Susceptibility of Klebsiella sp. isolated from septicemia patients to water soluble pigments of Pseudomonas sp. and Staphylococcus sp. isolated from hospital campus sp.

Smaranika Pattnaik,  
Dept. Of Microbiology, School Of Life Sciences, Jyoti Vihar, Burla 768019

Received on:10-11-2011; Revised on: 15-12-2011; Accepted on:12-01-2012

ABSTRACT

The soil sample was collected near the District Medical Campus, Sambalpur, Orissa and was analyzed for the pigment producing activity by following Serial Dilution method. Further routine microbiological diagnostic methods were carried out for the identification and characterization. The isolates, Pseudomonas sp. and Staphylococcus sp were identified and observed to be water soluble blue-green pigment (pyocyanin) producing as well as yellow pigment (staphyloxanthin) producing strains respectively. The pigment producing activity was observed during the stress condition (period between onset to completion of stationary period). Later both the purified forms of the pigments were assayed for the sensitivity test against the human pathogenic strain of Klebsiella sp. which was isolated from individuals who were hospitalized in V.S.S. Medical College, Burla, Odisha and suffered from severe underlying diseases. It was observed that the cell free purified pigments had cidal effect against the test bacterial strains at their minimum Inhibitory Concentrations. More over a study was also carried out regarding the growth inhibitory property of the pigments while the bacteria were in dynamic stage of pigment production.

Key words: Klebsiella, Nosocomial pathogen, Pigmented bacteria, Growth Inhibitory property

INTRODUCTION

The medically important Klebsiella sp., account for a significant proportion of hospital-acquired urinary tract infections, pneumonia, septicemias, and soft tissue infections [1]. The principal pathogenic reservoirs for transmission of Klebsiella are the gastrointestinal tract and the hands of hospital personnel. Because of their ability to spread rapidly in the hospital environment, these bacteria tend to cause nosocomial outbreaks [2]. The incidence of ESBL (β-lactamase) producing strains among clinical Klebsiella isolates has been steadily increasing over the past years[3]. Klebsiella pneumoniae is a Gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium.

The resulting limitations on the therapeutic options demand new measures for the management of Klebsiella hospital infections.

More than half of the clinical isolates of Pseudomonas bacteria produce pyocyanin, a blue-green pigment (a derivative of phenazine). The Pseudomonas fluorescens group are nonpathogenic saprophytes that also produce a pigment, particularly under conditions of low iron availability. This pigment is a soluble, greenish, fluorescent pigment that led to the group’s name. This ability is due to secondary metabolites produced by these bacteria such as antibiotics, siderophores, and hydrogen cyanide.

3,4,5 trihydroxy 6 methyl tradecanoate (oxymethyl) oxan2 hexamethyl tetracosa decanoate (C₃₀ H₆₆ O₈)

Pseudomonas spp. are capable of producing the very distinctive water soluble pigment Pyocyanin. Pyocyanin is a blue green pigment. Reports are there for inhibitory effect against microbial cells. Strains of Staphylococcus are also capable of producing Staphyloxanthin [4], a carotenoid pigment that acts as a virulence factor. Staphyloxanthin has an antioxidant action that helps the microbe evade death by reactive oxygen species used by the host immune system. Staphyloxanthin is responsible for its characteristic golden colour. There is an inverse relationship between pigment production and the concentration of iron in the medium. A small number of evidences suggest that the pigments Pyocyanin and Staphyloxanthin could be potential antimetabolite against pathogenic strains. The present per suit was taken to assess the bacterial induced pigments as antibacterial principles against the strain of Klebsiella sp. isolated from septicemia patients of V.S.S. Medical College, Burla, Odisha. Pseudomonas is ubiquitous in soil and water, and on surfaces in contact with soil or water. There are certain reports about antibacterial property of pigments isolated from Pseudomonas. As the strain is from new ecorace, the study was undertaken to appraise the inhibitory property of the pigment. In addition to this another bacterium, Staphylococcus aureus was also isolated from the same location having yellow pigment production. Both the pigments were assigned for the antibacterial activity. Pseudomonas Pyocyanin pigment had antibacterial activity against the test bacterium klebsilella however Staphyloxanthin pigment did not have antibacterial potentiality.

MATERIALS AND METHODS:

Media:
Nutrient agar, Nutrient, broth, Cetrimide Agar base and broth, Staphylococcus Mannitol agar and broth were taken as the media for the cultivation Pseudomonas and Staphylococcus respectively. The media were prepared according to directives of Hi-Media Company, Mumbai.

5-methyl 5 (H) phenazinone
Bacteria:
Klebsiella sp., isolated from septicemia patients and identified in the Deptt. of Microbiology, V.S.S. Medical College was taken for the study. The V.S. S. Medical College Hospital campus soil was in use as the source for isolation of soil bacteria. Pseudomonas sp. and Staphylococcus sp. could be isolated from soil by following method “Pour Plate Dilution Method”[5].

Production and Biochemical analysis of pigments:
The pigment producing activity of Pseudomonas sp. and Staphylococcus sp. was studied by performing growth curve analysis of the bacterial strains. The overnight broth cultures were subjected to incubation in a rotary shaker and Optical density was measured for viable cells as well as pigments at 630nm and 520nm [6] respectively.

Extraction and Purification of Pyocyanin and Staphyloxanthin Pigments:
The late stationary stage (72hrs) broth cultures of Pseudomonas sp. and Staphylococcus sp. were subjected to respective pigment extractions by following modified method of Rauf and Latif [6]. The purified pigments were dissolved in sterile distilled water maintaining a concentration of 1µg/µl (w/v) were assayed for anti bacterial vulnerability.

Test for antagonistic effect:
A “Tube dilution” method was followed to test for antagonistic activity of the Pyocyanin producing Pseudomonas cells and Staphyloxanthin producing Staphylococcus cells in opposition to Klebsiella. Overnight cultures (10^6 CFU/ml) of Klebsiella cells were inoculated in broths at a dilution of 1:100. Pseudomonas and Staphylococcus O/N cultures were put in each Klebsiella containing tubes maintaining dilutions of 1:10, 1:100, 1:1000 and 1:10,000 respectively and incubated for 72 hrs. Presence of visible turbidity and production of pigments by the hostile bacterium was studied.

Susceptibility testing of purified Pyocyanin pigments in opposition to Klebsiella sp:
Overnight Klebsiella broth cultures (10^6 CFU/ml) were subjected to “Susceptibility testing” of purified Pyocyanin pigments against Klebsiella sp. quantitatively by following “Tube Dilution” method. A range of concentrations of Pyocyanin starting from 1µg/ml to 100µg/ml were in use for the testing.

RESULTS:
Cultures Pseudomonas sp. produced a blue water soluble pigment and Staphylococcus sp. produced a yellow pigment respectively (Figure # 1 & 2). From the growth curve analysis of Pseudomonas and its Pyocyanin pigment production study it was observed that the initiation of pigment production started in an hiatus between onset and offset stationary periods (Figure # 3 & 4). As of the study of antagonistic outcome of Pseudomonas on the cells of Klebsiella, it was observed that the bacterium had remarkable inhibitory effect on growth of Klebsiella at a concentration of 80µl/ml (V/V) broth O/N culture of Pseudomonas (Figure # 5). In a test for susceptibility ordeal against the Klebsiella, with the purified pigments, it was observed that, Klebsiella cells were killed with a concentration of 40µg/ml (W/V) of purified Pyocyanin pigment. Subcultures were made onto agar plates showed no endurance of living cells. In contrast the Staphyloxanthin pigment could not cease the growth/multiplication of Klebsiella cells in antagonistic effect tests. Therefore consequent tests were carried out with Pyocyanin pigment only.
DISCUSSION:
The features may be due to osmotic imbalance induction and weakening of cell stringency by the pigment Pyocyanin. The possible mode of action of Pyocyanin against *Klebsiella* sp. cell commotion may be as a consequence of its interaction with the cell wall architecture enzyme(s) of the susceptible bacterium. However, there was no substantial evidence regarding the inhibitory property of Staphyloxanthin against *Klebsiella* sp. isolated from *Staphylococcus aureus*.

CONCLUSION:
Although this study is a succinct approach to establish Pyocyanin pigments as antibacterial principle, still it may provide some essentials in chemotherapeutic sphere.

REFERENCES:

Source of support: Nil, Conflict of interest: None Declared