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Effect of Mutation on Antimicrobial Activity of Actinomycetes from Western Ghat's of India

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Abstract. A total of 56 Actinomycetes strains were isolated from Indian western ghat's soil and their antagonistic activities were tested against six human pathogenic bacteria viz, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Shigella* sp.,*Klebsiella pneumonia* and *Bacillus subtilis*. Out of 56 strains isolated, only ten strains (WGA2, WGA7, WGA12, WGA15, WGA19, WGA21, WG29, WGA31, WGA37, and WGA49) showed high antibacterial activity against all the tested human bacterial pathogens. Among these, only 5 effective antagonistic strains (WGA7, WGA15, WGA21, WGA37 and WGA49) were selected for mutation study by using physical (UV radiation) and chemical (NTG) mutation methods. After the mutations, antibacterial activity of the strains viz WGA15, WGA49 (UV treated) WGA37 (NTG treated) was increased, but the WGA37 UV treated strain showed a decreased antibacterial activity. All the five strains possess LL-DAP and all the strains contained glycine in their cell wall. Presence of LL-diaminopimelicacid indicates that the cell wall having chemo type–1. So the cell wall property of the genes was identified the *Streptomycetes*.

Keywords: Indian western ghat's soil, Actinomycetes, Human Pathogenic bacteria, Antibacterial activity, Mutation.

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1 Introduction

The soil bacteria are responsible in producing various metabolites. *Streptomycetes* in particular have given us a number of useful compounds of various chemical structures, so called secondary metabolites, including antibiotics [19]. As a result of the increasing prevalence of antibiotic-resistant pathogens and the pharmacological limitations of antibiotics, there is an exigency for new antimicrobial substances. The results of extensive screening have been the discovery of about 4000 antibiotic substances from bacteria and fungi, many of which have found applications in medicine; most of them are produced by *Streptomyces* [9]. AA-1, AA-5, AA-10, AA-13, and AA-17 *Streptomycetes* shows a moderate antagonistic activity against human pathogens. These strains were mutated by using standard Physical (UV) and Chemical (NTG) mutation methods. After mutations, antibacterial activity of the strains via AA-13 (UV treated) and AA-10 (NTG treated) was increased. But the strain AA-17 (UV and NTG treated) showed a decreasing trend in the antibacterial activity against almost all the tested human pathogens [11]. Removal of *scb A* gene by UV mutation enhanced the antibiotic production in *Streptomyces lividans* [1]. In searches for bioactive antibiotics, *Streptomyces* strains have been isolated from various types of soils, including rice paddy, lake mud and water, deciduous forest, tropical forest, wasteland, and cave soils [8,10,14,15,18,20]. Therefore, the present study was undertaken to isolate the antagonistic Actinomycetes from the soil sample collected from Western Ghats and check their antibiotics production efficiency by mutation methods.

2 Metrials and Methods

The soil samples were collected from different places of Indian Western Ghats, the samples was brought to the laboratory in aseptic condition.*Streptomyces* were isolated by spread plate technique on Starch-casein agar and actinomycetes isolated agar. Dry colonies of *Streptomyces* were selected and isolated. Thus isolated pure cultures of the *Streptomyces* were transferred to Yeast extract Malt extract agar slants and preserved at 4 $\pm 2^{\circ}$ C. The anti-microbial activity was determined by streaking on Starch-casein agar and incubated at 28° C for 7 days.

2.1 Screening of Actinomycetes isolates for antimicrobial activities

Primary screening

A modified cross-streak method was used for primary screening of actinomycetes isolates. Modified nutrient agar plants were streaked at the center. The plates were incubated at 28 $\pm 2^{\circ}$ C for 5 days. After 5 days of incubation, the overnight bacterial strains, such as *E-coli, Staphylococcus aureus, Salmonella typhi, Shigella* sp., *Klebsiella pneumonia* and that were streaked. The plates were then incubated at 28 $\pm 2^{\circ}$ C and the incubation distance was measured after 24–48 hours. A control plate was also maintained without inoculating the actinomycetes, to assess the normal growth of the bacteria.

Secondary screening

The selected isolates were further tested in the secondary screening by shake flask studies to conform their antimicrobial activity. The spore suspension of the selected isolates were inoculated into ISP2 medium and kept in a shaker at 250rpm at 30° C

for 96 hours. After incubation, the culture broth was separated from the mycelium by centrifugation at 5000 rpm and the supernatant was used for testing the antimicrobial efficiency.

The tested bacteria *E-coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Shigella* sp., *Klebsiella pneumoniae* and *Bacillus subtilis* were placed on the surface of the modified nutrient agar and incubated for 24 hours at room temperature. After proper observation, good growths of tested organisms on the Petri plates were observed. Then the what man number 1 filter paper disc impregnated with 0.1ml of the specific supernatant were placed in the Petri plate with pathogens, and incubated at room temperature for 24 hours. The diameter of the inhibition zone for each strain was recorded.

2.2 Effect of mutation on antibacterial activity

The strains which showed efficient antibacterial activity were further selected to study the effect of mutation on their antibiotic production.

Physical mutation

The selected strains were cultured in the tubes containing 9 ml ISP 2 broth. The tubes were inoculated with one loopful of the strain and incubated in a rotatory shaker at 250 rpm at 30° C for 96 hours. After incubation, the tubes were removed from the shaker and 3ml of each culture was exposed to UV irradiation at a distance of 30 cm for 180 seconds. Then, 1 ml of the exposed cultures were transferred to 9 ml of glycerol–starch broth medium and the tubes were incubated for 96 hours on a rotatory shaker at 250 rpm at 30° C. After incubation, the tubes were removed from the shaker and the broth was centrifuged at 2000 rpm for 20 min and the supernatant was used to examine the post mutation effect on the strains for their antibacterial activity.

Chemical mutation

The selected strains were cultured in the tubes containing 9 ml ISP 2 broth. The tubes were inoculated with one loopful growth of the strain and incubated in a rotatory shaker at 250 rpm at 30° C for 96 hours. After incubation, the culture broth was centrifuged at 3000 rpm for 10 minutes and the pellets were collected. The pellets were suspended with 2 ml of tris buffer (pH 7.2) in the test tubes and 50μg/ml of NTG (N-methyl-N-nitro-N-nitro-N-nitrosoguanidine) was added to the test tubes. Then the test tubes were incubated at 30° C for 30 minutes. After incubation, 1ml of NTG treated culture transferred in to 9 ml of GS fermentation medium (Glucose—10g, Soya bean meal—10g, Nacl—10g, $CaCO_3$—1g, Distilled water—1 Lit, pH 7.5) and the tubes with culture for antibiotic production were incubated for 96 hours on a rotatory shaker at 250 rpm at 30° C. After incubation, the tubes were removed from the shaker and the broth was centrifuged at 2000 rpm for 20 minutes. Finally the supernatant was used to examine the post mutational effect on the strains for antibacterial activity.

The bacteria such as *E-coli*, *Staphylococcus aureus Salmonella typhi*, *Shigella* sp., *Klebsiella* and *pneumonia* were placed on the surface of the modified nutrient agar and incubated for 24 hours at room temperature. After observation a good growth of tested organisms on the Petri plates were observed and what man number 1 filter paper disc impregnated with 0.1ml of the specific supernatant were placed in the Petri plate and incubated at room temperature for 24 hours. The diameter of the inhibition zone for each strain was recorded.

Taxonomical Investigation

The gene level identification of the five Actinomycetes strains viz WGA7, WGA15, WGA21, WGA37 and WGA49 were made using cell wall composition analysis.

Results

Totally 56 Actinomycetes strains were isolated from Indian western ghat's soil samples and the isolated strains were tested for their antagonistic activity against potential human pathogens viz.*E-coli, Staphylococcus aureus, Salmonella typhi, Shigella* sp.,*Klebsiella pneumonia* and *Bacillus subtilis*. Out of 56 strains, only ten strains (WGA2, WGA7, WGA12, WGA15, WGA19, WGA21, WG29, WGA31, WGA37, and WGA49) showed higher antagonistic activity against all the tested human bacterial pathogens (Table 1). Among the ten strains, only 5 effective antagonistic strains (WGA7, WGA15, WGA21, WGA37 and WGA49) were selected for mutation study by using physical (UV radiation) and chemical (NTG) mutation methods.

Effect of Mutation on Antibacterial Activity

The strains viz WGA7, WGA15, WGA21, WGA37 and WGA49 which have showed antibacterial activity against tested human bacterial pathogen were treated with physical and chemical mutagens to study the effect of mutation on their antibacterial activity. Mutated strains were checked for their antibacterial activity against human bacterial pathogens. *E.coli, Staphylococcus aureus, Salmonella typhi, Shigella sp, Klebsiella pneumonia* and *Bacillus subtilis*.

Strains exposed to UV radiation showed variation in zone of inhibition against all the six tested bacterial pathogen non UV radiation exposed strains. As compared to the control and UV mutated strain WGA7 showed an increased trend in the inhibition zone against *E-coli* ($+2$ mm) *Staphylococcus aureus* ($+2$ mm) *Klebsiella pneumonia* ($+1$ mm) and *Bacillus subtilis* ($+1$ mm). The decreased inhibition zone was observed against *Salmonella typhi* (-2 mm). There was no change against *Shigella* sp. (Figure 1). Mutated strain WGA15 has showed an increase in the inhibition zone

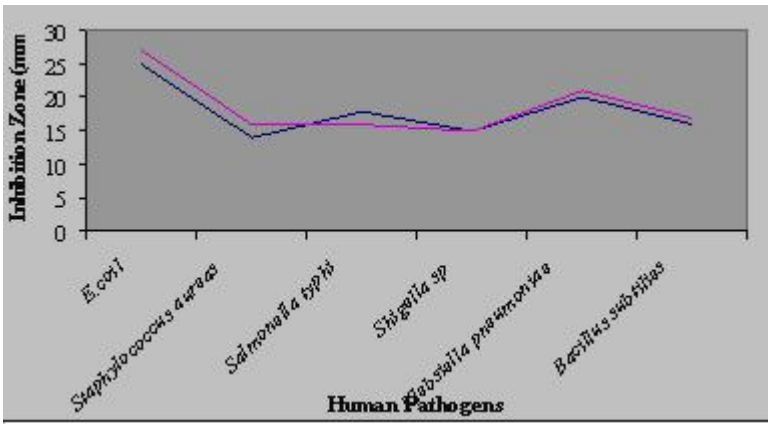

Figure 1: Variation of inhibition zone (in mm) of UV mutated strains WGA 7 and non-mutated strains WGA 7.

against *E-coli* (+4 mm) *Staphylococcus aureus* (+2 mm) *Klebsiella pneumonia* (+6 mm),*Bacillus subtilis* (+4 mm) *Salmonella typhi* (+8 mm) and *Shigella* sp. (+1 mm) (Figure 2).

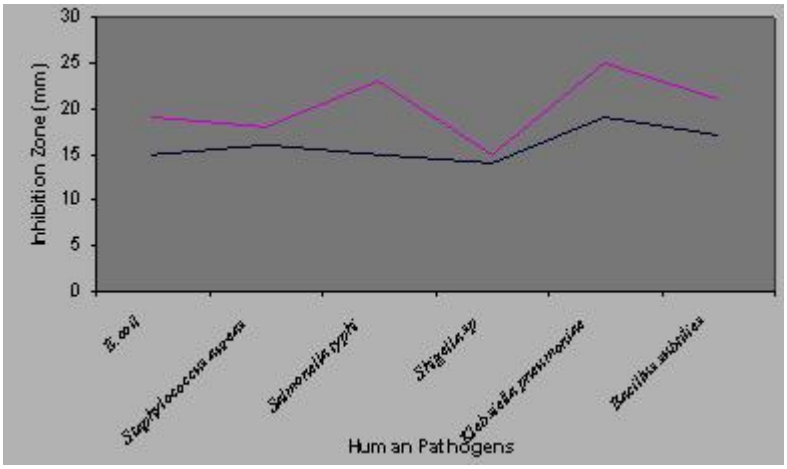

Figure 2: Variation of inhibition zone (in mm) of UV mutated strains WGA 15 and non-mutated strains WGA 15.

Mutated strain WGA21 has showed an increased trend in the inhibition zone against

Table 1: Antibacterial activity of Actinomycetes (WGA–Western Ghats Actinomycetes).

S.No	Strains	E.coli	Staphylococcus aureus	Salmonella typhi	Shigella sp	Klebsiella pneumonia	Bacillus subtilis
1	WGA2	11	19	7	10	9	–
2	WGA7	25	14	18	15	20	16
3	WGA12	8	19	4	–	–	12
4	WGA15	16	15	16	14	19	17
5	WGA19	12	9	–	4	–	17
6	WGA21	23	15	15	10	17	10
7	WGA29	7	14	6	–	9	11
8	WGA31	14	21	5	–	–	9
9	WGA37	16	15	19	17	16	14
10	WGA49	14	29	11	20	15	22

Staphylococcus aureus (+4 mm) *Klebsiella pneumonia* (+8 mm), *Bacillus subtilis* (+6 mm)*Shigella* sp. (+2 mm), where as decreased of inhibition zone was noticed against *E-coli* (−1 mm) and *Salmonella typhi* (−1 mm) (Figure 3).

Mutated strain WGA37 has showed an increased inhibition zone against *E-coli* (+10 mm), *Salmonella typhi* (+8 mm) but decreased inhibition zone was observed against (−2 mm), *Klebsiella pneumonia* (−1 mm). There was no change was observed against *Staphylococcus aureus* and *Shigella* sp. (Figure 4).

Mutated strain WGA 49 showed an increased inhibition zone against *E-coli* (+1 mm), *Staphylococcus aureus* (+12 mm), *Klebsiella pneumonia* (+4 mm), *Bacillus sub-*

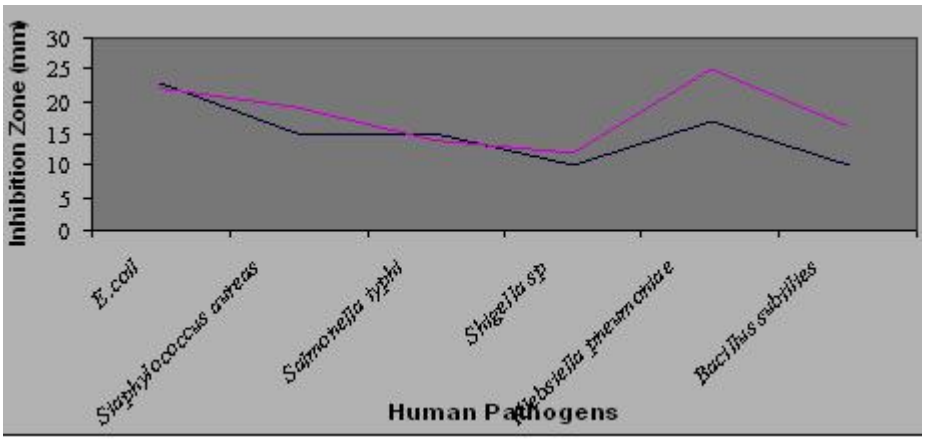

Figure 3: Variation of inhibition zone (in mm) of UV mutated strains WGA 21 and non-mutated strains WGA 21.

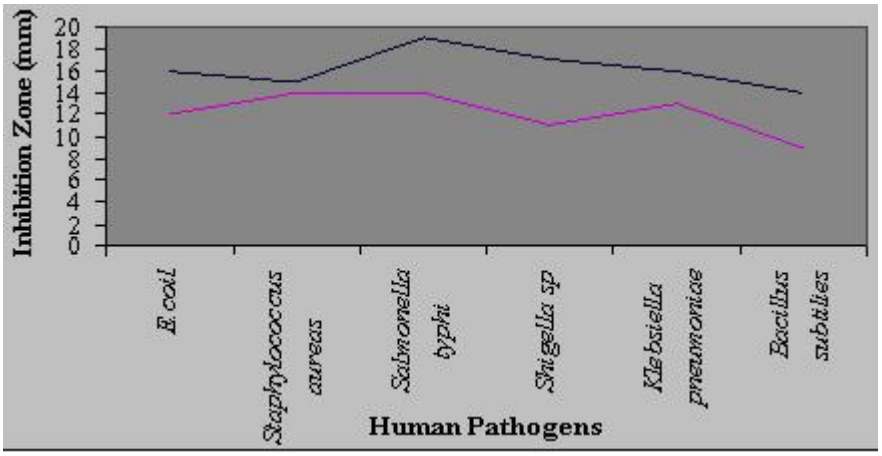

Figure 4: Variation of inhibition zone (in mm) of UV mutated strains WGA 37 and non-mutated strains WGA 37.

tilis (+2 mm), *Salmonella typhi* (+2 mm) and *Shigella* sp. (+1 mm) (Figure 5).

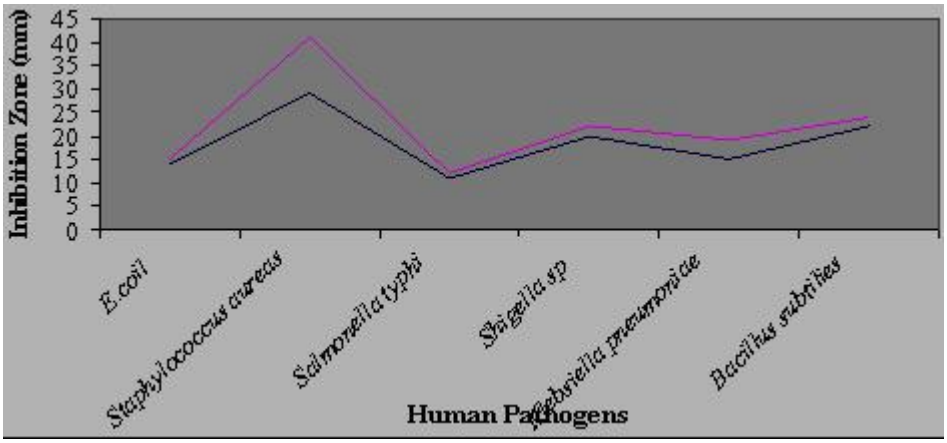

Figure 5: Variation of inhibition zone (in mm) of UV mutated strains WGA 49 and non-mutated strains WGA 49.

The chemical mutated Strains showed variation in the inhibition zone against all the six tested bacterial pathogens than the non-mutated strains. As compared to the control and chemical mutated strains WGA7 showed an increase in the inhibition zone against *E-coli* (+1 mm), *Staphylococcus aureus* (−1 mm), *Klebsiella pneumonia* (−3 mm),*Bacillus subtilis* (−4 mm),*Salmonella typhi* (+8 mm) and *Shigella* sp. (+6 mm) (Figure 6).

The increased inhibition zone was reported in mutated strain WGA 15 against *E-coli* (+4 mm), *Staphylococcus aureus* (no change), *Klebsiella pneumonia* (+2 mm), *Bacillus subtilis* (−4 mm), *Salmonella typhi* (no change) and *Shigella* sp. (+4 mm) (Figure 7).

Mutated strain WGA21 showed an increase in the inhibition zone against *E-coli* (−8 mm), *Staphylococcus aureus* (−4 mm), *Klebsiella pneumonia* (−3 mm), *Bacillus subtilis* (−2 mm), *Salmonella typhi* (−6 mm) and *Shigella* sp. (+2 mm) (Figure 8).

Mutated strain WGA37 showed an increase in the inhibition zone against *E-coli*

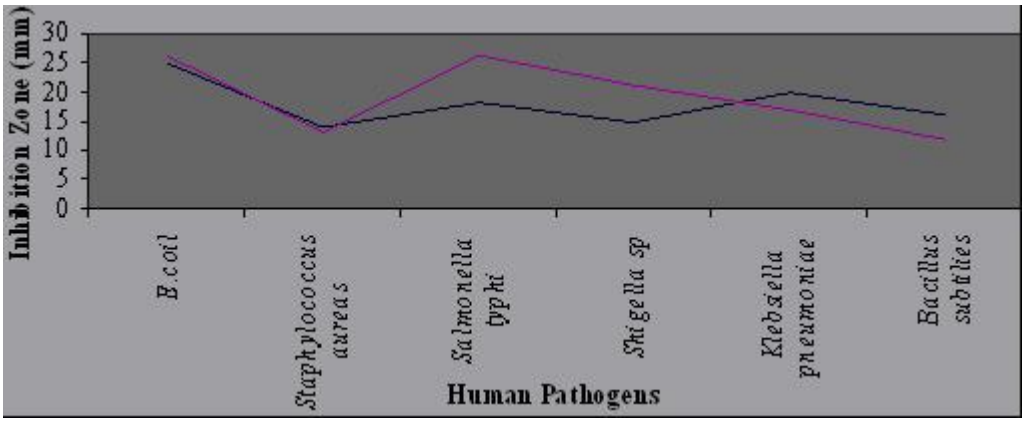

Figure 6: Variation of inhibition zone (in mm) of NTG mutated strains WGA 7 and non-mutated strains WGA 7.

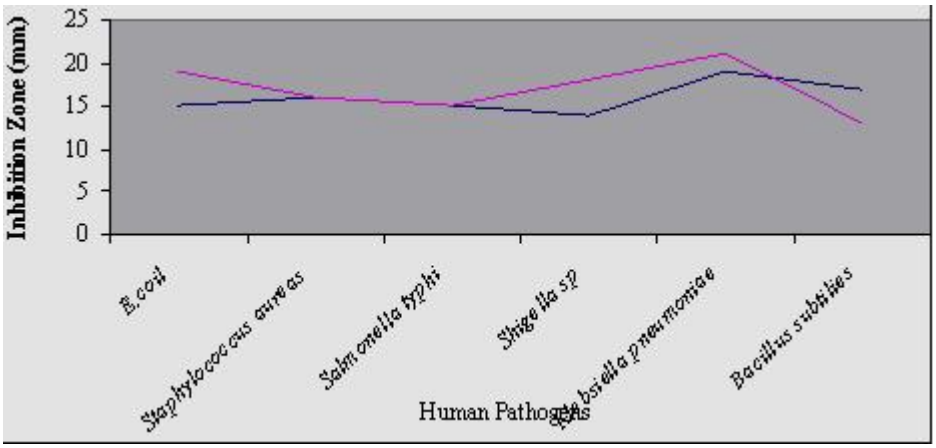

Figure 7: Variation of inhibition zone (in mm) of NTG mutated strains WGA 15 and non-mutated strains WGA 15.

(+14 mm), *Staphylococcus aureus* (+5 mm), *Salmonella typhi* (+4 mm), (+6 mm), *Klebsiella pneumonia* (+5 mm) and *Shigella* sp. (+8 mm), (Figure 9).

Mutated strain WGA 49 showed an increase in the inhibition zone against *E-coli* (+2 mm), *Staphylococcus aureus* (+6 mm), *Klebsiella pneumonia* (no change), (−2

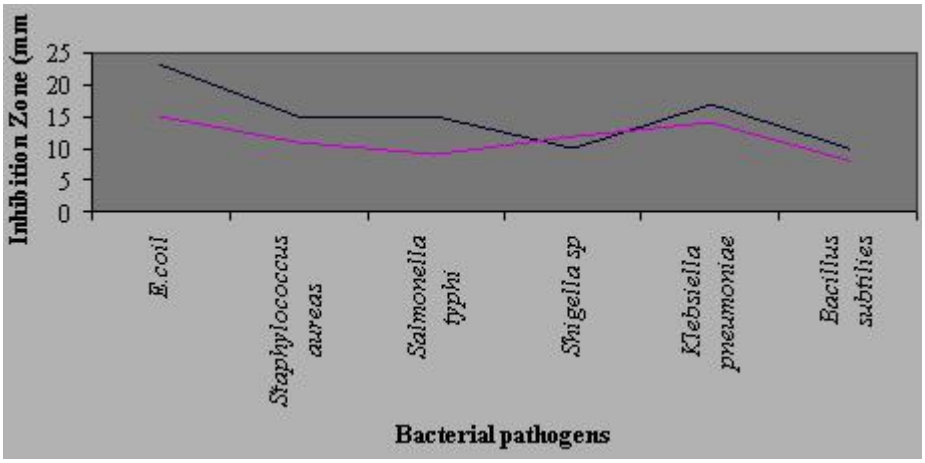

Figure 8: Variation of inhibition zone (in mm) of NTG mutated strains WGA 21 and non-mutated strains WGA 21.

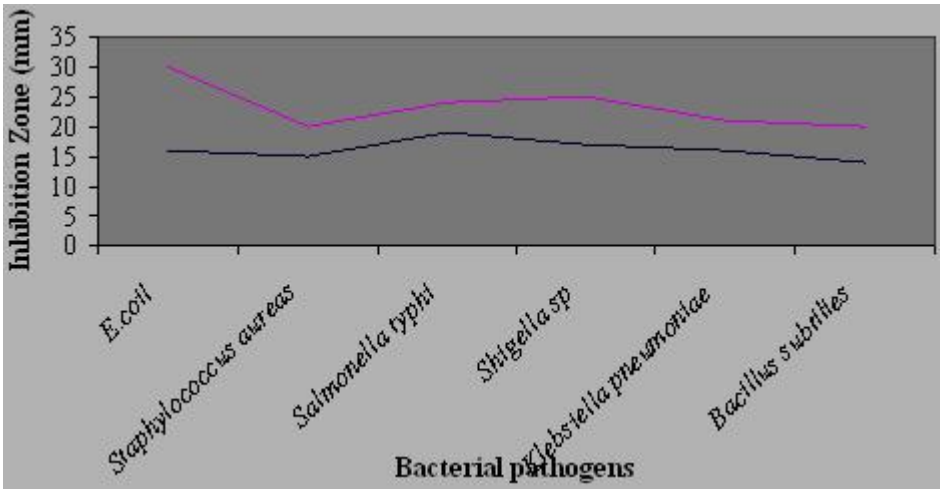

Figure 9: Variation of inhibition zone (in mm) of NTG mutated strains WGA 37 and non-mutated strains WGA 37.

mm), Salmonella*typhi* (no change) and *Shigella* sp. (−4 mm) (Figure 10).

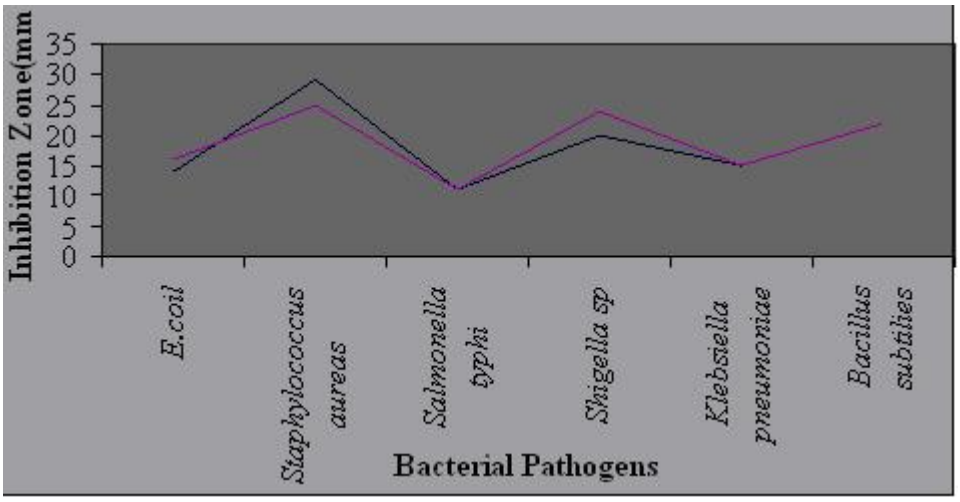

Figure 10: Variation of inhibition zone (in mm) of NTG mutated strains WGA 49 and non-mutated strains WGA 49.

3 Taxonomical investigation

All the five antagonistic strains were found to possess LL-DAP and all of them contained glycine in their cell wall, which indicates the cell wall chemo type 1, i.e., the wall property of the genus *Streptomyces*.

4 Discussion

Several species of *Streptomyces* from different soil and water samples are a virtually unlimited source of natural secondary metabolites, many kinds of which are used as pharmaceutical and agrochemical products [2, 6, 13] and they have a wide variety of chemical structures, including tetracyclines, macrolides, quinocyclines and meroparamycin.

These antibiotics show antibacterial activity against Gram positive and Gram negative bacteria [5, 16, 17].

Ellaiah et al., [4] have reported that the 156% fold increases in lipase yield of *Aspergillus niger* by UV mutagenic treatment where as the present investigation, the lipase yield of UV mutant BTUV3 was 164% higher than the parent strains (BTUV12) and wild strains (BTS-24). Caob and Zhanga [3] have reported an increase in lipase production of 3.25 fold by using *Pseudomonas* mutant with UV and NTG.

Philips [12] reported that UV-mutated actinomycetes increase antibiotics production than non mutated actinomycetes. He also reported that the *Penicillium chrysogenum* after mutation produced antibiotics 10,000 times higher than the non mutated strains.

In the present study, after mutation, the strains WGA15, WGA49 (UV treated) WGA37 (NTG treated) increased the antibacterial activity against all the tested human bacterial pathogens, due to the mutation partial activity gene of these strains which are responsible for the production of antibiotics could have been activated. But the strains UV treated showed a decreased antibacterial activity against almost all the tested human bacterial pathogens. This can be attributed to fact that the mutation of the active gene of this strain, responsible for the production of antibiotics might have been partially inactivated. This could be indicated that bacterial species is more resistant to the antimicrobial substances produced by the actinomycetes.

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