Sodium Azide Induced Mutation in Actinomycetes

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ABSTRACT

Objectives: The study was done with an aim to determine the gain and loss of functions among the actinomycetes mutants induced by sodium azide.

Methods: The study was carried out in the laboratory of the Sainik Awasiya Mahavidhayala, Bhaktapur, Nepal from 2016 December to 2017 March. A total of 30 soil samples were collected from Tokha, Bhaktapur area and Godawari area. Actinomycetes were isolated from the soil sample using pour plate technique on selective media; starch casein agar. The isolates were identified by using standard microbiological methods and each isolate was exposed to different concentration of sodium azide to generate mutants. The wild type and mutants were compared in morphology, biochemical reactions and antibiotic susceptibility to test organism to determine the gain and loss of functions.

Results: Among 30 samples processed, 20(67%) actinomycetes were isolated, in which 6 (20%) were identified as the *Streptomyces* spp. A total of 28 mutants were isolated from 6 wild types by exposed at 10ppm, 20ppm, 40ppm, 50ppm, 100ppm concentration of sodium azide. Out of 28 mutants formed, only 10 mutants from sample showed same pigmentation as its wild type while other 18 mutants showed change in their pigmentation. In sugar utilization test, 8 different sugars for 28 mutants each, 56 cases showed Gain of Function (GOF), similarly 44 cases showed Loss of function (LOF). Antibiosis remained unaffected against *Pseudomonas* i.e. no GOF or LOF was seen. Only 2 cases of LOF against *Staphylococcus aureus* were seen while there were no cases of LOF in other pathogens. 3 cases of GOF against *E. coli*, 4 against *S* Typhi and 4 against *S. aureus* were observed.

Conclusion: The potential of mutant actinomycetes has been realized, and hence opens exciting avenues in the field of biotechnology and biomedical research.

Key words: *Streptomyces*, Sodium azide, Wild type, Mutants, GOF, LOF

INTRODUCTION

Actinomycetes are gram positive, filamentous bacteria, with high G+C content (69-78%) in DNA exhibiting highly differentiated developmental cycle (Williams et al. 1989), inhabiting a wide range of habitats. Unlike bacteria, actinomycetes are unique in their morphology with extensive branching substrate and aerial mycelium bearing chain of arthrospores. Of the 22,000 known microbial secondary metabolites, 70% are produced by actinomycetes, and two thirds of them are contributed by the genus *Streptomyces* (Subramani and Aalbersberg 2012). Actinomycetes contain about 40 families and over 170 genera and about 2000 species have been validly described and published (Harwani 2013).

Actinomycetes are numerous and widely distributed in soil, compost etc. and are next to bacteria in abundance. The most common genus of actinomycetes in soil is *Streptomyces* that produces straight chains or coils of spores or conidia. More than one-half of the antibiotics used in human medicine, including aureomycin, chloromycetin, kanamycin, neomycin, streptomycin, and terramycin, come from soil actinomycetes. The smell of freshly turned soil is due to metabolic end products called geosmins that are produced by these organisms and move through soil as unseen volatiles (Dindal 1990; Sylvia et al. 2005). This study will be useful for determination of novel strains of actinomycetes and check the potency by mutated by sodium azide

of antimicrobial agents against gram positive and gram negative pathogens. This novel strains will be application in pharmaceutical industry and cope the challenge of MDR. Actinomycetes are of enormous importance since they possess a capacity to produce and secrete a variety of extracellular hydrolytic enzymes (Saadoun et al. 2007; Tan et al. 2009) about 60% of the new insecticides and herbicides reported in the past 5 year originate from *Streptomyces* (Tanaka and Omura 1993). Actinomycetes produce a variety of antibiotics with diverse chemical structures such as polypetides, B-lactams and peptides in addition to a variety of other secondary metabolites that have antifungal, anti-tumor and immunosuppressive activities (Behal 2000).

MATERIALS AND METHODS

The study was carried out in the laboratory of the Sainik Awasiya Mahavidhayala, Bhaktapur, Nepal from 2016 December to 2017 March. A total of 30 soil samples, 10 samples were collected from Tokha, 10 samples were collected from Bhaktapur area and others 10 were collected from Godawari area.First, the soil slurry was made by suspending 10 g of the collected dry soil in 90 ml distilled water.The pour plate technique was done in which 0.1 ml of sample and SCA broth was poured and incubated at 25^{O}C for 1 week. After growth of actinomycetes, the wild cultures were further streaked in other SCA plates for obtaining pure culture. Obtained pure culture was streaked in SCA incorporated with different concentration of Sodium Azide i.e. 10ppm, 20ppm, 40ppm, 50ppm and 100ppm. After obtaining the growth of actinomycetes in different concentration the mutants were compared with wild type by the help of different biochemical tests, gelatin hydrolysis test and antibiosis.

RESULTS

Out of 30 soil samples collected from different areas, 20 (67%) actinomycetes were isolated. Out of 20 (67%) actinomycetes, 6 (20%) isolates were identified as *Streptomyces* spp. which were further used in this study to generate mutagens using different concentration of sodium azide. A total of 28 mutants were isolated from 6 wild types exposed at 10ppm, 20ppm, 40ppm, 50ppm, 100ppm concentration of sodium azide.

Out of 28 mutants formed only 8 mutants from samples of Godawari showed same colony color (both dorsal and ventral) as its wild type. While other 20 mutants showed change in its colony color.

No changes in gelatin hydrolysis test of wild type and their respective mutants were observed. In sugar utilization test, different sugars i.e. arabinose, fructose, galactose, glucose, lactose, mannitol, maltose and sucrose were used where all the mutants showed color change from blue to either yellow or green indicating sugar utilization except in case of lactose where only one sample (i.e. Bhaktapur 1 mutants grown in concentration 50ppm) showed color change while others remained unchanged.

Among AST test performed against four organisms, *Pseudomonas* was not inhibited by the growth of actinomycetes. In case of *S. aureus* 18% (5 out of 28) developed resistance. 14% (4 out of 28) in case of *S.* Typhi and 11% (i.e. 3 out of 28) in case of *E. coli* developed resestance while rest of mutants were susceptible to those microorganisms.

In case of morphology, only three mutants showed GOF out of 28 mutants only 11%. In sugar utilization test, most mutants seem to metabolise glucose while in galactose and lactose, only one mutant out of 28 showed gain of function. In AST, mutants could not develop GOF. Out of 28 mutants, 2 mutants showed LOF on the basis of pigmentation. In case of glucose and lactose, no any mutants showed loss of function. While in AST, only two mutants failed to act against *S. aureus* while there is no LOF in other mutants.

Table 1: Comparison of AST between wild types and mutants

	Zone of inhibition given by antibiotics from/mm					
Test organism	**B1W**	**B1M10**	**B1M20**	**B1M40**	**B1M50**	
E.coli	6	6	6	6	6	
Pseudomonas	6	6	6	6	6	
S. Typhi	6	16	6	6	6	
S. aureus	16	16	16	16	16	
	B2W	**B2M10**	**B2M20**	**B2M40**	**B2M50**	**B2M100**
E.coli	6	6	6	6	16	6
Pseudomonas	6	6	6	6	6	6
S. Typhi	6	6	16	6	6	6
S. aureus	16	16	6	36	36	36

	Zone of inhibition given by antibiotics from/mm					
Test organism	B1W	B1M10	B1M20	B1M40	B1M50	
	G1W	G1M10	G1M20	G1M40	G1M50	G1M100
E.coli	6	6	6	6	6	6
Pseudomonas	6	6	6	6	6	6
S. Typhi	16	16	16	16	16	16
S. aureus	6	6	6	6	6	6
	G2W	G2M10	G2M20	G2M40	G2M50	G2M100
E.coli	6	6	6	6	26	6
Pseudomonas	6	6	6	6	6	6
S. Typhi	6	6	6	26	6	6
S. aureus	6	16	6	6	6	6
	T1W	T1M10	T1M20	T1M40	T1M50	T1M100
E.coli	6	6	6	6	16	6
Pseudomonas	6	6	6	6	6	6
S. Typhi	6	6	6	6	16	6
S. aureus	26	6	6	6	6	16
	T2W	T2M10	T2M20	T2M40		T2M100
E.coli	6	6	6	6		6
Pseudomonas	6	6	6	6		6
S. Typhi	6	6	6	6		6
S. aureus	6	6	6	6		6

Note: 6mm= diameter of well, 1 and 2 are the different samples where B is bhaktapur, G is Godawari, T is Tokha and 10, 20, 40, 50 and 100 are sodium azide concentration.

DISCUSSION

In this study, out of 30 samples processed 6 isolates were identified as *Streptomyces* spp on the basis of microscopy and sugar fermentation tests. Studies carried out by Iwami et al. (1986); Kavita and Vijayalakshmi (2007); Wijittra et al. (2006) also suggest that *Streptomyces* spp. exist as the major component of actinomycetes population isolated from soil.

In this case, out of 28 actinomycetes isolates were exposed to five different concentrations of sodium azide, i.e.10ppm, 20ppm, 40ppm, 50ppm, 100ppm. Out of which, 26 (92.9%) isolates were tolerant except B1M100 and T1M50 which account for 7.14%. These two mutants were found to be sensitive to sodium azide. However, in study done by Shrestha et al. (2015), out of total 38 actinomycetes isolates, 2 (5.3%) were highly sensitive, 23(60.5%) were moderately sensitive and 13 (34.2%) were tolerant to sodium azide. Out of total isolates two (2/38; 5.3%) were highly sensitive and couldn't grow even on 10 ppm.

On doing antibiosis, these mutants couldn't develop antagonistic activity against *Pseudomonas*. Out of 28 mutants only 12 mutants showed antagonistic activity against pathogens which accounts for 42%. While in case of Kumar et al. (2011) out of 56 strains, only ten strains showed higher antagonistic activity against all the tested human bacterial pathogens which counts for 17%.

All the mutants in our case have shown sugar utilization in arabinose, fructose, galactose, glucose, sucrose, maltose and mannitol except in lactose while in case of Subedi et al. (2015) all mutants had one or more positive mutations (GOF) of fructose, mannitol, arabinose and salicin utilization.

CONCLUSION

From the finding of this study carried out by collecting soil samples from Bhaktapur, Godawari, Tokha and their periphery, it can be concluded that species of *Streptomyces* were the major among actinomycetes present in the soil. Furthermore, mutants obtained by treating those *Streptomyces* with mutagen i.e. Sodium azide at different concentrations viz. 10, 20, 40, 50 and 100 ppm can affect their colony morphology, enzymatic activities, sugar utilization pattern and antibiosis.

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