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OPTIMIZITION OF CONDITION FOR PRODUCTION FOR PRODIGIOSIN BY SERRATIA MARCESCENS

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ABSTRACT

Serratia marcescens species are gram negative bacteria, classified in the large family of Enterobacteriaceae. Serratia can be distinguished from other genera by its production of three special enzymes DNAase, lipase and gelatinase. Another characteristic feature of the Serratia among the Klebsiellaea is the production of cell associated red color pigment. Serratia, like other Enterobacteriaceae, grow well on ordinary media under anaerobic and aerobic conditions. They grow well on synthetic media by adding maltose sugar for their feed as there nutrients using various compounds as a single carbon source. Optimum growth of all strains of Serratia has been observed at pH 9 and at temperatures from 20–37°C.

KEYWORDS: Enterobacteriaceae, Prodigiosin, Serratia marcescens.

INTRODUCTION

Once considered a harmless saprophyte, Serratiamarcescens is now recognized as an important opportunistic pathogen combining a propensity for healthcare-associated infection and antimicrobial resistance.Serratia marcescens is a member of the genus Serratia, which is a part of the family Enterobacteriaceae. Currently 14 species of Serratia are recognized within the genus, eight of which are associated with human infection. Of the eight species implicated in clinical infection S. marcescens, S. liquefaciens and S.odorifera are best known of all Serratia species, S. marcescens is the most common clinical isolate and the most important human pathogen.S. marcescens is credited with a long fanatical history dating back to antiquity, when, because of its ability to produce a red pigment it was described as having ‘masqueraded’ as blood. Early in this century, this distinctive red pigmentation of S. marcescens, combined with an apparent low level of virulence, led to its use as a biological

marker of infection. Consequently, S. marcescens was used in a number of classic bacterial transmission experiments, which led to improved understanding of the epidemiology of infection. Under more controversial settings, S. marcescens was also used by the US military in a series of biological warfare test experiments conducted on the general population. From 1960 onwards, however, non-pigmented isolates of S. marcescenspredominated over pigmented strains in the clinical setting and were increasingly implicated in healthcare-associated infection particularly among compromised patients.As members of the Enterobacteriaceae family, Serratia spp are motile, nonendospore forming Gram-negative rods. In the laboratorySerratia are routinely isolated from bloodstream and wound sites using blood agar culture or from. Respiratory and urinary sites using selective culture methods. Common selective agar cultures include MacConkey agar which categorizes Serratia isolates with the other non-lactose fermenting Enterobacteriaceae or chromogenic agars, which classifies them into a broad Klebsiella, Enterobacter, Serratiand Citrobacter (KESC) grouping.

Prodigiosin – Red pigment

Many microorganisms have the unique property of producing a secondarymetabolite which is characterized by a unique color. Other than the fact that thesearenatural pigments which are safer when compared to their chemical counterparts as anadditive especially in the food industry, many of these pigments have unique properties which play an important role in the pharmaceutical industry as well as in being a good source of nutrients such as vitamins and minerals. However, most microbial pigments are water insoluble, have poor stability and show different intensities of color at varying pH, temperature and nutrient sources **(Joshi et al., 2003)**.

MATERIALS AND METHOD

Method for prodigiosin red pigment extraction

1. Collect the sample from nearby my home, college, and sakkardara lake in a sterile sample container for the isolation of pigment producing organisms.
2. Next day 1gm of soil sample was serially diluted (10^2 -10^5).
3. Differential isolates were isolates and after that make their slant and stored at 4^0C until further use.
4. For screening 100ml conical flasks containing 50ml of nutrient broth (g/l) was prepared and used.

5. 48hrs inoculum was added to each of the flask and incubate at the 27^0 c in a sterile condition.
6. After 96hrs of incubation period broth was taken for pigment extraction.
7. After 96hrs of incubation the 1ml medium was centrifuge at 10,000rpm for 10min at 4^0c.
8. Pigment was extracted from cell pellet by shaking in 1ml of acidic acetone (96 ml acetone and 4ml HCL) for 35min at 30^0c.
9. Debris was removed by centrifugation at 10,000 rpm for 20min at 4^0c. theacetonic extract bof the pigment was evaporated at room temperature and it was dissolved in 3ml of chloroform and transfer into fresh sterile micro tube.
10. It was again evaporated at room temperature to concentrate the pigment.

ESTIMATION OF PRODIGIOSIN

The absorption pattern over various wavelength was initially checked and it was found that absorption maxima waere at 499nm where prodigiosin also absorb maximally. At this wavelength the absorption were recorded. The result were studies after above mentioned time intervals. The bacterial cell absorption to extraction was noted at every steps. Isolated prodigiosinwas estimated by using the following formula (Makhael and Yousif, 2009).

Prodigiosin unit/cell = {OD499 – (1.381 x OD620)} x 1000 /OD620
OD – Optical density
OD499 – pigment absorption
OD620 – bacterial cell absorption
1.381 – constant

RESULT AND DISCUSSION

From the above observation it was found that the isolated bacteria were obtained from soil sample was identified as S.marcescens. The morphological characters are still widely used for characterizing genera. Isolate were tested by biochemical tests for the identification of S.marcescens. Gram negative, pink color, rod shape, serratiamarcescens bacteria were observed in microscopic study of gram staining slide.

OBSERVATION

MEKHAEL and YOUSIF 2009, these two scientist had done there work in nutrient broth as well as in peptone glycerol broth. They found maximum prodigiosin production in 72hrs and the optical density was found to be 1.980 in nutrient broth and peptone glycerol broth. The

lowest prodigiosin unit per cell was found after 96hrs that is in nutrient broth it was 957.1 and in peptone glycerol broth that is 885.38.

ABSORBANCE AT 499nm

Hours	Nutrient Broth	
	Agitated	Stationary
24hr	00	00
48hr	1.76	1.74
72hr	1.89	1.88
96hr	1.98	1.96

Culture of S.marcescenswas inoculated on above mention media, Prodigiosin production can be visualized in culture flask. From the above observation table it was found that the maximum prodigiosin production in 96hrs is 1.98 unit per cell in agitation phase and 1.96 in stationary phase at 499nm. The lowest prodigiosin production unit per cell was found after 96hrs.

ABSORBANCE AT 620nm

Hours	Nutrient Broth	
	Agitated	Statitionary
24hr	00	00
48hr	1.49	1.47
72hr	1.45	1.44
96hr	1.50	1.48

From the above observation table it was found that the in agitation and stationary phase maximum prodigiosin production unit per cell in nutrient broth was found to be 1.50 and 1.48 respectively at 620nm. The lowest prodigiosin production was found after 96hrs.

Total Prodigiosin in Nutrient Broth

Hours	Total prodigiosin in nutrient broth mg/lit	
	Agitated	Stationary
24hr	00	00
48hr	1379.24	1379.26
72hr	1379.11	1379.12
96hr	1379.02	1379.02

The culture of S.marcescens was innoculated on above mentioned media. Prodigiosin pigment can be visualized in culture flask. Optical density of the extracted prodigiosin production was estimated periodically after intervals of 24hrs, 48hrs, 72hrs.prodigiosin production was nil after 24hr in stationary phase determined spectrophotometrically at

499nm. (Makhael and Yousif, 2009). Prodigiosin unit per cell after 48hrs was found to be 1.76 at 499nm and 1.49 at 620nm in nutrient broth. Maximum production were reported in 72hr. in stationary phase the prodigiosin unit per cell in nutrient broth was found to be 1.88 at 499nm and 1.44 at 620nm. The lowest prodigiosin unit per cell was found after 96hr in nutrient broth it was 1.98 at 499nm and 1.50 at 620nm.

CONCLUSION

bacterial strain isolated from surrounding area was identified as Serratiamarcescens and named Biopigments produced by bacteria possess enormous effieciency as medicinally important product. We have been successful in designing a economically feasible medium supporting the enhanced growth of Serratiamarcescens and simultaneously supportinga high yield of medicinally important biopigmentprodigiosin. The selected as Serratiamarcescens MBBO5 based on the morphological and 16srRNA gene sequence. The optimum condition for prodigiosin production was when incubated at 30^0c, with a pH of 7.0, at 36hr of incubation with 5% innoculum supplemented with glucose, yeast extract, andcystein as a best carbon and maltose, nitrogen and amino acid source for the selected bacterial strains. Prodigiosin production using basal media at optimized condition showed 1.7 time higher than the standard media and among the substrate tested, peanut powder was found to be best natural substrate at a concentration of 2.0% in distilled water. The production was 4.5 times higher than the formulated for prodigiosin production would be a boon to pharmacitical industries for large scale production of the medicinally potential drug prodigiosin. Over the last four decades, S.marcescens has emerged as an important health care associated pathogen. Infection with this organisms represents a tangible cost in term of patients morbidity and antibiotic usage. In the light of the multiple antimicrobial resistance demonstrated by S.marcescens, it essential that the clinician evaluates the antimicrobial susceptibility of clinical isolates on the basis of the data supplied by the microbiology laboratory and on the clinical setting of the infection, prior to the selection of appropriate therapy.

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