Science and Technology Journal, Vol. 7 Issue: I ISSN: 2321-3388

In-vitro Evaluation of Actinobacteria for its Potential in Bio-control of Fungal Plant Pathogens

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Abstract—Infections caused by fungal plant pathogens are recently recognized as a threat to food security worldwide and its control strategies need to be taken care where naturally synthesized fungicides such as those obtained from actinobacteria are becoming an area of great interest. A total of 68 isolates of actinobacteria were evaluated for their antagonistic potential against four fungal plant pathogens viz., *Fusarium oxysporum* CABI-293942, *Fusarium udum* MTCC-2755, *Fusarium proliferatum* MTCC-286 and *Fusarium graminearum* MTCC-1893 by dual culture *in-vitro* assay. It was found that 83.8% of the isolates showed inhibitory activity against at least one of the tested plant pathogens with the percentage of inhibition ranging from 20–87.2. Thirteen *Streptomyces* isolates and one *Nocardiposis* isolate exhibited inhibition activity against all the tested pathogens. Overall, this study gives a basic understanding of the potential aspect of freshwater sediments derived actinobacteria against fungal phytopathogens.

Keywords: Anti-fungal, Phyto-pathogens, *Nocardiopsis*, *Streptomyces*

INTRODUCTION

Fungal diseases are one of the most common problems to certain crops upon which humanity depends (Godfray *et al.* 2016) and compounds derived from actinobacteria represent a promising agent to tackle the problems (Wang *et al.* 2018; Qi *et al.* 2019). Actinobacteria are Gram +ve bacteria, ubiquitous in nature, typically soil dwellers (Goodfellow and Williams 1983), found commonly in freshwater ecosystems (Sibanda *et al.* 2010). Various habitats have been explored in search of actinobacteria and several useful compounds have been expansively reported from different ecosystems (Maldonado *et al.* 2005; Passari *et al.* 2015). However, there has been a significant decline in the rate of discovery of novel actinobacteria in recent years and there has been increasing isolation of known organisms besides re-isolation of known compounds (Zotchev 2012).

Mizoram, Northeast India, is a large bio-prospecting area identified as the Indo-Burma mega-biodiversity hotspot by Conservation International (Myers *et al.* 2000). All the lakes and rivers of Mizoram are freshwater (Zothanpuia *et al.* 2015). Few actinobacteriological research has been reported from Mizoram such as freshwater sediments derived actinobacteria for its potential as antimicrobial agent and secondary metabolites producer (Zothanpuia *et al.* 2018), endophytic actinobacteria as biologically active compounds and phytohormone producers (Passari *et al.* 2015) and plants growth promoters (Passari *et al.* 2016). Investigation on the antifungal potential of actinobacteria from freshwater sediments may give a basic understanding and will provide baseline data for further studies that are significant for biotechnological exploitation especially in the management of food security.

MATERIALS AND METHODS

Isolation and Characterization of Actinobacteria

Serial dilution and spread plate technique were used for the isolation actionobacteria from freshwater sediments of Tlawng river, Tuirial river and Tamdil lake (Yuan *et al.* 2014;

http://doi.org/10.22232/stj.2019.07.01.11

Zothanpuia *et al.* 2018). Seven different nutritional media were used for the isolation such as starch-casein agar (SCA), yeast-extract malt-extract agar (ISP2), Actinomycetes-isolation agar (AIA), Streptomyces agar (SA), glycerol-asparagine agar (ISP5), tyrosine-agar medium (ISP7), and tap-water yeast-extract agar (TW-YE), the isolated organisms were characterized as exactly reported in the previous studies (Zothanpuia *et al.* 2018).

SCREENING FOR ANTIFUNGAL ACTIVITY

The actinobacterial isolates were evaluated for their antagonistic potential against four fungal phytopathogens collected from microbial type culture collection (MTCC), Chandigarh, India *viz.*, *Fusarium oxysporum* CABI-293942, *Fusarium udum* MTCC-2755, *Fusarium proliferatum* MTCC-286 and *Fusarium graminearum* MTCC-1893 by dual culture *in-vitro* assay (Khamna *et al.* 2008). Colony growth inhibition (%) was calculated by using the formula: C - T/C × 100, where C is the colony growth of the fungal pathogen in control, and T is the colony growth of the fungal pathogen in presence of actinobacteria. All isolates were tested in triplicate and mean values were calculated.

RESULTS AND DISCUSSION

Actinobacteria remains an important source of important biologically active compounds effective against certain plant and animal diseases (Goodfellow and Fiedler 2010; Yuan *et al.* 2014). The present investigation isolated 68 actinobacteria from freshwater sediments of Mizoram, Northeast India; 30 isolates from Tamdil Lake, 19 from Tlawng River, 19 from Tuirial River, and were earlier discussed in the previous article (Figure 1) (Zothanpuia *et al.* 2018).

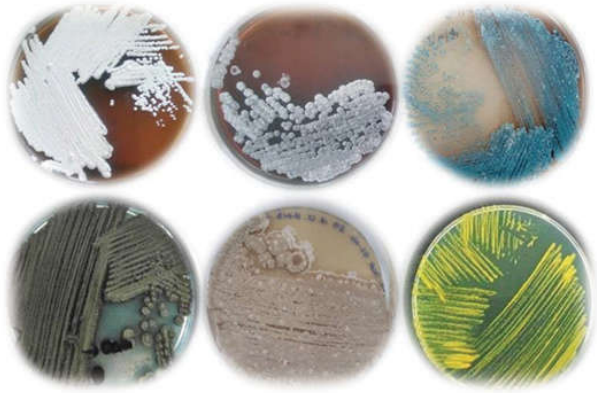

Fig. 1: Morphological Features of Actinobacteria Isolated from Freshwater Sediments

Streptomyces represent the most dominant genus among the isolated organisms (72%), followed by *Nocardiopsis*, *Saccharopolyspora, Rhodococcus, Prauserella, Amycolatopsis, Promicromonospora, Kocuria* and *Micrococcus,* the 16S rRNA gene sequences of all the isolates were deposited in NCBI GenBank database and accession numbers were given as cited (Zothanpuia *et al.* 2018). This study revealed that Lakes and rivers are important reservoirs of actinobacteria (Leiva *et al.*, 2004).

Aactinobacteria represents one of the most potential candidates to tackle the problems associated with the fungal plant pathogens that were largely reported as a potent biocontrol agent (Nafis *et al.* 2018; Qi *et al.* 2019). Diseases and the problems caused especially by the genus *Fusarium* were reported worldwide (Lamprecht *et al.* 2011) which include wilting, chlorosis, necrosis, premature leaf fall, stunting, etc. The antifungal activity was checked to understand the inhibition ability of the isolated actinobacteria against four fungal pathogens by dual culture *in-vitro* assay, which is a method widely used for preliminary screening (Zothanpuia *et al.* 2018a). The phytopathogens include *F. udum, F. oxysporum, F. graminearum* and *F. proliferatum* which were reported as pathogens maintained in the microbial type culture collection. From a total of 68 actinobacterial isolates tested, 83.8 % (n=57) of the isolates showed inhibition activity against at least one of the tested pathogens (Table 1). This justified the antifungal potential of actinobacteria from freshwater sediments supported by the findings of Rifaat (2003) that demonstrated the anti-mycotic activity of 114 freshwater actinobacteria from Nile River. 14 isolates *viz.* ***Streptomyces cyaneofuscatus*** DST15, ***Streptomyces*** sp. DST16, ***Streptomyces*** sp. DST25, *Saccharopolyspora* sp. DST31, *Streptomyces* ***griseoplanus*** DST53, ***Streptomyces*** sp. DST54, *Streptomyces* ***cyaneofuscatus*** DST64, *Streptomyces albidoflavus* DST71, ***Streptomyces*** sp. DST86, *Streptomyces albidoflavus* DST102, ***Streptomyces*** sp. DST104, *Nocardiopsis* sp. DST105, ***Streptomyces*** sp. DST116 and *Streptomyces* sp. DST119 showed activity against all the tested four *Fusarium* pathogens. Maximum inhibitory activity of the actinobacterial isolates was found against *F. graminearum* (46.4%) [Figure 2 (I)], followed by *F. proliferatum* (30.4%) [Figure 2 (II)], *F. oxysporum* (29.5%) [(Figure 2 (III)] and *Fusarium udum* (26.9%) [(Figure 2 (IV)] with percentage of inhibition ranging from 20-87.2. It was earlier reported the antifungal potential of two actinobacterial isolates ***Streptomyces*** sp. DST23 and ***Streptomyces parvus*** DST24 against *F. oxysporum, F. proliferatum*, and *F. oxy. ciceri* from freshwater Tuichang river (Zothanpuia *et al.* 2015)

which was in accordance with the reports of *Streptomyces* from Krishna river showing antibacterial and antifungal activity (Ellaiah *et al.* 2002). A similar investigation was also executed in Lake Baikal, the largest freshwater lake worldwide that described more than 70% of the isolates having an antifungal activity (Protasov *et al.* 2017). Among all the isolated organisms, *Streptomyces* sp. DST25 showed the maximum percentage of inhibition against *Fusarium Udum* (87.20%) which justified the potential of Actinobacteria especially *Streptomyces* in fighting the diseases caused by fungal pathogens supported by the findings of Nafis *et al.* (2018) and Qi *et al.* (2019). *Streptomyces* are among the largest contributors of antibiotics in the microbial world, widely distributed in soil and also colonize water and other natural environments (Goodfellow and Fiedler 2010). Most research including the present study is an early experimental stage but revealed the ability of *Streptomyces* in the control of fungal plant pathogens which was also supported by the review of Bubici (2018). Upon further investigations on the lead compounds, isolation, identification, and bio-formulation of freshwater sediments derived actinobacteria may help in the development of control strategies of fungal plant diseases which remains a great concern worldwide.

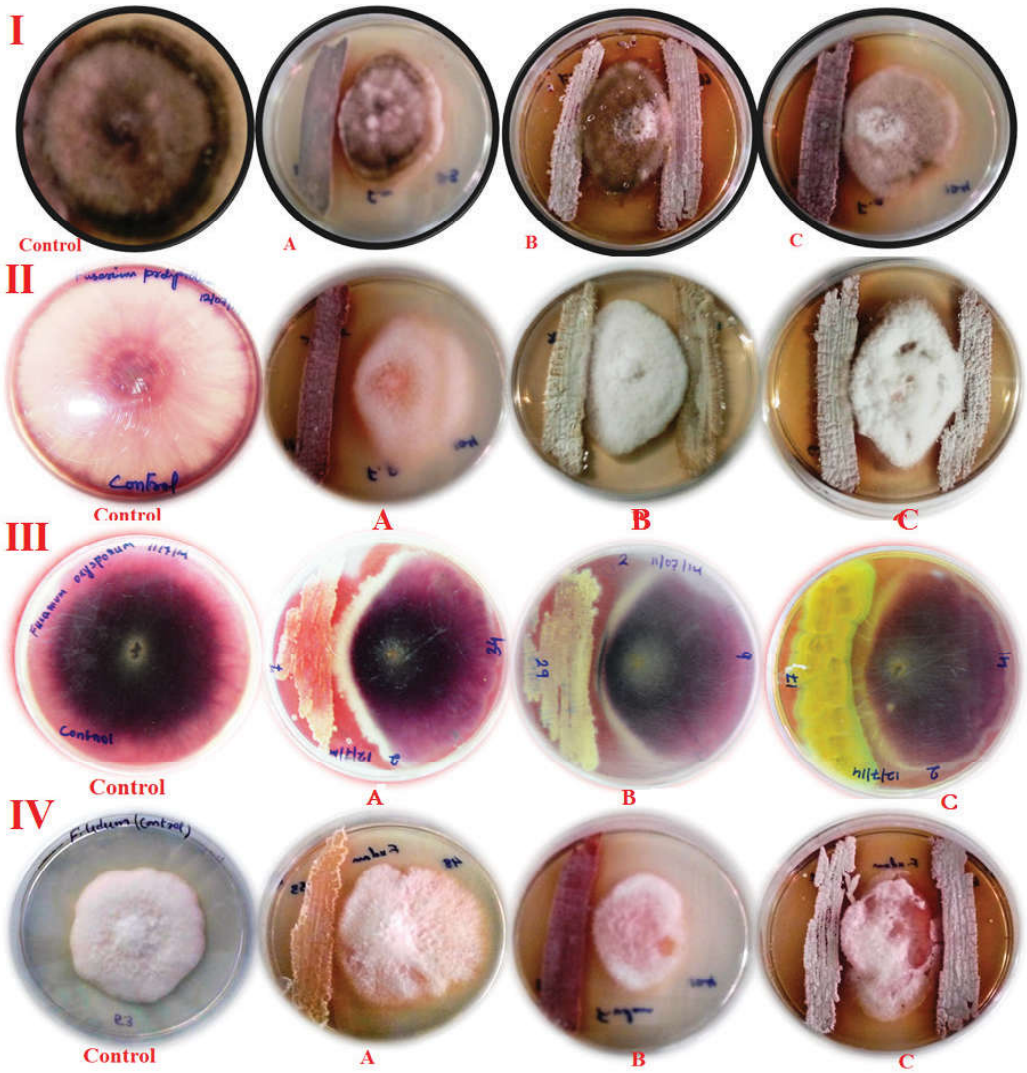

Fig. 2: (I) Antifungal Activity of Actinobacterial Isolates Against *F. graminearum* (II) *F. proliferatum* (III) *F. oxysporum* and (IV) *F. udum.* Control-Fungal Pathogens without Actinobacteria and A, B and C Denotes the Dual Culture; Growth of Pathogens Inhibited by Actinobacteria

Table 1: *In-vitro* Antagonistic Activity of Actinobacterial Isolates Against Four Fungal Phytopathogens

Sl. No.	Isolate Name	Isolate No.	Antifungal Properties C-T/CX100			
			F. udum	*F. oxysporum*	*F. graminearum*	*F. proliferatum*
1	*Streptomyces* sp.	DST13	-	48.50±0.29	20.40±0.65	46.00±0.30
2	***Streptomyces cyaneofuscatus***	**DST 15**	**74.40±0.16**	**54.60±0.42**	**42.00±0.25**	**55.00±0.63**
3	***Streptomyces* sp.**	DST 16	61.00±0.69	**45.50±0.44**	**52.00±0.69**	**62.20±0.33**
4	*Streptomyces* sp.	DST 17	59.00±0.03	-	61.29±0.98	-
5	*Streptomyces* sp.	DST 18	-	-	-	46.50±0.93
6	*Streptomyces* sp.	DST19	61.54±0.49	39.40±0.13	-	47.00±0.38
7	*Prauserella* sp.	DST22	48.71±0.14	-	35.50±0.41	37.84±0.29
8	*Streptomyces* sp.	DST23	-	73.68±0.01	-	57.89±0.36
9	Streptomyces parvus	DST24	-	50.68±0.00	-	67.12±0.18
10	***Streptomyces* sp.**	**DST25**	**87.20±0.37**	**69.69±0.41**	**54.84±0.84**	**72.97±0.075**
11	*Kocuria* sp.	DST27	73.68±0.01	45.20±0.09	-	48.65±0.53
12	Streptomyces cellulosae	DST28	43.59±0.46	-	32.25±0.69	-
13	Streptomyces intermedius	DST29	57.89±0.36	35.48±0.28	-	52.63±0.00
14	Streptomyces flavogriseus	DST30	-	48.49±0.24	-	-
15	***Saccharopolyspora* sp.**	**DST31**	**58.97±0.33**	**51.52±0.68**	**48.39±0.24**	**56.76±0.35**
16	*Streptomyces* sp.	DST35	-	-	23.60±0.29	-
17	*Rhodococus* sp.	DST38	-	54.55±0.5	-	45.95±0.25
18	Streptomyces pactum	DST44	-	-	22.58±0.64	-
19	Streptomyces koyangenesis	DST48	-	-	-	45.94±0.21
20	*Streptomyces* sp.	DST50	-	-	35.48±0.41	-
21	*Rhodococcus* sp.	DST51	54.00±0.85	45.45±0.49	41.94±0.38	-
22	Streptomyces flavogriseus	DST52	48.72±0.49	-	19.35±0.79	32.43±0.59
23	***Streptomyces griseoplanus***	**DST53**	**58.97±0.63**	**58.00±0.33**	**48.50±0.39**	**56.76±0.44**
24	***Streptomyces* sp.**	**DST54**	**58.97±0.98**	**57.58±0.62**	**48.40±1.39**	**56.76±0.44**
25	*Streptomyces* sp.	DST56	-	-	41.94±0.71	-
26	Streptomyces somaliensis	DST58	43.59±0.14	-	-	-
27	Streptomyces cyaneofuscatus	DST59	-	39.39±0.36	22.58±0.39	45.95±0.38
28	*Streptomyces* sp.	DST60	-	39.39±0.61	29.03±0.5	40.54±0.96

Table 1 (Contd.)...

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Sl. No.	Isolate Name	Isolate No.	Antifungal Properties C-T/CX100			
			F. udum	*F. oxysporum*	*F. graminearum*	*F. proliferatum*
29	*Streptomyces* sp.	DST63	59.00±0.12	-	48.39±0.56	59.46±0.99
30	***Streptomyces cyaneofuscatus***	**DST64**	**58.97±0.02**	**39.39±0.35**	**35.48±0.52**	**59.46±0.76**
31	Streptomyces lavendulae	DST65	-	-	54.84±0.28	-
32	Streptomyces olivaceus	DST66	-	-	35.48±0.16	-
33	Streptomyces griseoplanus	DST67	-	-	41.94±0.73	-
34	*Streptomyces* sp.	DST69	-	-	35.50±0.34	43.24±0.73
35	***Streptomyces albidoflavus***	**DST71**	**53.8±0.76**	**45.45±0.44**	**35.48±1.29**	**51.35±0.57**
36	Streptomyces rubiginosohelvolus	DST72	38.46±0.44	-	-	-
37	Streptomyces atratus	DST73	-	40.35±0.81	9.68±0.45	43.24±0.73
38	Streptomyces atroolivaceus	DST74	53.85±0.16	39.40±1.39	41.94±0.06	-
39	Streptomyces koyangenesis	DST75	61.53±0.53	42.50±0.43	51.61±0.56	-
40	***Streptomyces* sp.**	**DST86**	**66.67±0.37**	**39.39±0.61**	**41.93±0.38**	**37.84±0.48**
41	*Saccharopolyspora* sp.	DST89	53.85±0.09	45.45±0.55	35.48±0.62	-
42	Nocardiopsis	DST95	-	-	-	35.14±0.64
43	Streptomyces albidoflavus	DST96	-	37.88±0.25	-	-
44	*Saccharopolyspora* sp.	DST97	-	-	22.60±0.27	-
45	Streptomyces cyaneofuscatus	DST99	-	51.52±0.32	35.48±0.71	-
46	Streptomyces albidoflavus	DST100	-	-	41.94±0.37	-
47	***Streptomyces albidoflavus***	**DST102**	**49.52±0.39**	**51.52±0.25**	**48.45±0.17**	**40.54±0.71**
48	Streptomyces cyaneofuscatus	DST103	48.72±0.19	54.55±0.25	-	45.95±0.95
49	***Streptomyces* sp.**	**DST104**	**66.67±0.84**	**51.52±0.38**	**48.40±0.54**	**64.86±0.44**
50	***Nocardiopsis***	**DST105**	**60.26±0.26**	**51.52±0.29**	**49.50±0.77**	**45.95±0.42**
51	Streptomyces atroolivaceus	DST106	-	-	23.00±0.52	45.95±0.56
52	***Streptomyces* sp.**	**DST116**	**48.71±0.48**	**51.52±0.21**	**29.03±1.06**	**45.95±0.63**
53	Streptomyces griseus	DST118	-	39.39±0.69	-	43.24±0.42
54	***Streptomyces* sp.**	**DST119**	**49.00±0.39**	**45.45±0.52**	**51.61±0.39**	**56.76±0.55**
55	Streptomyces fulvissimus	DST120	50.50±0.48	39.39±0.43	-	51.35±0.57
56	*Streptomyces* sp.	DST142	42.50±0.19	-	-	-
57	*Nocardiopsis* sp.	DST145	28.00±0.08	-	-	-

CONCLUSION

In this study, sixty-eight actinobacteria were isolated from the sediments of three freshwater systems using serial dilution and spread plate technique, where the genus *Streptomyces* was found to be dominant. They were evaluated for their anti-fungal activity against four *Fusarium* plant pathogens collected from microbial type culture collection and found that 83.8% of the isolated organisms showed inhibitory activity against at least one of the tested plant pathogenic fungi and 13 *Streptomyces* isolates were found to inhibit all the tested pathogens which undoubtedly presented the anti-fungal potential of actinobacteria and might be a good candidate as biocontrol agent especially disease caused by *Fusarium* pathogens.

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