http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences, 2 (1): 214-216, 1999 Research Article

An *in vitro* Antibacterial Activity of Different Effective Microorganism Cultures Against Pathogenic Species

Sajjad-ur-Rahman, M. Siddique, Tahir Hussain*, Sarfraz Hussain** and M. Ansar*
Faculty of Veterinary Science, *Faculty of Agriculture, **Faculty of Agricultural Engineering,
University of Agriculture, Faisalabad-38040, Pakistan

Abstract

Five different effective microorganisms (EM) were studied for their antimicrobial activity against various pathogenic bacteria *in vitro*. Type EM_3, EM_4 and EM_5 showed good antibacterial activity against animal pathogens including *Escherichia coli*, *Salmonella* spp., *Pasteurella* spp. and *Staphylococcus* spp. There was an inverse correlation between the antibacterial and the higher dilution of the culture. All biologically active EM_5, may be used efficiently at the highest dilution of 10^4, while the EM_2 and EM_3 proved ineffective against the pathogenic organisms under study.

Introduction

The use of antibiotics as therapeutic agent has been intensively practiced in various sectors of life. There are many reported side effects of antibiotics even at the recommended dose level. Due to the introduction of effective microorganisms (EM) culture by Higa (1991), it became possible to control pathogenic microbes effectively without any hazard of side effects. The EM has been formulated into various culture groups of organisms pooled together on the basis of their biological characters. These include, EM_1, EM_2, EM_3, EM_4 and EM_5. The biological activity of every EM culture is well established and is still applicable under the special set of circumstances.

All categories of EM's have got their unique ability to minimize the risk factors which may contribute towards the onset of pathological problems in plants and animals. Hussein *et al*. (1992) further illustrated that these EM's are rich in their enzyme activity and may be adopted to the soil under all environmental conditions.

In the present study, all the five modified EM's were trailed to detect the *in-vitro* antibacterial activity against specific pathogenic species of bacteria isolated from various diseased animals. Qualitative as well as quantitative analysis were also conducted to establish the role of each EM's towards the control of pathogenic organisms.

Materials and Methods

All the five types of EM cultures detailed in Table 1 were procured from the Nature Farming Research and Development Foundation (NFRDF), Department of Soil Science, University of Agriculture, Faisalabad. Each EM culture was collected in the sterilized test tubes and stored at refrigeration temperature.

Isolation of Pathogenic Organisms: Four pathogenic spp. of bacteria were isolated from the post-mortem cases of birds and Lab. animals at the Department of Veterinary Microbiology, University of Agriculture, Faisalabad. Three species of bacteria belonged to gram's negative while on was gram's positive group. All species were found pathogenic to laboratory animals and birds (Buxton and Fraser, 1977) as detailed in Table 2.

Purification and suspension Preparation: All the pathogenic spp. were purified and their cultural characteristics were detected and found to be well matching with the morphological and cultural characteristics as detailed by Merchant and Packer (1983) and Krieg and Halt (1984).

All spp. were grown separately on the specific media. The concentration of organisms were determined as describe by Cruickshank *et al*. (1974), through Breed's smear method and the final concentration of the organism culture was maintained on nutrient broth and stored in refrigerator (Table 3).

Preparation of Different Dilutions of EM's: All the five type of EM's as detailed in Table 1 were diluted at the 10-for serial dilution up to 10^4 in the sterilized phosphate buffer saline (pH = 7.2) and stored at temperature below 10°C the refrigerator.

Antibacterial Activity of EM's: All diluted and undilutid EM's were soaked into the sterilized filter paper discs (7 m diameter) and applied on to the surface of nutrient agar plate which were already spreaded with 0.2 ml of specific pathogenic species of the microorganisms separately.

All the plates were incubated at 37°C for 24 hours and the results were recorded. The zones of inhibition we measured cross wise with the help of scale and the average zone of inhibition was calculated. The activity of individual EMs and at its various dilutions were determined on the basis of highest zone of inhibition as described by Cruickshank *et al*. (1974).

Results and Discussion

Present study revealed scientific background regarding the

Table 1: Biological characteristics of five different EM cultures showing various working dilutions in the study

EM's culture	Biological Activity	Concentration/ml
EM_1	Nitrogen Fixing Photosynthetic, lactic acid producing bacteria	10^1 to 10^4
EM_2	Photosynthetic, Streptomyces.	10^1 to 10^4
EM_3	Photosynthetic with yeast & Actinomycetes	10^1 to 10^4
EM_4	Lactobacillus, Streptococcus & Photosynthetic	10^1 to 10^4
EM_5	Photosynth., Streptomyces & Yeast	10^1 to 10^4

Table 2: Characteristics of various pathogenic microorganisms isolated from animal sources

Microorganism	Morphological characters	Groups staining character	Pathogenic character
E. coli	cocci-bacilli	G -ve	caused death in mice/rabbit
Sallmonella spp.	cocci-bacilli	G -ve	caused death in poultry
Pasteurella spp.	rod shaped	G -ve	caused death in rabbits
Staphylococcus spp.	cocci/bunches	G +ve	caused death in mice

Table 3: Purification and suspension count of pathogenic microorganisms used in the study

Microorganism	Purification on specific media	Culture suspension count/per 100 ml
E. coli	MacConky's Agar	1.6×10^5
Sallmonella spp.	MacConky's Agar	1.2×10^5
Pasteurella spp.	Blood Agar	2.0×10^5
Staphylococcus spp.	Staph-110, medium	10.8×10^5

Table 4: Average zone of inhibition in (mm) at various dilutions of EMs against various pathogenic bacteria

EMS	Dilution	*E. coli*	*Salmonella* spp.	*Pasteurella* spp.	*Staphylococcus* spp.
EM_3	10^1	12.0	14.2	12.8	12.5
	10^2	10.0	10.0	10.8	10.2
	10^3	8.5	10.5	9.8	10.5
	10^4	8.0	8.6	8.8	9.5
EM_4	10^1	12.5	13.5	12.8	12.8
	10^2	10.8	12.2	11.4	11.6
	10^3	9.2	10.6	10.8	10.5
	10^4	8.5	9.5	9.4	8.8
EM_5	10^1	10.6	10.5	11.2	11.4
	10^2	9.8	9.5	9.6	8.8
	10^3	8.5	8.4	8.2	8.2
	10^4	7.8	8.0	8.0	7.5

Average zone of inhibition were found less than 7.5 mm or negative through out the four dilutions of EM_2 and EM_3

antibacterial activity of different EM's against various pathogenic organisms. It was found that the EM_1, EM_4 and EM_5 showed much better antibacterial activity against all Pathogens under study while the EM_2 and EM_3 were found to be completely ineffective against the pathogens.

The EM_1 showed the highest zone of inhibition against *Sallmonella* spp, which was 14.2 mm followed by 12.8 mm against *Pasteurella* spp. 12.5 mm against Staph. spp and 2.0 mm against *E. coli*. As EM, consists of nitrogen fixing as well as lactic acid producing and photosynthetic category of bacterial microflora. Present results indicated predominant activity of lactic acid producing bacteria, which are responsible for extra-ordinary low pH of EM_1 culture.

The four experimental dilutions of EM_2 and EM_3 showed the zones of inhibition consistently below 7.5 mm in diameter, therefore the antibacterial activity was considered as insignificant. Parr *et al*. (1991) described the availability of predominantly photosynthetic yeast and streptomyces categories of microorganisms in EM_2 and EM_3, which express growth promoting activity in plants.

In EM_4, the highest zone of inhibition was 13.5 mm against *Salmonella* spp. followed by 12.8 mm against *Pasteurella* spp. and *Staphylococcus* spp. and 12.5 mm against *E. coli* (Table 4). As EM_4 contains the *Lactobacillus* spp. yeast and streptomyces its activity is well determined towards suppressing the activity of harmful insects and pathogen microflora (Hussein *et al*., 1992).

The EM_5 comprises of active fermentation products in the form of vinegar and alcohols etc. which suppress pathogens and insects (Hussein *et al*., 1992). In the present study, the

EM_5 showed the maximum activity against Staph spp., followed by *Pasteurella*, *E. coli* and *Salmonella*, with the inhibition zone of 11.4, 11.2, 10.6 and 10.5 mm, respectively, as detailed in Table 4.

Overall results of EM cultures which are biologically active against the pathogenic microorganisms suggest that most of the diarrhoea cases caused by *E. coli*, typhoid cases due to *Salmonella* spp., skin wound infections due to *Staphylococcus* spp. and respiratory infections due to *Pasteurella* spp. may also be treated successfully with EM cultures in human as well as in animal species. Such biologically active EMs may be helpful in reducing the incidence of such zoonotic problems in the country and their use will be quite safe and free from any side effects. Furthermore, present study strongly justify that the EM cultures may be utilized and introduced in the country on a wide scale for the improvement of human and animal health. As all the EM's are imported and there are every chances of low adaptability of the cultures to the local environment, it is suggested that every effort, should be made to construct the EM'S on the similar pattern that include the combination of our native bacterial isolates which may prove to be much more active and potent against various other diseases of plants, animals and human population in the country.

Aknowledgements

We feel much pleasure to extend our gratitude to the team of Scientists working in the Nature farming Research and Development Centre, University of Agriculture, Faisalabad for providing every facility in the completion of above research.

References

Buxton, A. and G. Fraser, 1977. Animal Microbiology: Immunology, Bacteriology, Mycology, Diseases of Fish and Laboratory Methods. Vol. 1, 1st Edn., J.B. Lippincott Co., Canada, ISBN: 9780632006908, Pages: 830.

Cruickshank, R., J.P. Duguid and B.P. Marmion, 1974. Medical Microbiology: The Practice of Medical Microbiology. Vol. 2, 12th Edn., Churchill Livingstone, USA., ISBN: 9780443012037, Pages: 587.

Higa, T., 1991. Effective microorganisms: A biotechnology for mankind. Proceedings of the 1st International Conference on Kyusei Nature Farming, October 17-21, 1991, Khon Kaen, Thailand, pp: 8-14.

Hussein, T., G. Jilani and T. Higa, 1992. Principles of nature farming with effective microorganisms (Part I). International Nature Farming Research Centre, Faisalabad, Pakistan.

Krieg, N.K. and J.G. Halt, 1984. Bergey's Munnal of Systematic Bacteriology. Vol. 1, Will and Wilkins, London.

Merchant, A.L. and R.A. Packer, 1983. Veterinary Bacteriology and Virology. 7th Edn., CBS Publishers and Distributors, Delhi, Pages: 752.

Parr, J.F., S.B. Hornick and C.E. Whitman, 1991. First International Conference on Kyusei Nature Farming. U.S. Department of Agriculture, Washington DC., Pages: 175.