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Antibacterial activity Monascus purpureus (red pigment) isolated from rice malt

Ajay kumar*, Usha verma, Heena sharma

Department of Microbiology, Indian Institute of Education Hari Devi GhannahttiShimla-II-171011, Himachal Pradesh
(Affiliated from Himachal Pradesh University Shimla-II)

E-mail : jkmr156@gmail.com, heeni.sharma31@gmail.com Contact No. : +8801756707535

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Abstract

Biopigments are such microbial product which has greater impact over chemically synthetic pigments. Certain organism like *Monascus purpureus* has the ability to produce monascidin A compound which are red coloured pigments, having antimicrobial properties. The aim of this work was to produce biopigments from organic waste to bring down the production cost and to reduce the organic load from the environment and use the product as antibacterial agent. Rice malt was used as substrate for the production of red pigment using *Monascus purpureus*. Pigment production was achieved at 30 °C on 10 days of incubation and at pH of 6. The pigments were obtained effectively using ethanol as a solvent and check out the antibacterial activity of pigments against pathogenic and nonpathogenic bacteria i.e. *Escherichia coli, Bacillus subtilis, Bacillus megaterium and Pseudomonas aeruginosa.*

INTRODUCTION

Natural pigments are obtained from plants and micro organisms which are produced as secondary metabolite. Secondary metabolites are organic compounds and are not directly involved in the normal growth, development or reproduction of an organism, benefit to human kind in various aspects. The dye stuff industry is suffering from the increases in costs of feedstock and energy for dye synthesis, and they are under increasing pressure to minimize the damage to the environment [1-4]

Biopigments are such microbial product which has greater impact over chemically synthetic pigments. The pedagogical value of bacterial pigments is highlighted by the wide range of concepts and methods in chemistry, biology, and art. The use of additives in food plays a significant role to enhance its quality [5]. However, color and flavors are often sensitive to heat, oxygen, light and acid and thus changed or lost during processing and storage. Natural colorants and flavors mainly derived from plants and chemosynthetic compounds are used by the food industries to replenish and sometimes raise the genuine stock [6,7].

The natural products made by bacteria have laid the foundation for engineering these molecules and for developing cost-effective ways to manufacture them. Whereas our primary interest in these molecules is due to their antibiotic properties, many of these natural products have distinct colors. *Monascus* pigments, which are produced by various species of *Monascus*, have been used as a natural colorant and as traditional food [6,7]. *Monascus purpureus* is a species of mold that is purplish red in colour. It is also known by the names angkak rice mold, corn silage mold and maize silage mold. Solid-state fermentation of *Monascus* on rice has a long tradition, since first century A.D. For centuries angkak has been consumed extensively in Asia as a natural food coloring of fish, Chinese cheese, red wine and sausages [8]. These are also used in meat processing industry in Western countries [1].

Monascus pigments have been used in food industries and have the potential for therapeutic use [4]. The antimicrobial effect of Monascus culture, due of monascidin A, confirmed by scientific investigations, was proved against some bacterial and fungal strains. Monascus sp. produce a complex mixture of three categories of pigments, viz. orange, red, and yellow, each with two components of polypeptide origin. These secondary metabolites are having a common azaphilone skeleton [2-3]. The initial moisture content, temperature and incubation time plays a greater role in the growth of Monascus as well as in the production of pigment. Pigment production was achieved at 30 oC on 10 days of incubation and at pH of 6. The pigments were obtained effectively using ethanol as a solvent and check out the antibacterial activity of pigments against pathogenic and nonpathogenic bacteria i.e. *Escherichia coli, Bacillus subtilis, Bacillus megaterium, and Pseudomonas aeruginosa.*

The extract of Monascus purpureus was prepared in 95% alcoholic extract of Monascus purpureus (red pigment) as 20%, 40%, 60% & 80% in sterilized water. The antibacterial screening of Monascus purpureus (red pigment) carried out by four replicates to avoid the technical error. Distilled water and alcohol were also used as a control.

The test organisms were isolated, compared with the standard ATCC culture of IMTECH, Chandigarh and maintained at 40°C by sub culturing.

MATERIALS AND METHODS

Collection of Culture:

A culture of *Monascus purpureus* (MTCC 410) was obtained from the Institute of Microbial Technology, Chandigarh (IMTECH, Chandigarh, India) It was maintained on potato dextrose agar medium (Hi-Media, Mumbai, India); preserved at 4°C and sub-cultured once in every three weeks.

Production of pigment from Monascus purpureus:

Inoculum of Monascus purpureus was prepared in 100ml potato dextrose broth. The broths was sterilized at 15 psi for 20 minutes and the culture of Monascus purpureus inoculate in

sterilized broth and incubate at 30°C for 6-8 days for growth of pigments.

Pigment production on Substrate:

Rice malt was chosen as a substrate. It was collected from the household's material.10ml of pre-cultured inoculum was added in 200ml of sterilized rice malt and incubate at 30°C for 10-12 days.

Extraction of pigment:

From the 10-12 days pigmented substrate, the cells were removed by filtration using Watmann's filter paper #1.The residue (cells) were collected and discard the filtrate. The collected cells were dried at 55°C in a hot air oven for one and half hour and then powdered it. The red pigment was extracted using 99% ethyl alcohol. The solvent fraction was collected and concentrated by keeping in rotary shaker at 200rpm over night.

Antibacterial activity of *Monascus purpureus* (Red Pigment):

Antibacterial activity of red pigment was done by disc diffusion method (Kirby-Bauer Method) on NAM against 4 test organisms i.e. *Escherichia coli, Bacillus subtilis, Bacillus megaterium, and Pseudomonas aeruginosa.*

7g of NAM was dissolved in 250ml distilled water in air dried conical flask and it was boiled on a hot plate to dissolve the components and then cotton plugged. The media was sterilized in an autoclave for 15 minutes at 15 psi. 5 mm of filter paper discs were sterilized at 15 psi for 15 min in autoclave to avoid contamination. Now these discs were then dipped in the extract of *Monascus purpureus* in a sterilized watch glass. These discs were kept for about 40 min for evaporation of ethanol. The sterilized nutrient agar was inoculated with the test organism and poured in the petriplate. Plates were kept for solidification. After solidification place the disc dipped in extract in center position and places the control on the top and distilled water disc on bottom position. Plates were left for the diffusion for half hour. They were allowed for 24 hrs incubation to allow the growth of microorganisms. After 24 hours the zones of inhibition of plant extract were measured.

Comparison of Antibacterial activity of Diluted Red Pigment with Antibiotic:

Antibacterial activity of diluted red pigment was done by disc diffusion method (Kirby-Bauer Method) on NAM against 4 test organisms i.e. *Escherichia coli , Bacillus subtilis, Bacillus megaterium* and *Pseudomonas aeruginosa.* For the preparation of dilution 500mg of ciprofloxacin was diluted in 100ml of water. 1ml was again diluted in 9ml of sterilized water to make the actual volume of antibiotic 1mg/ml. Sterilized paper discs were dipped in dilutions of 20%, 40%, 60%, and 80%. The sterilized Nutrient agar media was inoculated with test organisms and poured to the petriplate. Solidify the media and place the disc of dilutions i.e. 20%, 40%, 60% and 80% on the petriplate i.e. one on left, right, top, bottom and centre was antibiotic. Let the plates diffused for half an hour. Incubate the plates at 27°C for 24-48 hrs in an inverted position for observation of zone of inhibition against the test organisms. The zones of inhibition were measured in mm (diameter).

RESULT & DISCUSSION

The present study clearly indicates that alcoholic extract of *Monascus purpureus* possesses antibacterial activity. Data presented in Table 1&2 shows the antibacterial effect of alcoholic extract of *Monascus purpureus* (red pigment). It is clearly revealed that on increasing the concentration of extract the size of inhibition zone increased markedly.

CONCLUSION

It was concluded that pure *Monascus purpureus* (red pigment) showed antibacterial activity *against Bacillus subtilis* 12mm, against *Bacillus megaterium, against Escherichia coli* i.e. 13mm, *14mm and against Pseudomonas aeruginosa* 18mm. The maximum zone of inhibition of *Monascus purpureus* (red pigment) showed against *Pseudomonas aeruginosa* i.e. 19*mm*.The minimum zone of inhibition of *Monascus purpureus* (red pigment) shows against *Bacillus subtilis* 12mm (Table 1).

It was also concluded that diluted *Monascus purpureus* (red pigment) i.e. 20%, 40%, 60% & 80% showed antibacterial activity against *Bacillus subtilis* was 5mm, 6mm, 9mm & 12mm respectively, against Bacillus megaterium was 6mm, 10mm, 12mm& 14mm respectively, *against Escherichia coli* was 4.8mm, 8.5mm, 10.8mm &13mm respectively and against Pseudomonas aeruginosa was 7mm, 10mm, 14mm & 17.8mm respectively. The maximum zone of inhibition of *Monascus purpureus* (red pigment) showed against *Pseudomonas aeruginosa* was 8mm, 10mm, 14mm & 19mm respectively. The

Table 1. Antibacterial effect of pure alcoholic extract of *Monascus purpureus* (red pigment)

Test organism	Pure Alcoholic Extract Replicates					Control Alcohol (mm)	Control Water(mm)	Effective Zone of Inhibition (mm)
	1	2	3	4	Mena (mm)			
Bacillus subtilis	17.2	17	17	17.5	17.1	5	5	12.1
Bacillus megaterium	19	19.5	19.7	19	19.3	5	5	14.3
Escherichia coli	18	18.4	18.7	18.5	18.4	5	5	13.4
Pseudomonas aeruginosa	23	23.5	23.4	23	23.2	5	5	18.2

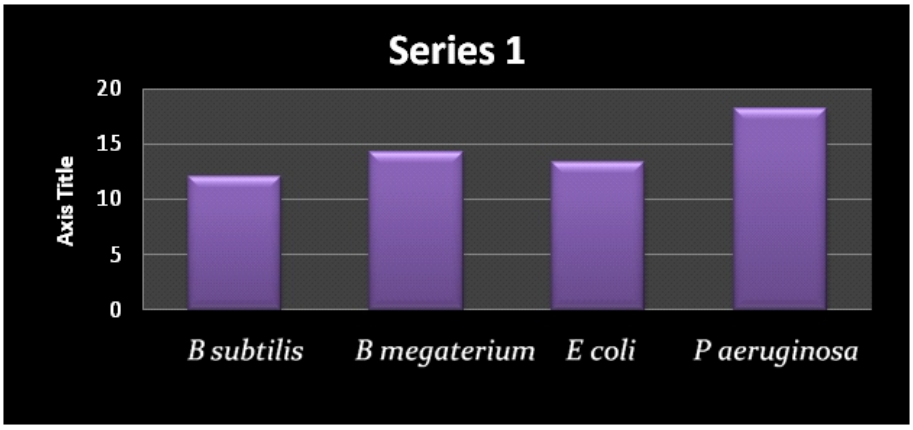

Fig. 1. Antibacterial effect of pure alcoholic extract of *Monascus purpureus* (red pigment)

Table 2. Antibacterial effect of diluted alcoholic extract of *Monascus purpureus* (red pigment)

Test organisms	Dilution of red pigment (A) (mm)				Control Alcohol (B) (mm)	Effective Zone of inhibition (A-B) (mm)				Antibiotic (Ciprofloxacin) (a) (mm)	Control Water (b) (mm)	Effective Zone of inhibition (a-b) (mm)
	20%	40%	60%	80%		20%	40%	60%	80%			
Bacillus subtilis	10	11	14	17	5	5	6	9	12	25	5	20
Bacillus megaterium	11	15	17	19	5	6	10	12	14	25	5	20
Escherichia coli	9.8	13.5	15.8	18	5	4.8	8.5	10.8	13	30	5	25
Pseudomonas aeruginosa	12	15	19	22.8	5	7	10	14	17.8	27	5	22

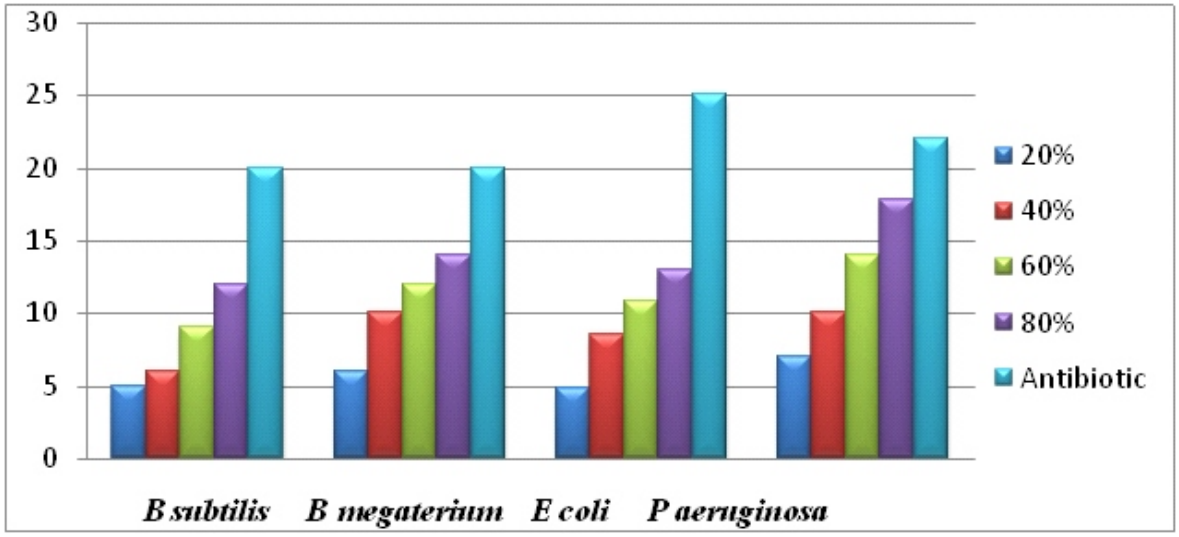

Fig. 2. Antibacterial effect of diluted alcoholic extract of Monascus purpureus (red pigment)

minimum zone of inhibition of *Monascus purpureus* (red pigment) shows against *Bacillus subtilis* was 5mm, 6mm, 9mm & 13mm respectively (Table 2).

Antibiotic (ciprofloxacin) gave of inhibition of 20mm against *Bacillus subtilis,* 20mm against Bacillus megaterium, 25mm against *Escherichia coli* and 22mm against *Pseudomonas aeruginosa.* The maximum zone of inhibition of ciprofloxacin showed against *Escherichia coli* i.e. 25mm. The minimum zone of inhibition of ciprofloxacin showed against *Bacillus subtilis* and *Bacillus megaterium*. The extract of *Monascus purpureus* was found 81% effective as compared with antibiotic (ciprofloxacin).

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