *Streptomyces* (Remya and Vijayakumar 2008) followed by the rarer genera *Glycomyces, Nocardiopsis* (11%), *Nocardia.*

## Conclusion

Actinomycetes isolates with salt-tolerant activity were obtained from *jhum* fields. With this potential soil microbial activity may improve across the crop's soils of *jhum* cultivation, which may enhance sustainability in agriculture. Moreover, along with the changes of soil property, it may restore its vitality of ecosystem.

**Table 2.** *In-vitro* screening of actinomycetes strains for their salt-tolerance -, +, ++, +++ indicative of no growth, low medium and high of growth.

| Sl. No. | Isolate code | Concentrations g/L | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 50 | 70 | 100 | 150 | 180 | 200 | 250 | 300 |
| 1. | SKT8 | - | - | + | + | - | - | - | - |
| 2. | SKT9 | - | - | - | + | + | - | - | |
| 3. | SKT12 | + | + | + | +++ | +++ | +++ | ++ | ++ |
| 4. | SKT24 | - | - | - | + | - | - | - | - |
| 5. | SKT27 | - | - | - | +++ | +++ | ++ | - | - |
| 6. | SKT29 | - | - | + | | | | - | - |
| 7. | SKT33 | - | | - | +++ | +++ | ++ | ++ | - |
| 8. | SKT36 | - | - | - | + | - | - | - | - |
| 9. | SKT40 | - | - | + | + | - | - | - | - |
| 10. | SKT48 | - | - | - | + | + | - | - | - |

Therefore, potential microbes may lead into diverse applications in various industries, pharmaceuticals, agricultural and biotechnological and environmental eco-friendly. Thus, there is crucial requirement to isolate potential microbial strains and screening of their activities severity of problem confronting agriculture. The present study encompasses the opening for further investigations. Further, molecular techniques are necessary for their genotypic characterizations of salt-tolerant actinomycetes strains.

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