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ANTAGONISTIC ACTIVITY OF BACTERIAL SPECIES ISOLATED FROM SOIL AGAINST FUNGI

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ABSTRACT

Isolation of different bacterial colonies from soil sample was carried out. All the isolated bacterial colonies were screened for their antagonistic activity against the pathogenic fungi. Antagonistic activity test was performed against pathogenic fungi (*Aspergillus niger, A. flavus, Penicillium notatum* and *P. chrysogenum*) and the best Zone of inhibition were occurs in *Aspergillus flavus* and *Penicillium chrysogenum* both (11 mm). Different test were performed to characterize the microbes. Microbes have anti-microbial properties that characterization of various antimicrobial substances as organics acids (lactic acid and formic acids), diacetyl, and hydrogen peroxide alone or in combination. Strain 1 (*Escherichia coli)* was more effective to inhibit the growth of *Aspergillus niger, A. flavus, Penicillium notatum* and *P. chrysogenum*.

KEYWORDS: Antagonistic activity, *Escherichia coli*, Microbes, Fungi, Soil screening, Zone of inhibition.

INTRODUCTION

The present research paper focused on the isolation of bacterial strain that having antibiotics producing capability from soil samples collected from the campus of Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan, (India). The antibiotics are broadly distributed in the nature, where they play main role in managing the microbial population of soil, water, compost, and sewage.[1] Antibiotics are low molecular-weight molecules that produced secondary metabolites, mainly by microorganisms that found in the soil[2]. Microbes which produce antibiotics in large amount that can survive for longer time than the other microbes which produced antibiotics in less amount. These antibiotics which produce by the microbes

have been very beneficial and helpful for the treatment of many human and animal diseases caused by microbes like bacteria, fungi and protozoa.The bacteria present in soil can be rods, (bacilli), cocci (spherical), and spirilla (spirals) of which, bacillus are more in numbers than others bacteria. They are one of the major groups of soil bacteria and are widely distributed.[2] *E. coli* is a Gram negative straight rod-shaped (bacilli) bacterium arranged singly or in pairs that is commonly found in the lower intestine of warm-blooded organisms.[3,4,5] These are the Some important examples of antibiotics used in medical treatments are bacitracin, Gramycidin S, polymyxin, and tyrotricidin[6] produced by different bacillus species. Microbes have anti-microbial properties that characterization of various antimicrobial substances as organics acids (lactic acid and formic acids), diacetyl, and hydrogen peroxide alone or in combination.[7] And other antimicrobial substances with antagonistic properties include biocides, probiotics, and sterilants.

MATERIAL AND METHODS

Sample Collection

Soil sample was collected from the campus of the Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan The soil sample was sieved to extract in very small size of soil particles which were used to serially dilute for isolating microorganisms that are possible to antibiotic producers.

Isolation of Microorganisms from Soil Samples

Processing of Soil Samples

Soil samples were processed by dissolving 1gm of soil sample in 10ml of distilled water to make soil suspension in test tube. The test tube was vortex for 1 to 2 minutes to separate soil, stones and debris. Supernatant was transferred in to another test tubes and ten folds serial dilution were prepared. Then spread 1ml of supernatant from each dilution on nutrient agar plates and put in incubator for incubation at 37ºC overnight. After 24-48 hrs zone of inhibition were observed in all agar plates.

Isolation and Screening of Bacteria

Selected colonies were plated onto nutrient agar slant for isolation of bacteria. After an overnight incubation, separated bacterial colonies were selected based on their colony characters and streaked on new agar slants according to previous process.[8] The slants were incubate at 37ºC for 24 hrs.

Characterization of Pure Antibiotics Producing Bacterial Isolates

Gram-staining was used for pure bacterial isolates selected for characterization. Next biochemical tests, which including the Catalase test, Simmons citrate test, and some selective and differential media- MacConkey agar, EMB agar media test were performed for the identification of pure bacterial isolates. The characteristics of growth on NA for the pure bacterial isolates that include the forms, colours, of the bacterial colonies, and the microscopic characters were cell shapes and arrangements under light compound microscope were observed and noted. The bacterial isolates identification was based on Bergey's Manual of Determinative Bacteriology.[9]

Gram staining: A smear of isolated strain was prepared on a clean glass slide and the smear was air-dry and then heat-fixed. The heat-fixed smear was flooded with crystal violet and leave for one min. after one min., it was washed with water and flooded with mordant Gram's iodine. The smear was decolorized with 95% ethyl alcohol and washed with water and then counter-stained with safranin for 45 sec. After washing with water, the smear was dried with tissue paper and examined under 40X and oil immersion (100 X).[10]

Catalase test: Catalase test used for presence of catalase which converts hydrogen peroxide to water and oxygen. This test was carried out by putting a drop of hydrogen peroxide on a clean glass slide and add the colony of the microorganism. And allowed to contact to hydrogen peroxide. Presence of bubbles indicates positive reaction and absence of bubble indicates negative reaction.[11]

Citrate utilization test: This test determines That, bacterium can use sodium citrate as a sole source of carbon. This is carried out by inoculating the test organism in culture tube containing Simon's citrate medium and this was incubated for 24 hours to 72 hours. The medium was converting into deep blue colour after incubation indicated a positive results.[12]

After identification, sample culture was subjected to antagonistic activity test, for determining its antagonistic nature against four fungal species (*Penicillium notatum, P. chrysogenum, Aspergillus niger, and A. flavus*).

RESULT AND DISCUSSION

The natural soil microorganisms have maximum ability to produce novel antibiotics. In this study, a total of 3 bacterial isolates from the one depth was successfully isolated via

preliminary screening and selection from soil. According to Sewell, bacteria can be presents mostly first three inches in the soil surface When the depth reached six feet the bacteria decreasing in numbers. Among these isolates, only 3 bacterial isolates were selected for the secondary screening. All of these three strains-1, 2, 3 were showed some biochemical tests and Morphological characterstics. Along with these three Strains, the Strain-1*(E.coli)* which is isolated from the Sample-1 was more effective as compare to the remaining two Strains. Due to the high effectiveness of Strain-1 (*E. coli)*, it was selected to test its antagonistic activity against four fungi *(Aspergillus niger, A. flavus, Penicillium notatum, P. chrysogenum).*

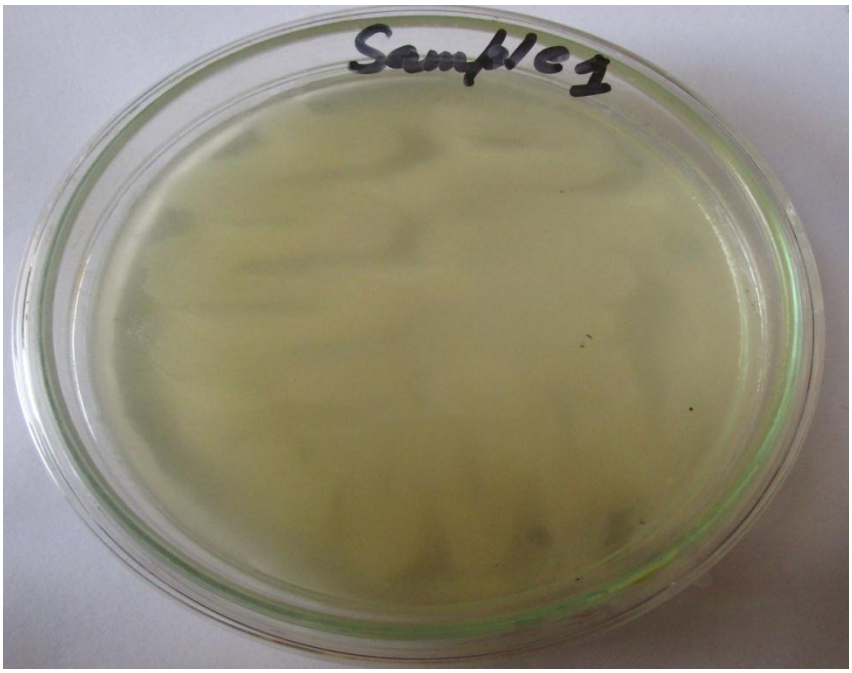

Figure 1: Pure sample of Strain-1 (*E. coli*).

Staining results: Strain-1 was gram negative bacteria that retain pink colour.

Catalase test result: Strain-1 was producing bubbles after adding H_2O_2 that means result was Catalase positive.

Citrate test result: Strain-1 was producing royal blue colour in the Simon's citrate medium so it was citrate positive test.

Mac Conkey agar, EMB agar media results: In the Mac Conkey agar media Strain-1 produce pink colonies. And in EMB agar media E.coli produce pinkish red colony.

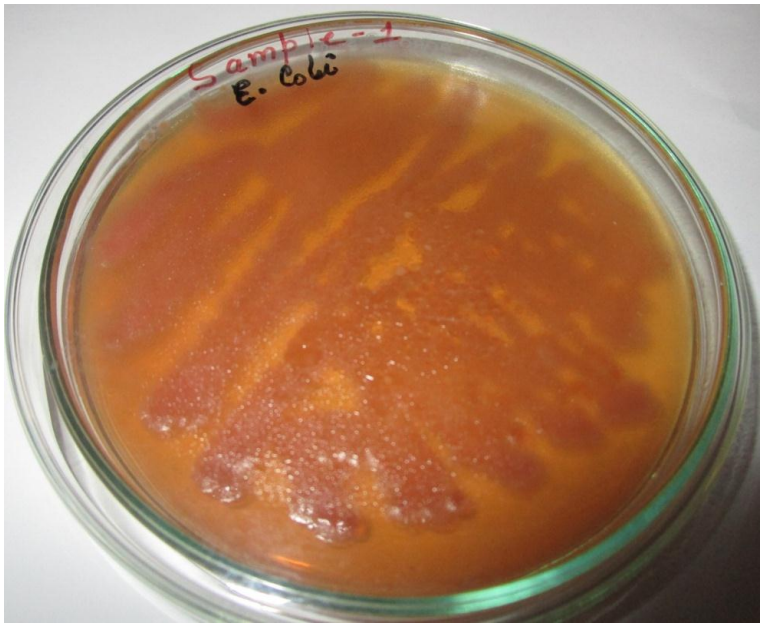

Figure 2: Strain-1 (*E. coli*) colony grow on MacConkey agar media.

Table 1: Morphological and Biochemical tests for the identification of Strain-1.

S. No.	Test	Morphological and Biochemical Test
1.	Grams staining	**-ve**
2.	Shape	Bacilli
3.	Spore formation	-
4.	Citrate utilization	+
5.	Catalase	+

Antagonistic Activity of Strain-1 (*E. coli*) against Fungi

For all the above study, Strain-1 was chosen as a model organism. Strain-1 was isolated from soil sample. Also Antagonistic activity test was done to check the effect of fungal spp. on the growth of Strain-1 cells and comparative study of fungal species was done. In soil isolate, the maximum inhibition was observed in *Aspergillus flavus* and *Penicillium chrysogenum* (11 mm) followed by *P. notatum* (9 mm) and while the *A. niger* exhibited least effect (8 mm). In soil isolates, the maximum inhibition was observed in *Aspergillus flavus* and *Penicillium chrysogenum* both (11 mm) and least in *Aspergillus niger* (8 mm).

Table 2: Antagonistic activity of Strain-1 (*E. coli*) against Fungi.

S. no.	Antagonistic Microorganism	Fungi Used	Diameter of Zone of Inhibition (Iz)
1.	Strain-1 *(E. coli)*	*Aspergillus niger*	8 mm
2.	Strain-1 *(E. coli)*	*Penicillium chrysogenum*	11mm
3.	Strain-1 *(E. coli)*	*Aspergillus flavus*	11mm
4.	Strain-1 *(E. coli)*	*Penicillium notatum*	9mm

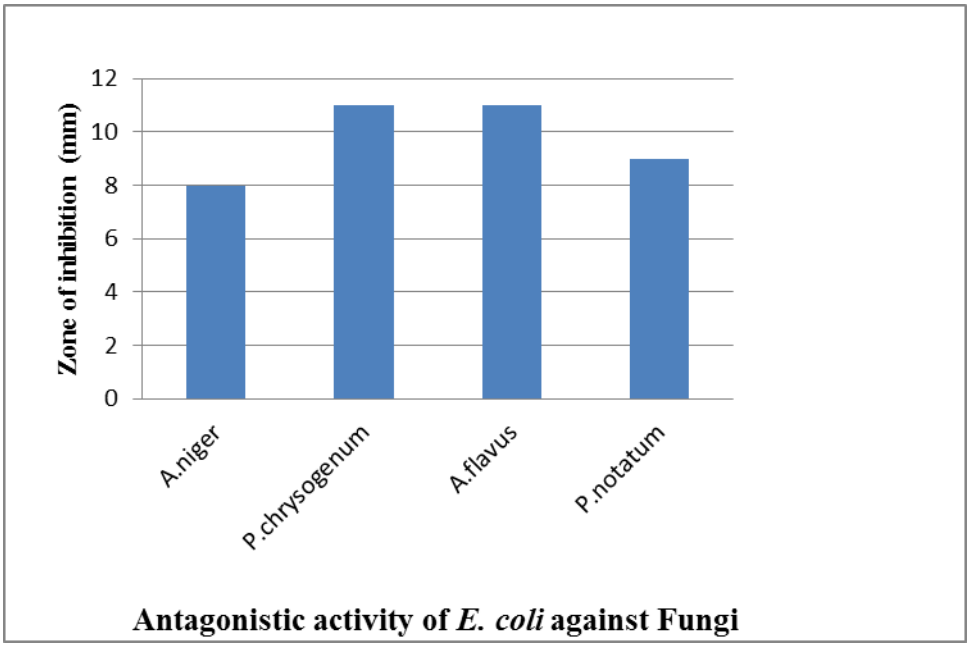

Figure 3: Zone of inhibition of Strain-1 (*E. coli*) against fungi represent by the graph.

Antibiotic production is a quality of several types of soil microbes and may represent a survival mechanism where organisms can eliminate competition and colonize a niche.[13] According to Shinde, the *E. coli* ATCC 8739 with 49.77% antagonistic activity show against ten fungal pathogens.[14] Some of the soil bacteria showed antifungal properties because they produce chitins which may be a part of a lytic system that capable bacteria for living on hyphae as real growth substrate.[15] *E. coli* was produced chitinase enzyme because of this chitinase *E. coli* dissolve the cell wall of the pathogenic fungus *F. oxysporum* which cause the wilt disease in *Cucumber*.[16] Along with the three strains of the bacteria, *E. coli* was found to be more effective as compare to remaining two bacterial strains. It was observed that besides antagonistic properties of Strain-1 (*E. coli*) also exhibited the capabilities to suppress the growth of test fungal species (*Penicillium notatum*, *P. chrysogenum*, *Aspergillus niger* and *A. flavus*) used in this study. This showed that Strain-1 (*E. coli*) might have great ability to become an effective anti-fungal agent which is clearly shown in the figure 3. So the Strain-1 is an anti-bacterial as well as anti-fungal agent. Strain-1 could be applied in agricultural industries, husbandry and medical fields. Even, more species of test fungi were needed in this testing in order to show the anti-fungal properties exhibited by Strain-1.

CONCLUSION

In all the studies, the inhibition zone was measured which around the discs. It is concluded that *E. coli* isolated during the work of this study from the soil samples possessed

antimicrobial activity against pathogenic fungi. *E. coli* can be used to prepare medicine and due to technology antibiotics can be easily produced for human beings.

REFERENCES

1. Zinsser H. Antimicrobial agents, 9th chapter. In: Zinsser H, Joklik WK, Willett HP, Amos DB, Wilfert C (ed.), Zinsser Microbiology, Prentice Hall International, UK, 1988: 128-160.
2. Brock TD and Madigan MT. Biology of Microorganisms. 6th edn. USA; Prentice-Hall International Inc, 1991. ISBN: 0130838179.
3. Ahmed S and Cowden J. (An outbreak of E. coli O157 in central Scotland. 3rd International Conference and Workshop on Shiga Toxin (verotoxin) Producing E. Coli Infections), 1997; 22.
4. Nataro JP and Kaper JB. (Diarrheagenic *Escherichia coli*). Clinical Micro Reviews, 1998; 11(1): 142-201.
5. Stephan R, Schumacher S, Corti S, Krause G, Danuser J, and Beutin L. (Prevalence and Characteristics of Shiga Toxin-Producing Escherichia coli in Swiss Raw Milk Cheeses Collected at ProducerLevel). J Dairy Sci., 2008; 91(7): 2561-5.
6. Drablos F Nicholson DG, Ronning ME. (Study of zinc coordination in bacitracin A). Biochim. Biophys. Acta, 1999; 1431(1999): 433-442.
7. Piard JC, Desmazeaud M. (Inhibiting factors produced by lactic acid bacteria. 2. Bacteriocins and other antibacterial substances). Le Lait, INRA Editions, 1992; 72(2): 113-142.
8. Iqbal MN, Anjum AA, Ali MA, Hussain F, Ali S, Muhammad A, Irfan M, Ahmad A, Irfan M and Shabbir A. (Assessment of microbial load of un-pasteurized fruit juices and in vitro antibacterial potential of honey against bacterial isolates). Open Microbiol. J, 2015; 9: 26-32.
9. Holt JG, Krieg NR, Sneath PHA, Stanley JT, Williams ST. Bergey's manual of determinative bacteriology 9th ed. (Baltimore, Williams & Wilkins Company); 1994; 71.
10. Williams ST, Locci R, Beswick A, Kurtboke DI, Kuznetsov VD, Lemonnior FJ, and Long PF. (Detection and identification of novel *actinomycetes*). Res Microbiol, 1993; 144(8): 653-656.
11. Chester B. (Semiquantitative catalase test as an aid in identification of oxidative and non saccharolytic gram-negative bacteria) Journal of clinical microbiology, 1979; 10(4): 525-528.

12. Abdulkadir M, and Waliyu S. (Screening and isolation of the soil bacteria for ability to produce antibiotics). European Journal of Applied Sciences, 2012; 4(5): 211-21.
13. Jensen MJ, Wright DN. Chemotherapeutic agents. Microbiology for the health sciences. New York: Prentice Hall, 1997.
14. Shinde VN. Biocontrol of fungal diseases. Lulu publication, 2015; 123.
15. De Boer W, Paulien JA, Gunnewiek K, Lafeber P, Janse JD, Spit BE and Woldendorp JW. (Anti-fungal properties of chitinolytic dune soil bacteria). Soil Biol. Biochem, 1998; 30(2): 193–203.
16. Soohee C, Dal KS. (*Escherichia coli* can produce recombinant chitinase in the soil to control the pathogenesis by *Fusarium oxysporum* without colonization). J. Microbiol. Biotechnol, 2007; 17(3): 474–480.