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**INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES**<http://doi.org/10.5281/zenodo.804040>Available online at: <http://www.iajps.com>**Research Article****EVALUATION OF ANTIBACTERIAL EFFECT OF
PRODIGIOSIN PIGMENT PRODUCED BY SERRATIA
MARCESCENS AGAINST STAPHYLOCOCCUS AUREUS AND
ESCHERICHIA COLI ISOLATED FROM PROCESSED FOOD.****Parya karbaschi¹, Bahareh Rahimian Zarif^{2*} and Zahra Houshmandi³**^{1,2,3} Department of Biology, Sanandaj Branch, Islamic Azad University, Sanandaj, Iran**Abstract:**

prodigiosin pigment is three Pyrrole that is secondary metabolite produced by some bacterial species like Serratia marcescens . prodigiosin activities and frequent drug shown to inhibit bacterial And has attracted the attention of many researchers in the field of medicine and pharmacy should be. The aim of this study was Evaluation of antibacterial effect of prodigiosin pigment produced by Serratia marcescens against Staphylococcus aureus and Escherichia coli isolated from processed food.

Method: Standard strains of Serratia marcescens PTCC 1111 were prepared from collection of microbial strains by Scientific and Industrial Research Organization. Staphylococcus aureus and Escherichia coli strains was isolated and identified by dilution and standard microbiological methods from processed foods. Pigment produced by Serratia marcescens was extracted by the acidic methanol. Finally, by well diffusion, MIC and MBC methods antimicrobial effects of pigments were investigated.

Results: Inhibition zone against Staphylococcus aureus and E. coli was 10 mm and 5 mm, respectively. The MIC was 0.78 mg/ml and 3.126 mg/ml for Staphylococcus aureus and E. coli, respectively. MBC was 3.126 mg/ml and 6.25 mg/ml for S. aureus for E. coli, respectively.

Conclusion: The S. marcescens prodigiosin produced by showed bactericidal and bacteriostatic effect showing promising antimicrobial activity and suggesting future studies regarding its applicability in antibiotics therapies. In addition, results showed that prodigiosin could be use in foods industry.

Key words: *Prodigiosin, Serratia marcescens, Staphylococcus aureus, Escherichia coli, processed food, antimicrobial*

Corresponding author:**Bahareh Rahimian Zarif,**

Department of Biology,

Sanandaj Branch, Islamic Azad University,

Sanandaj, Iran. Email: bahareh_r_z@yahoo.com

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INTRODUCTION:

Serratia is a gram negative, facultative anaerobic, motile, oxidase positive bacteria with pigments which is present in fresh water and is classified in to the Enterobacteriaceae family. Serratia genus was first identified in 1819 by Bartoleo Bizio. He selected this name in honor of the Italian physicist Serafino Serratia. Different varieties of this genus can be Serratia plymuthica, Serratia rubidaea, Serratia, and Serratia odoriferous [1].

In old literature, thus this bacterium was mentioned as in other names such as monas prodigious or bacillus prodigosus. In 1950, Serratia marcescens was released by the U.S. Air Force in the California's atmosphere which caused severe urinary tract infection and a case leading to death and an increase in the number of people suffering from pneumonia long after the pollution [2].

Serratia marcescens growth has been investigated in an environment of water, disinfectants and plastics such as blood bags. This bacterium has unique extracellular products. Since Serratia marcescens was known as the infectious agent, its pigment biosynthesis came under investigation) [2].

The bacteria can be found in natural environments such as soil, water, on the surface of parts of plants as

well as an opportunistic pathogen in humans. The bacteria are rod-shaped and measures about 0/5-0/8 and 0/-2 microns and their arrangement is in a peripheral form (Trish Perry). Its colonies on agar are opaque, circular, convex, and in full margins and are in white, pink and red colors. Methyl red is negative and can decompose casein, tryptophan and parse citrate. Serratia marcescens produces lactic acid through fermentation and oxidation [2].

The bacteria grow in the temperature range 5-40. Pigment cannot be observed in the old culture of Serratia and the color is borne out of this feature of paling. Some species are lacking pigment and at least one species (D1) produces red-orange color that does not fade out [2].

Prodigiosin structure, primary prodigionine was found in the early 1960s by a partial and general chemical synthesis and by revealing of Pyrrole Promethan core skeleton. Three pirvali rings of Prodigiosin have been named as pirvali ring A, ring B and ring C (figure, 1.1). Prodigiosin exists in two rotameter (structure) convertible, cis (or β) and trans (α) (figure 1.1). The balance between these forms is dependent on the pH solution because the trans form loses proton more conveniently [3].

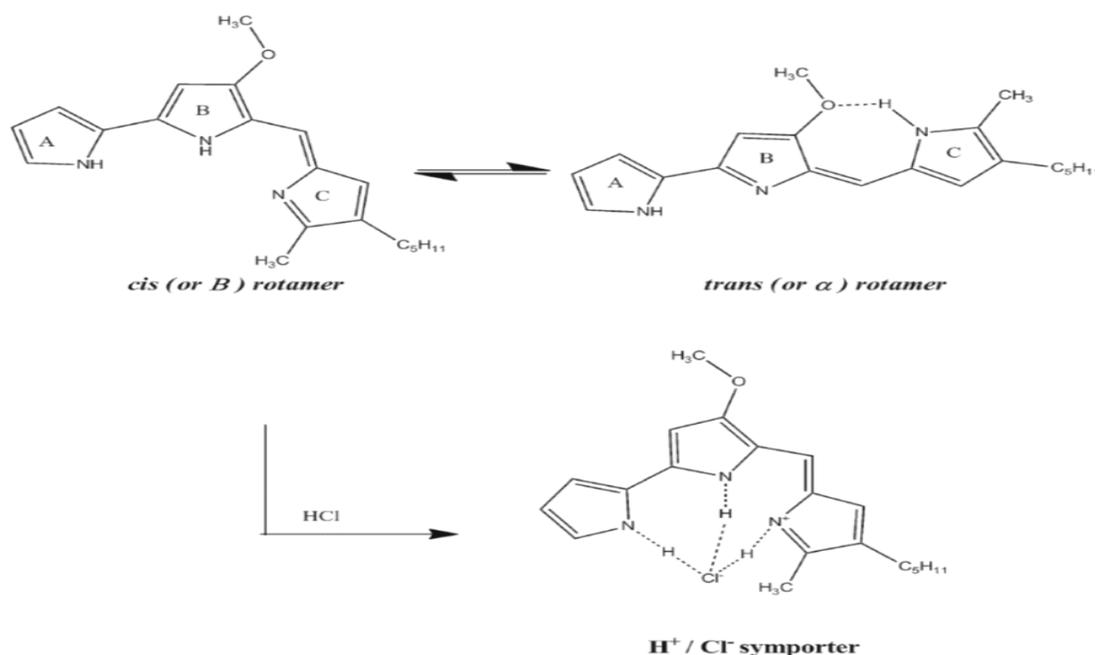


Fig 1: primary prodigionine , prodigiosin . prodigiosin in a solution, depends on pH solution as a combination of cis. (or β) and trans (or α) in a ration [3].

Three pyrrole rings of prodigiosin have been named rings A, B and C. the lower structure shows the connection of tree pirvali nitrogen of prodigiosin with chloride ion, when it serves as a symporter (inner membrane protein) H^+ / Cl^-

MATERIAL AND PROCEDURE:

Serratia marcescens standard strain

Serratia marcescens standard strain PTCC 1111 was prepared from a microbial collection of industrial and Scientific Research Organization. Then Serratia marcescens standard strain PTCC 1111 ampoules was sterilized with a flame with alcohol cotton at 70 degrees; thereafter, it was placed in sterile gauze, broken gently and then strain Lyophilized powder was poured in an LB environment and placed for 24-48 hours at the 37 degrees temperature so that the strain is activated.

Isolation and identification of Staphylococcus aureus and Escherichia coli from processed foods

Successive dilutions were prepared from different samples of processed food by way of standard microbiological methods. Then, dilutions were cultured on Mannitol salt agar and EMB environments. Colonies of Staphylococcus aureus and Escherichia coli are identified in these primary environments; such that Escherichia coli has metallic luster on the EMB environment and Staphylococcus aureus was placed on mannitol salt agar of the yellow colony where the periphery of colonies have been yellow colored. Initially, intended separated colonies were stained with early detection and biochemical tests were performed to confirm them.

Production and extraction of prodigiosin by Serratia marcescens strain

First, the standard strain was grown on nutrient agar. After seeing the colonies in pink (which shows the production of pigment), colonies are washed with physiology serum and are entered into a falcon. Then, it was centrifuged in 4000 rounds for two minutes so that bacterium cells are deposited. The supernatant (normal saline) was discarded. Now, as much as 30 ml nutrient broth was poured into a falcon in which bacteria cells are deposited and cells were mixed well. This falcon was incubated in 28degrees temperature for 48 hours.

The strain was centrifuged for 15 minutes in 13000 rounds at the 4 degrees temperature after the incubation of the environment and supernatant (normal saline) was discarded. Prodigiosin pigment was extracted by adding 30 ml acidified methanol (96 ml methanol +4ml HCl) in a shaking incubator at 30 degrees centigrade for 35 minutes from the cells extracted. This time supernatant (normal saline) containing pigment was retained. The methanol extracts pigments and pigment powder remains dry at room temperature

Investigating the antimicrobial property Prodigiosin

- **Preparation of a new culture (in logarithmic phase) of microorganisms**

Staphylococcus aureus and E. coli stored a few days before the MIC and MBC testing are

cultured on laboratory environments (nutrient agar) so that microorganisms are placed in the exponential phase after an overnight incubation while preparing microbial suspension.

- **Preparing the culture environment**

A culture environment applied for the testing is usually Mueller-Hinton agar. The thickness of the environment in lower parts of the plate should be homogenous around 4 mm. pH of this environment should be tuned between 7/2 to 7/4. Plates can be stored for 7 days at 4 degrees centigrade. Should plates are stored in plastic bags which prevent water evaporation in a large part; they can be used for longer period of time.

- **The preparation of half-McFarland standard**

To prepare appropriate microbial suspension, in order to determine its antibiotic sensitivity, the number of bacteria existing in the inoculated samples should enjoy an acceptable criterion. The number of these bacteria for the conduct of antibiogram method should be arbitrarily $1/5 \cdot 10^8$ bacteria cell in each ml of inoculation. For this, the following is performed:

1. 0/5 ml barium chloride (BaCl_2), 0/048mol/l was added to 99/5 ml of sulfuric acid 0/18 mol/l and the suspension as obtained with stirring.

2. Correct density of the standard turbidity is identified by using spectrophotometric measurements at optical path length of 1cm. absorption in 625nm must be between 08/0 to 13/0 in.

3. Barium sulfate suspension must be poured for as much as 4-6ml in screw cap tubes fitted with bacterium suspension tubes.

4. The cap of these pipes must be fastened firmly and kept in room temperature in darkness.

5. Barium sulfate standard should be strongly stirred prior to each tike of use (preferable with a mechanical vortex) so that uniform opacity is created. In case of seeing large particles, new standards should be provided.

6. Barium sulfate standard should be monthly replaced or its absorption be measured.

- **Preparing microbial suspension**

First, TSB environment was prepared and in each experimental tube, 4 ml is poured and then autoclaved. In each tube, some bacteria colony was inoculated so that it has opacity similar to 0/5 McFarland. Only one special colony should be used.

To compare the opacity of the culture tube with the McFarland tube, it is advised to use white background with black lines and sufficient light. The absorption should also be obtained in 625 Nm for the samples that need to fit the size of McFarland standard absorption.

- **Microbial suspension inoculation into the culture environment**

The microbial suspension having been prepared in accordance with the McFarland 0/5 standard opacity, a sterile swab is dipped in to microbial suspension and after taking its extra liquid by pressing the swab to the interior wall of the tube, the wet swab at the Mueller-Hinton Agar environment surface which had reached the room temperature previously was uniformly inoculated in all directions with the angle of 60 degrees.

- **Well diffusion**

In this stage, wells at the standard size (6mm) are created on agar Muller-Hinton and 20 micro liters of the 15 mm solution of prodigiosin pigment which was dissolved in 1 ml DMSO are poured into the well (one control well was filled with 20 micro liters DMSO). Plates were incubated in 37 degrees for about 24 hours. After 24 hours, the diameter of the inhibitory zone formed around the wells under light was seen and it was measured using caliper, and was then reported in form of millimeter [4].

G) Determining the amount of the least growth inhibitory concentration and the least bactericidal concentration

1. This method is used for determining the minimum inhibition concentration and minimum bactericidal concentration of different material. MIC and MBC testing is done like the following: a series of 11 testing tubes with successive dilutions are prepared.

2. Ten tubes are selected and are specified from one to ten; one milliliter of the liquefied culture environment is added to each of the tubes numbered 1-10.

3. One milliliter of the intended pigment solution prepared in tube 10 is added (pigment is solved in DMSO)

4. one milliliter of tube one is taken and added to tube 2 and after mixing, thus action is carried out to tube 10; thereafter, 10 milliliter us taken from tube 10 and is discarded, and thus 2* dilutions are prepared.

5. one milliliter of the prepared bacterial mixture is added to each of tubes 1-10 and the content of the tubes are stirred well.

6. All tubes are incubated for 18-24 hours at the temperature of 35-37 degrees centigrade.

7. After the initial incubations, the tube indicating

visible inhibition is selected to be the one with the least inhibitory concentration.

8. To calculate MBC amount, it is imperative to culture 0/01 milliliter of each tube in which there is no visible culture inside a plate containing agar.

9. After tallying colonies on the agar plate, MBC is defined as the least concentration of the material under study which leads to the death of 99/9% of all bacteria. Thus, the tube having 5 colonies or less than that can be selected as MBC provided that 0/01 milliliter of the tube under testing is exactly taken.

10. Tube No. 11 is used for controlling, which is expected to grow there without problems and DMSO not be inhibitory [4].

RESULTS:

The results indicated that canned mushrooms and beans produced by one of the local companies and shortly after its expiry date got their said E. coli and Staphylococcus aureus isolated and identified in accordance to the said microbiological methods. In the end, they were retained in stored cultures until the day of experiment.

Standard strain prepared was cultured on agar nutrient having been activated so that pigment production is nominally examined and it is made clear whether this strain produces pigment or not. The stages of production and extraction were performed in the part of material and methods and pigment powder was obtained.

Pigments produced by the said method were examined in the well diffusion which were created around the well of 10 mm inhibitory zone against and Staphylococcus and 5 mm against E. coli. In the following, inhibitory zone photos are brought.

Results pertaining to MIC and pigment MBC determination

MIC and MBC amount of produced prodigiosin is brought as a graph in the following.

MIC and MBC amount of produced prodigiosin against said E. coli and Staphylococcus

DISCUSSION:

Prodigiosin's anti-microbial functioning is related to damaging the plasma membrane. As Suryawanshi et al. have pointed out in 2014 in their own studies [5].

Prodigiosin has a hydrophobic property which suggests prodigiosin may perform its own anti-microbial activity through chaotropicity-mediated stress, while soluble chaotrops can reduce water activity and add an extra stress to the cell. Prodigiosin is not sufficiently soluble to do this.

In addition, chaotropicity-mediated stress will cause

changes in the cells in different surfaces and in different places. Thus, this kind of performance may be the basis for inhibiting proton pump and inability in maintain pH slope of the bacterium by prodigiosin.

That which is clear is that in microbial cells, the main target of prodigiosin is the plasma membrane instead of Cytosolic enzyme systems [6].

In 2012, Gulani *et al.* conducted a study with the heading of assessing parameters affecting increasing production of *Serratia marcescens* prodigiosin and assessing its anti-microbial, anti-oxidant and dyeing property. The findings showed that prodigiosin's anti-microbial property produced by Disk diffusion against *Staphylococcus aureus* bacteria, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida parapsilosis*, *Cryptococcus* were 17/5, 10/5, neutral, neutral, 11/3 and 15 mm[7].

The findings also suggested that prodigiosin produced by the existing strain in this study has a lower anti-microbial property against *aureus* compared to prodigiosin produced by the strain existing in the Gulani's study; however, in relation to *E.coli*, the pigment in our study has more effect. In the current study, 10 and 5 mm zone were obtained for *E. coli* and *staphylococcus aureus* respectively, while in the study by Gulani, 17/5 mm was achieved for *staphylococcus* and the rate for *E.coli* was neutral. These discrepancies could be related to the difference in *Serratia marcescens* strain, extracting pigment, solution used and test conditions.

In the Ananda Priya *et al.* study, in 2013, they concluded that prodigiosin showed higher antifouling activity against Marine sediment bacteria such as *Alteromonas* and *Gallionella*. The minimum inhibitory concentration (MIC) and the minimum bactericide concentration (MBC) of the pigment in regards to *Alteromonas* were 6/75 and 12/5 microgram/ml respectively.

Prodigiosin significantly caused *Cyanobacteria's* inhibition of adhesion on glass surface which in turn resulted in increasing the use of bacterial pigments as a source of antifouling combinations for controlling fouling problems in marine settings [8]. There are differences between the two studies. They achieved higher MIC and MBC. Though the bacterial genus differs (*Alteromonas*), warm bacteria were negative and compared to our results in relation to negative warm bacteria, *E.coli* yielded better findings.

Vora *et al.* conducted a study in 2014 and extracted prodigiosin from *Serratia marcescens* strain and then investigated its Anti-microbial and antioxidant property. This pigment has displayed an anti-bacterial activity against *B. cereus*, *S.aureus* and *E.coli* with an inhibition zone of 12 and 7 and 6 mm respectively[9]. In comparison to studies done up to now, Vora *et al's* findings are more similar to those of the current

research.

In 2015, Lapenda *et al.* sought to seek anti-microbial activity of *Serratia marcescens* prodigiosin, strain of UFPEDA 398. Oxacillin resistant to antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus spp* and *Staphylococcus aureus* resistant to oxacillin was tested.

A significant area of growth inhibition disk diffusion test for *Staphylococcus aureus* ($0/6 \pm 35$), *Enterococcus faecalis* (1 ± 22) and *Streptococcus pyogenes* ($0/6 \pm 14$) is shown. Minimum inhibitory concentration observed for the three bacteria were 1, 2 and 4 micrograms / ml, while minimum bactericidal concentration was in the range of 2, 4, 8 and 16 micrograms / ml [10]. In the study by Lapenda, much antimicrobial was seen against property against *Staphylococcus aureus* which was approximately three times as much as in our study.

CONCLUSION:

Prodigiosin produced by *Serratia marcescens* bacteria showed promising bactericide and bacteriostatic effects and more studies about its application are recommended Findings, also, suggested that prodigiosin can be used in food industry.

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