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Research Paper

ISOLATION, IDENTIFICATION, EXTRACTION AND PRODUCTION OF ANTIBACTERIAL VIOLACEIN PIGMENT BY PSYCHROTROPHIC BACTERIUM MTRI7 STRAIN

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Abstract

Chromobacterium violaceum (MTRI7) a bacterium belonging to Rhizobiaceae (Soil born Phytopathogen) family is found in soil in tropical and subtropical areas. The isolation ,identification, extraction and production of antibacterial violacein pigment studied. Antibacterial violet pigment is a mixture of Violacein and deoxyviolacein, isolated from psychrophophic bacterium MTRI7 strain was examined, and optimum condition for the effective production of violet pigment were studied. The antibacterial activity of violet pigment was confirmed for several bacteria such as *Pseudomonas aeruginosa, Sthaphylococcus aureus, Klebsiela pnemoniae, Proteus vulgaris* and *Salmonella typhi.* The high concentration of violet pigment, about 100ul caused not only growth inhibition but also death of cells. The growth properties of MTRI7 strain were clarified under incubation conditions such as pH, temperature and tryptophan concentration. The minimum inhibitory concentration was obtained and zone of inhibition shows the bactericidal effect. The optimum condition found pH 6, temperature 20^0C and 1mg/L tryptophan concentration for growth and violacein production.

Key words: Antibacterial activity, growth inhibition, optimum condition, violet pigment.

INTRODUCTION

Natural Colours are generally extracted from fruits, vegetables, roots and microorganisms and often called " Biocolors" because of their biological origin [1]. For a decade, both natural pigments and synthetic dyes have been extensively used in various field of everyday life such as food production, textile industries, paper production, agricultural practices and researches, water science and technology [2].

The disinfection, sterilization, inhibition, and other effective method for preserving the septic action of food, and it has been reported that some pigments produced by a microorganism has a physiological activity for the prevention of bacterial contamination [3]. The natural pigment are comparatively often used as antibacterial

reagent for food preservation because of their high decomposition nature and low toxicity compared with synthetic pigment [4, 5]. Today, pigment are used in many areas such as medicine, animal feed, paper, ink, food, textile. Pigment production is very useful for bacteria. There are many reasons for pigment production to produce the pigments for photosynthesis, UV protection, Defense mechanism, secondary metabolites for storage of energy. Some pigment are active against phytopathogens and human pathogens. Extremophiles are very colorful. Pigment of extermophiles required for respiratory and photosynthetic function.

Violacein (Fig 1), the main pigment produced by Chromobacterium violaceum, is a bactericide, trypanocide, tumoricide pigment and in addition it has anti-viral activities [6].

Violacein consist of three structural units: 5- hydroxyindol, 2-oxoindol, and 2-pyrolidone which are poorly water soluble and during formation rapidly precipitates either as discrete particals or on cells or cell clumps [6].

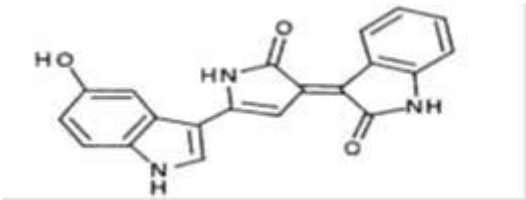

Fig 1- General structure of violacein (3-(1,2-dihydro-5-(5-hydroxy-1H-indol-3-yl)-2-oxo-3H-pyrrol-3-ilydene)-1.3-dihydro-2H-indol-2-one)

Natural pigments not only have the capacity to increase the marketability of products but they also display advantageous biological activities as antioxidants and anticancer agents [7]. Pigment of various color are synthesized to protect the cells of microorganism from injurious effect of light rays of visible and near ultraviolet range [8].

However, since some antibacterial reagents has a bad influence not only to a human body but also to environment, the usage of natural pigments with antibacterial activity is desired technology of bacterial contamination. In this work, the antibacterial activity of violet pigment produced by the bacterium MTRI7 strain and the optimum condition of this bacterium for efficient production of violet pigment investigated. The growth inhibitory and lethal effects of violet pigment on various putrefactive bacteria were clarified and the antibacterial activity of violet pigment was estimated quantitatively.

MATERIAL AND METHOD

Isolation and identification

Chromobacterium violacein was isolated from soil. Samples were collected from various places of Shri shivaji college then cultured on nutrient agar plates with optimum condition like pH 6, Temperature 20^0C and Tryptophan concentration 1mg/mL as a precursor for growth and violacein production (Fig 5). Identified on the basis of laboratory test like Colony morphology, Gram's staining and Biochemical tests. It was found that *Chromobacterium violaceum* (MTRI7) is a Gram negative, facultative anaerobic, rod shaped bacterium that is considered being non –pathogenic [9]. Its colonies are lightly convex, not gelatinous, regular and violet, although irregular and non- pigmented colonies also found in anaerobic condition as violacein is produced only in the presence of oxygen [10].

Microorganism
The MTRI7 strain isolated from soil and then identified by 16s r RNA technique used in this work (Fig3,4).

Media
The media used for enrichment and isolation of pigmented bacteria were Nutrient Agar with 0.1% Tryptophan , Nutrient broth and Mueller-Hinton Agar which were obtained from Himedia, India.

Optimum Conditions
For growth and production of violacein pigment in broth medium as well as on nutrient agar the Temperature required 20^0C, pH 6 and 1gm/mL Tryptophan used as precursor enhanced growth and violacein production [11, 12].

Production Violacein
Due to vast application of Violacein it enhanced the production of pigment. It required the optimum condition for growth culture for *C. violaceum* in Nutrient broth and Nutrient agar.
Growth of bacterium by using soil extract agar (SEA) was reported by Innis and Mayfield at 20^0C and 0^0C. The results showed that the colonies which develope at 20^0C were totally pigmented where as colonies grown at 0^0C were non pigmented. When colonies grown at 0^oC incubated at 20^0C pigment production was at 20^0C [12].
The same observation was observed Nutrient Broth at 0^0C, 15^0C, 20^0C, and 25^0C. Growth was measured spectrophotometrically at 650 nm. Since result showed that violacein production at 0^0C, then experiment performed in which various concentration of tryptophan, a known precursor of violacein, were added to Nutrient broth in flask and Nutrient agar plates, growth and pigment production determined. The optimum and suitable pH for this experiment reported around 7.4 where the temperature of medium was 20^0C [12].

Extraction of Violacein pigment
Extraction of violacein from reaction mixture with ethyl acetate and evaporated the solvent under reduced pressure while filtered out the pigment, dried it and extracted it with alcohol [13].
Centrifugation is another possible way to extract the pigment as [14]centrifuged the cell first at 8000g for 5 minutes and the supernatant was discarded. The cell pellets were then rinsed with deionized water, followed by centrifugation to recover the cells by discharing the supernatant again. The recovered cells were fully mixed with ethanol. The mixture of the cells and ethanol was treated by ultra-sonication that there was no residual pigment in the cell pellet after the extraction.

Anti-bacterial Activity of the Violacein Pigment
The antibacterial activity of the violacein pigment was tested by Agar-Cup Diffusion Method. Briefly 20 ml of Mueller Hinton Agar was poured into the Petri-dish and 8 mm well bored in the agar. 100µl of violacein extract was poured into the wells. The plates were incubated for 24 h at 37ºC and the zone of inhibition was measured in mm. The test was performed by swabbing of the growth inhibition zone of the plate and then swab was streaked onto Mueller Hinton agar plate and incubated aerobically at 37ºC for 48 hours. The presence of growth in Mueller Hinton agar plate was interpreted as an inhibitory activity.

OBSERVATION

Table No 1 : Anti-bacterial activity of violacein pigment

Sr. no.	Bacterium	Zone of Inhibition
1.	*B. subtilis*	21mm
2.	*S. aureus*	29.5 mm
3.	*E. coli*	26 mm
4.	*S. typhi*	24.5 mm
5.	*K. pneumonia*	53.5 mm

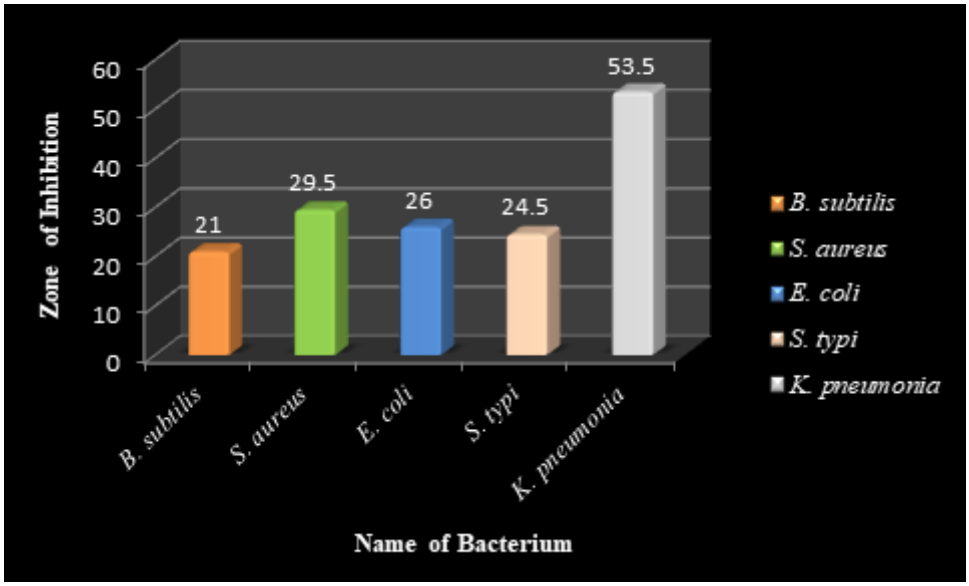

Fig- 2 Graphical representation of anitibacterial acivity of Violacien pigment. Table No 1

Fig3-*Chromobacterium Violaceium*

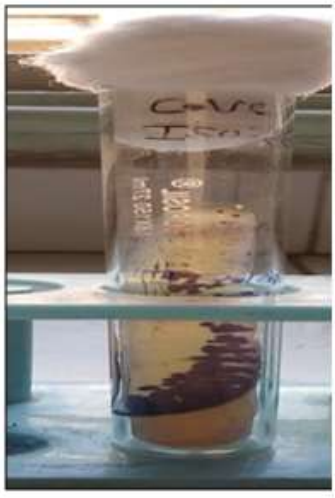

Fig4 - *Chromobacterium Violaceium(slant)*

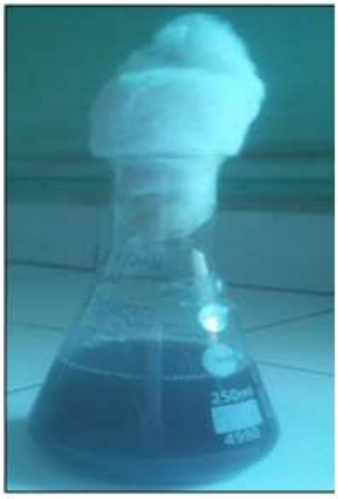

Fig5- *Vilocein pigment production*

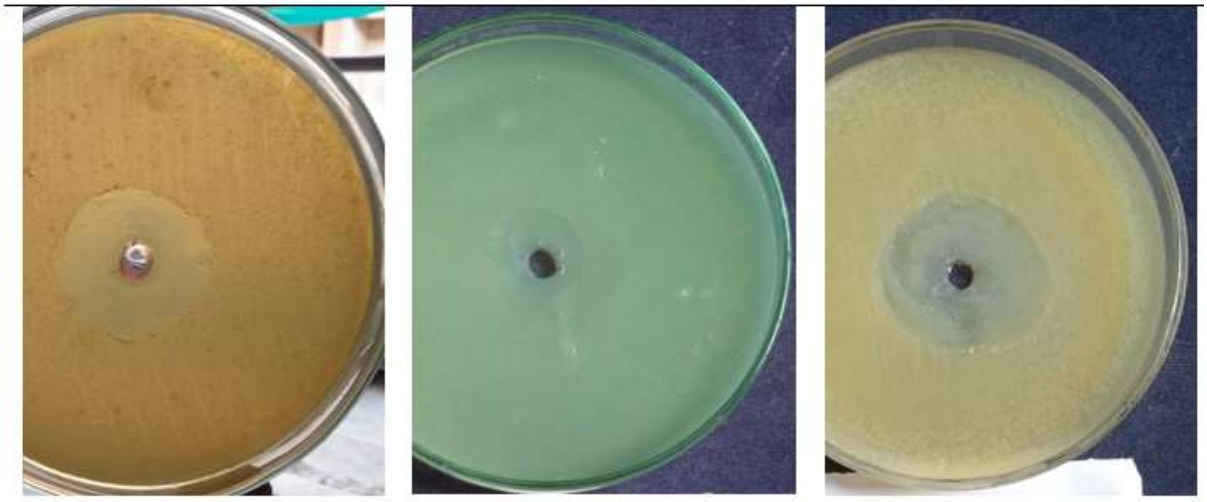

Fig 6- Antibacterial activity of Violacein against Bacterium

RESULT AND DISCUSSION

Many studies have been made about investigation of antimicrobial property of violacein pigment, The studies conducted about antibacterial effect of violacein showed that violacein inhibited growth of gram positive as well as gram negative bacteria at 100ul concentration (Table1). The antibacterial action of a mixture of violacen and deoxydiolacein isolated from the bacterium MTRI7 strain was demonstrated against gram positive as well as gram negative bacterium it showed bactericidal effect. It was determined that the most sensitive bacteria is *K. pnemonae* which showed 53.5mm zone, while *B. subtilis* showed 21 mm zone, *S. aureus 29.5mm zone*, *E. coli* showed 26mm zone and *S. typhi* showed 24.5mm zone which were moderately sensitive to violacein pigment(Fig6). Antibacterial activity showed that there was no uniform response among bacterial strains in susceptibility can be attributed to differences in cell wall composition. The reason was referred to be difference in the structure of the cell walls [15]. Selective antibacterial activity may be due to several factors, including the charge density, structure of lipopolysaccharides and lipid composition of the cytoplasmic membrane in Gram-negative and Gram-positive bacteria [16]. Our results indicated that the chemistry of the pigments has significant influence on its antimicrobial activity.

As per same result obtained in study of carotenoid extracted from *Rhodotorula glutinis* on some food pathogen, in which Salmonella enteritis B42 most sensitive while most resistant species was *Bacillus* [17].

Also Violacein pigment from RT102 strain was demonstrated against Gram positive *B. lichenniformis, B. subtilis, B. megatherium, Staphylococcus aureus* and Gram negative *Psedomonas aeruginosa*, at a high concentration of violet pigment could kill completely all the bacterium [18].

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