ANTIBIOTIC EXTRACT OF STREPTOMYCETES IN PATHOGEN BACTERIA

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Abstract

Streptomycetes usually inhabit soil and are important decomposers. They also produce more than half of the world's antibiotics, and are consequently invaluable in the medical field. The isolation of Streptomycetes has done for testing the antibiotic effect. In this study are used two kinds of soil from the locality of village Zhur, South of Kosova. Five extracts have been isolated. From the obtained samples the substance has been extracted, and it was tested in order to define the scale of inhibition for the development of some microbial cultures. Escherichia coli, Staphylococcus aureus and Pseudomonas have been chosen as testing cultures. The extraction of substances with microbial properties has been done by means of Augustine et al's method while the test of the obtained extract has been done according to the gel diffusion method. The inhibition zones have been established as well. Thus we may suppose that those results will encourage further researches in this field in the other localities of Kosova.

Key words: Streptomycetes, extraction, Escherichia coli, Staphylococcus, Pseudomonas.

1. Introduction

Streptomycetes is the largest genus of Actinobacteria and the type genus of the family Streptomycetaceae. Over 500 species of Streptomycetes bacteria have been described. As with the other Actinobacteria, streptomycetes are gram-positive, and have genomes with high GC-content. Found predominantly in soil and decaying vegetation, most streptomycetes produce spores, and are noted for their distinct earthy odor which results from production of a volatile metabolite, geosmin. Streptomycetes are characterized by a complex secondary metabolism [10]. They produce over two-thirds of the clinically useful antibiotics of natural origin (e.g., neomycin, chloramphenicol). The now uncommonly-used streptomycin takes its name directly from Streptomyces. Based on the fact that the most of the antibiotics which are using now days are losing its effect against pathogen microorganism and because of the great resistance of those microorganisms against antibiotics we were encouraged for further studies to find new substances with microbial properties which will be use for testing of antibiotic effects against pathogen bacteria [7, 8, 10]. Our study denotes that extract of streptomycetes isolated from soil of South Kosova has been more bactericide against Enterobacteriaceae family [5, 7, 10].

2. Materials and methods

Soil samples has used like material in our study. The samples have been taken in two kinds of land and two different depths in the region of South Kosova. The first land was paddocking another one was tillage. One depth was 15 cm meanwhile another one was 30 cm. In the laboratory it has stayed two weeks in room temperature to kill all the vegetative cells and to survive just sporogenous cell [7, 8, and 10]. The sample used for study was on dilution 10^3, 10^4 and 10^5. For isolation of colonies has used the pour-plate technique meanwhile the pure colonies are yield using the slant agar. Culture media used was Czapek's medium. Incubation of the soil samples was done in room temperature for 14 days. There are selected 5 pure colonies of streptomycetes which then are sub culturing in nutrient Slant Agar. Using the Slant agar the subcultures are incubated in room temperature for eight days. This step is done to increase the biomass of desirable colonies [4].

The methods used for extraction of streptomycetes' extract were according to Augustine et al 2004 [2, 3]. We have eroded the biomass, put it in 2 ml eppendorf tubes and add 0.5 ml sterilized H_2O.

After one hour the tubes are centrifuged in 10.000 rpm for 20 min to separate the biomass from liquid (to obtain the pellet and supernatant). The supernatant is removed in new tubes and than on it is added petroleum ether like extract in same volume 1: 1. They are homogenized for 45 min to extract and bind the antibiotic substance. Then it has centrifuged in 5000 rpm for 15 min. In the end of this the extract was ready for testing the antibiotic effect. The discs used were 12.7 mm. The colonies of pathogen bacteria used for testing were *Escherichia coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The method used for testing of antibiotic activity was Agar gel diffusion meanwhile the method used to determine the inhibition zone was according to Kirby-Bauer [4].

3. Results and Discussion

The results have been read after 24 and 48 hours. The obtained results are presented in the table 1.

Table 1. Isolates and Inhibition zones at E. coli, S. aureus and Psudomonas

Isolates		*1*	*2*	*3*	*4*	**5**
Inhibition Zone[a] (mm)	*E. coli*	14.8	16.1	13.7	17.4	16.9
	S. aureus	16.5	13.7	0 (12.7)	13.9	0 (12.7)
	Pseudomonas	0 (12.7)[b]	0 (12.7)	0 (12.7)	13.1	0 (12.7)

[a] Inhibition zone in millimeter
[b] 12.7 millimeter indicates the dimensions of the disc meanwhile the value 0 (12.7) mm indicates that the inhibition zone was zero.

Figure1. Some pictures with Inhibition zones

The active metabolites produced by Streptomycetes' culture exhibited various degrees of activities against pathogen bacteria. Based on the results all extracted has shown antibiotic effect against E. coli which was more sensitive [5, 7, 9, 10]. In the other side Pseudomonas exhibited resistance on four isolates even if the rest of isolates shown small antibiotic activity. The case mention above shows variability between two families of bacteria Gram negative (Gr^-). [10]. Meanwhile the Staphylococcus aureus as bacteria Gram positive (Gr^+) exhibited sensitivity on three isolates and resistance on two isolates [7, 8, 9, 10]. Samples from depth 15 cm have shown greater antibiotic effect. This result is because of the microbial activity which declined with increasing soil depth. The next fact is that compared to the samples from 30 cm the 15 depth, samples has higher organic C, total N, bicarbonate-extractable P, Ca+, Cu, Fe and Mn and supported higher populations of bacteria, fungi, actinomycetes, *Pseudomonas* spp., *Bacillus* spp., cellulolytic bacteria, cellulolytic fungi, nitrifying bacteria etc [10].

In statistical way the results for samples from 15 cm depth are: 75% of samples have shown antibiotic effect meanwhile 25% didn't show. From the percentage of samples that have shown antibiotic effect, more than 90% shown antibiotic effect on E. coli and the rest of samples on *S. aureus*.

The results from samples of depth 30 cm are: 35% shown antibiotic effects while 65% didn't show. From the percentage with antibiotic effect 70% shown antibiotic effect on Pseudomonas, 25% on E.coli and 5% on S. aureus.

4. Conclusions

Referring obtained results the following conclusions can be drawn:

- In general most of the isolates have shown antibiotic effect.
- Samples from depth 15 cm have shown greater antibiotic effect.
- E.Coli has shown to be more sensitive in those isolates.
- Pseudomonas was more resistant than other bacteria.
- Isolate nr 4 was the isolate which shown greater antibiotic effect.

5. References

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