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Antagonistic Activity of Actinomyces Isolated from Azerbaijan's Soils

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Abstract: Nowadays the idea of existence of acidophil actinomyces as a microbial community in soil does not raise doubt and they are one of the most important subjects of research. They have proved their own existence by growing in low-pH medium and formation of aerial mycelium and spores. In addition not only neutrophil but also acidophil actinomyces are considered to be the producers of antibacterial agencies and repressors of bacteria and fungi growth. They are famous for their second metabolic product (antibiotic). It has been proved that acidophil strains differ from neutrophil in their antagonistic property in relation to bacteria and fungi. This research compares the number and antagonistic property of neutrophil and acidophil actinomyces, isolated from Azerbaijan's soil.

Keywords: neutrophil, acidophil, actinomyces, antimicrobial activity.

1 Introduction

Among bacteria, the actinomycetes are important producers of bioactive compounds [1] and constitute a potential as biocontrol agents [2, 3, 4]. Actinomycetes represent a high proportion of soil microbial biomass, and appear to be of importance among the microbial flora of the rhizosphere [5]. Associations between actinomycetes and plant organs can be deleterious or beneficial for the host. While some actinomycetes secrete herbicidal compounds [6] or cause plant diseases [7], others can symbiotically fix atmospheric nitrogen [8] or protect plants against fungal infections (Cao et al. 2005). They protect to various degrees several different plants from soil-borne fungal pathogens [9, 10].

The possible existence of acidophilic and alkaliphilic, psychrophilic and thermophilic, halophilic and haloalkaliphilic, and xerophilic actinomycetes does not raise doubt in specialists [11, 12, 13, 14, 15). The terms *acidophilic* and *acidotolerant* actinomycetes appeared in the literature at the turn of the last century [16,17]. The works presenting them have shaken the existed opinion that all actinomycetes are neutrophilic species [18].Great number of acidophilic actinomycetes were representatives of the *Streptomyces* genus [19, 20, 21, 22]. Probably, this fact is related to their significant predominance in almost all the soils [11].

The major emphasis in the systematics of sporoactinomycetes has been on establishing relationships between neutrophilic strains which, in culture, grow between pH 5.0 and 9.0 with an optimum close to neutrality [14, 23]. which share key chemotaxonomic and morphological properties with neutrophilicstreptomycetes, grow in the range from about 3.5 to 6.5, with optimum rates at pH 4.5 to 5.5.

Streptomycetes have established themselves as the most potent group of microorganisms capable of forming awide variety of antibiotics [24]. On the contrary, antifungal antibiotics effective against fungal disorders are relatively few mainly because of their solubility and toxicity problems [25, 26]. The ever increasing incidence of fungal infections in plants, animals and human being has directed the attention towards the search for actinomycetes producing novel antifungal antibiotics having a broad spectrum of activity and lesser toxicity [27, 28].

Indeed, different Streptomyces species produce about 75% of commercially and medically useful antibiotics [29]. According to [30], RhizovitR from Streptomyces rimosus is used in the control of a wide range of fungi such as Pythium spp., Phytophthora spp., Rhizoctoniasolani, Alternariabrassicola, and Botrytis sp. Liu et al. [31] also reported that S. rimosus showed a high antagonism activity against *Fusarium solani*, *F. oxysporium* f sp. *cucumarinum*, *Verticilliumdahliae*, *R. solani*, *Fulviafulva*, *Botrytis cinearia*, *A.* alternat, Sclerotiniasclerotiorum and

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Bipolarismaydis.

2 Materials and Methods

For screening and isolating of actinomycetes was used culture media Gause1 which is composed of: Starch (2%), K_2HPO_4 (0.05%), $MgSO_4$ (0.05%), NaCl (0.05%), KNO3 (0.1%), $FeSO_4$ (0.001%), agar(2%). Actinomycetes colonies with different morphologies were selected and transferred to Gause1 slants for further studies.

2.1 Isolation actinomycetes from soil

Soil samples were collected from different regions of Azerbaycan: 4 Soil samples were analyzed in the first day - it is necessary to keep them in the cold place (in the fridge) during 2 days [13]. Samples (10g) of air-dried soil were mixed with sterile distilled water (100 ml) and then portion (1 ml) of soil suspensions (diluted 10^{-1}) were transferred to 9 ml of sterile distilled water and subsequently diluted to 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} and cultivated on the solid medium, were incubated at 30 °C for up to 20 days [32].

2.2 Characterization of the isolates

The selected *Actinomycetes*via antibacterial tests were characterized through morphological and biochemical tests. Morphological methods consisted of macroscopic and microscopic methods. The mycelium structure, color and arrangement of spores on the mycelium, and other properties such as the color of colonies, soil pH and etc. were observed. The observed structures were compared with Bergey`s Manual of Determinative Bacteriology, Ninth edition (2000) and the organisms were identified. Moreover several biochemical tests such as Casein hydrolysis, starch hydrolysis and urea hydrolysis, acid production from various sugars, NaCl resistance and temperature tolerance were done.

2.3 Isolating in pure culture

To study physio-biochemical properties and bacterial development cycle and also determine their type, it is necessary to work with pure culture of microorganisms. For isolating to pure culture of aerobic microorganisms, it was seeded on Petri dishes from accumulated culture. Its drop was carried on the culture medium and rubbed by sterile spatula on the surface of medium, after this, by the same spatula were rubbed the other 3-4 dishes and incubated. Grown isolated colonies were taken out by loop to the test tube with solid sterile medium or in liquid medium [33].

3 Result and Discussion

3.1 Antimicrobial activity of isolated strains

About 160 strains of actinomyces (129 neutrophil and 39 acidophil strains) were isolated from different types of Azerbaijan's soils(Figure 1).All isolated acidophil and neutrophil actinomyces belonged to Streptomyces genus. For subsequent analyze, 15 neutrophil and 15 acidophil actinomycetes were selected and their antimicrobial activity was studied by disk-diffusion method. The isolated cultures were screened against some microorganisms – *Staphylococcus aureus*, *Bacillus mezentericus*, *Escherichia coli*, *Aspergillusniger* and their antagonistic property was valued by appearance and measuring lysis zones around agar blocks of actinomyces. The result is featured in table1 and figure 2.The analyses of antagonistic effect on test culture revealed considerable differences between acidophil and neutrophil soil actinomycestowards bacteria and fungi.

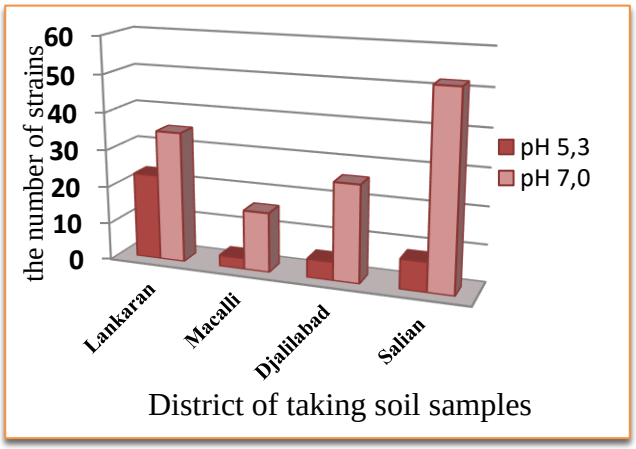

Fig.1: Distribution of actinomycetes in a neutral (pH 7, 0) and acidic medium (pH 5.3).

According to presented information on diagram (figure 1), the number of neutrophil strains in all soil samples were significantly more than acidophil strains. While the largest numbersof neutrophil actinomyces were isolated from Salian, the largest numbers of acidophil stains were isolated from Lankaran. In contrast the least numbers of acidophil and neutrophil actinomyces were isolated from Masalli.

Table 1: The percentage of active acidophil and neutrophil actinomyce against test-cultures

Test –culture	pH 5.3	pH 7
Staphylococcus aureus	20%	26%
Bacillus mezentericus	40%	73%
Escherichia coli	53%	26%
Aspergillusniger	33%	13%

Among selected strains (neutrophil and acidophil) most of acidophil strains suppress the growth of *Escherichia coli* (53%) and they were less active against *Staphylococcus aureus* (20%). A great percentage of neutrophil strains (73%) show antagonistic property against*Bacillus mezentericus.* microscopic fungi *Aspergillusniger*, was more resistant against neutrophils, which just 13% of them prohibit its growth. While acidophil strains grown on the acidic medium proved to inhibit fungal growth [16] and gram negative *E.coli*, those grown on the neutral medium

suppress the growth of gram positive *St.aureus* and *B.mezentericus.*

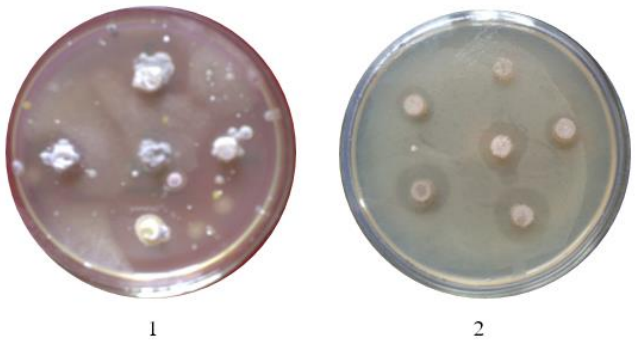

Fig.2: antagonistic property of acidophil (1) actinomyces against *E.coli* and neutrophil (2) actinimyces against *B.mezentericus.*

4 Conclusions

To conclude the number of neutrophil actinomyces were considerably more than acidophils, although acidophils were more active against *Escherichia coli* and *Aspergillus niger*. It proves their antagonistic character in relation to fungi and bacteria.

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