Research Article

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Characterization of *Serratia marcescens*: Prodigiosin Synthesis under Various Environmental Conditions and Biofilm Formation

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Abstract

Serratia marcescens is known for its high production of Prodigiosin, a pigment that serves as a virulence factor and possesses beneficial biological, antibacterial, antifungal, and antimalarial characteristics. Another virulence factor of *Serratia marcescens* is the ability to produce biofilms, which are aggregations of microorganisms adhering to surfaces surrounded by a self-produced matrix. These biofilms pose many health risks in the milk production and processing industry, such as milk spoilage, poor-quality milk products, and other health risks. After 24 hours of incubation at 30°C, colonies on Chrome agar appeared pink with a dark center, while on nutrient agar, colonies appeared red at 28°C due to *Serratia marcescens'* ability to produce pigment. The ability of these local isolates to produce pigment was evaluated using NB Medium with different incubation times. A total of 11 isolates from milk samples showed the ability to produce a high concentration of Prodigiosin when incubated for 72 hours. All isolates were found to produce Biofilm at different rates. In isolate (6), we observed the highest production of Biofilm.

Keywords: *Serratia marcescens,* Prodigiosin, pigment, Biofilm.

Introduction

Serratia marcescens is one of the types of opportunistic bacteria that belong to Gram-negative. It has a structure of rods. Due to recent bacterial taxonomy revision, it belongs to the newly classified Yersiniaceae family under the Enterobacterales order (1). These organisms have been isolated from several environmental samples such as water, soil, and different plant materials, indicating that these are spread widely worldwide (2). *Serratia marcescens* has one of the most important virulence Factors in the ability to form biofilms, which are the

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complex, structured microbial communities that develop on the surface and are enclosed in the self-produced extracellular matrix. *Serratia marcescens* is known to use biofilm production as one of its key strategies for enhancing its strength and tenacity in specific environments, including clinical environments (3,4). *Serratia marcescens* biofilm is complex with microcolonies and water channels, which facilitate nutrition and oxygen transport that enables the living cells of the bofilm (3). Serratia marcescens forms biofilms that are highly resistant to antimicrobial agents and the host immune system, making them cumulative and difficult to eradicate (5). *Serratia marcescens* has long been the subject of focused interest in scientific circles because of its ability to produce an intense red dye called prodigiosin (6). Although the compound has been revealed to possess antibacterial potential, it also shows anticancer and immunosuppressive activities of clinical interest (7). The biosynthesis of Prodigiosin in *Serratia* is regulated by the PhoBR two-component system operons, which are under the influence of changes in phosphate concentration (7). Nevertheless, the fact that Prodigiosin has bacteriostatic ability in the generating organism enables us to understand that it has a natural role in defense against microbes since its ability to inhibit microbial growth has been confirmed (7).

In addition, the production of siderophores, which are organic compounds that increase iron solubility, has been observed in *Serratia* strains. This observation suggests a possible link between the presence of iron and the production of pigments (8). Interestingly, not all the *Serratia* strains are colored red, and it is evident that the production of Prodigiosin is not so diverse in different species (9). The difference in pigmentation has implications in the clinical context because even non-pigmentogenic *Serratia* strains have been reported as a potential threat in hospitals (9). This pigment was found to absorb the red and blue light of the spectrum, and its synthesis depends on many factors, such as incubation time, pH, carbon and nitrogen sources, and inorganic salts (9). Current studies Pare concerned with the discovery of other types of Prodigiosin and the enhancement of their production as the typical diet condition may not provide the right condition for pigment formation (10). Scholars have managed to enhance the production of Prodigiosin in *S. marcescens* strains by analyzing diverse carbon sources and culture factors (10).

Materials and Methods

Isolation and identification: *Serratia marcescens* was isolated from milk samples collected from different areas of Basrah. Before milk sample collection, the udder was cleaned with tap water and dried with a clean towel. The teat was then dipped in a 1:1000 iodine solution and allowed to dry. The teat was then dipped in 70% alcohol and allowed to dry. One or two streams of milk were sampled and discarded. The milk was collected in a sterile container (60 ml), and the samples were transferred into an ice box.

The samples were incubated on chrome *Serratia* agar (Himedia/India) at 30°C for 24 hours. Subsequently, the morphology and

color of the colonies were examined. The suspected colonies were subsequently cultivated on nutrient agar (Himedia/India) at 28°C for 24 hours to identify the presence of the red pigment known as Prodigiosin.

Molecular identification: Identification and confirmation of organisms were done by 16SrRNA sequencing. DNA was isolated from the organism using a DNA extraction kit (Trans/China) according to the manufacturer's instructions. Following isolation and quantification, DNA samples were stored at -20°C until use.

PCR amplification: The large fragment of the 16S rRNA gene was amplified by PCR using the universal primers, according to (11). In the PCR reactions, we used the GoTaq® G2 green master mix (Promega, USA). Each PCR tube contained the following reagents: 12.5 µl of Green Master Mix, 1.5 µl of both forward and reverse primers, 100 ng of DNA template, and the volume was adjusted to 25 µl using nuclease-free water. The thermocycling conditions for the amplification of the 16S rRNA gene included an initial denaturation at 95°C for 5 minutes, followed by 34 cycles that included melting at 95°C for 20 seconds, annealing at 59.5°C for 30 seconds, elongation at 72°C for 1.5 minutes, and a final extension at 72°C for 7 minutes.

Analysis of the PCR Products: The PCR products were resolved by electrophoresis on a 1.5% agarose gel in 1x TBE buffer (Promega/USA). Ten microliters of DNA and five microliters of the ladder were introduced into the wells of the agarose gel. After electrophoresis, the gels were analyzed using a UV transilluminator.

Sequencing: To validate the PCR results, PCR products were sequenced at Macrogen Company in Korea, utilizing the same forward and reverse primers employed in amplifying the 16S rRNA gene for the forward and reverse sequencing, respectively.

Prodigiosin production by *Serratia marcescens*: *Serratia marcescens* was cultivated on nutrient agar and placed in an incubator at 28°C for 16 hours. One bacterial colony was selected and introduced into a 9 ml nutrient broth. The optical density of the culture was subsequently adjusted to 0.4, and the adjusted bacterial culture was diluted 100x using nutrient broth. The diluted culture was then incubated at 28°C for 24, 48, and 72 hours (12,13).

Estimation Of Prodigiosin: To estimate prodigiosin production, use the formula below (12):

$$\text{Prodigiosin units/cell} = \frac{([\text{OD}_{499} - (1.381 \times \text{OD}_{620})]) \times 1000}{\text{OD}_{620}}$$

$$\text{Where, } \text{OD}_{499} = \text{pigment absorption in culture}$$

$$\text{OD}_{620} = \text{bacterial culture absorption}$$

$$1.381 = \text{constant}$$

The presence of Prodigiosin in bacterial culture is detected by observing a solitary peak at 499 nm (OD499) (12). The value (1.381 × OD620) corresponds to the number of bacterial cells, while OD499-(1.381× OD620) indicates the absorption of Prodigiosin. To express prodigiosin units on a per-cell basis, the absorbance value of Prodigiosin is divided by the bacterial cell absorbance value (OD620). To prevent working with small numbers (<1), a factor of 1000 is used in the formula.

Evaluation of Biofilm Production by *Serratia marcescens*: To assess biofilm production, overnight cultures of *Serratia marcescens* were diluted with nutrient broth (NB) to an optical density (OD) of 0.1 at 600nm (14). Then, 200 µL of bacterial suspension aliquots were transferred to sterile 96-well polystyrene microplates and incubated overnight at 37°C. After aspirating the non-adherent planktonic cells, the wells were washed thrice with 300 µL of sterile water. The adherent cells were fixed with 99% methanol for 25 minutes and then stained with 200 µL of 1% crystal violet for another 25 minutes. After washing off the unattached dye and air-drying the plates, the bound crystal violet dye was extracted with 200 µL of 33% glacial acetic acid. The mean optical densities were calculated at a wavelength of 590 nm (15).

Results

Cultural characteristics: *Serratia marcescens* was isolated from a milk sample. Identification was done based on its cultural characteristics. After 24 hours of incubation at 30°C, colonies on Chrome agar appeared pink with a dark center (Figure.1A), while on nutrient agar, colonies appeared red at 28°C due to *Serratia marcescens* ability to produce pigment (Figure.1B).

16S rRNA Gene PCR results: The 16S rRNA gene was amplified in all the isolates, and the PCR bands showed 1500 bp (Figure.2).

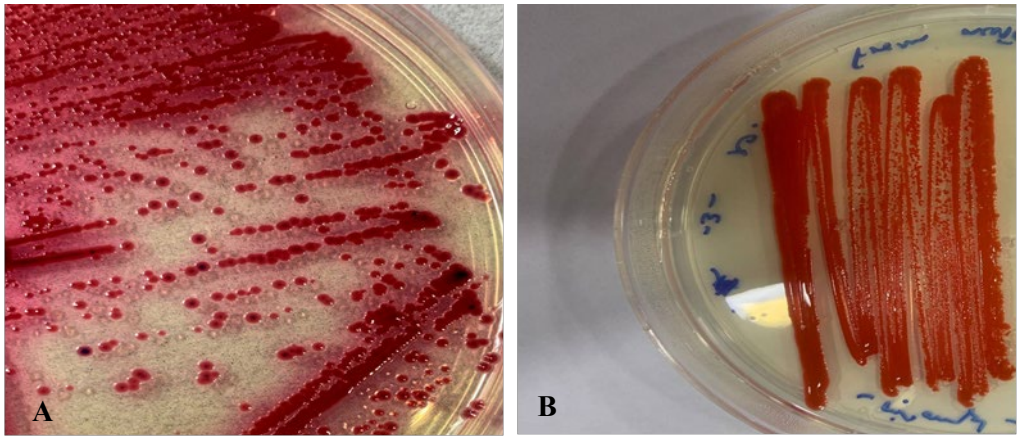

Figure.1 *Growth of Serratia marcescens* on agar: (A) on chrome agar, (B) on nutrient agar

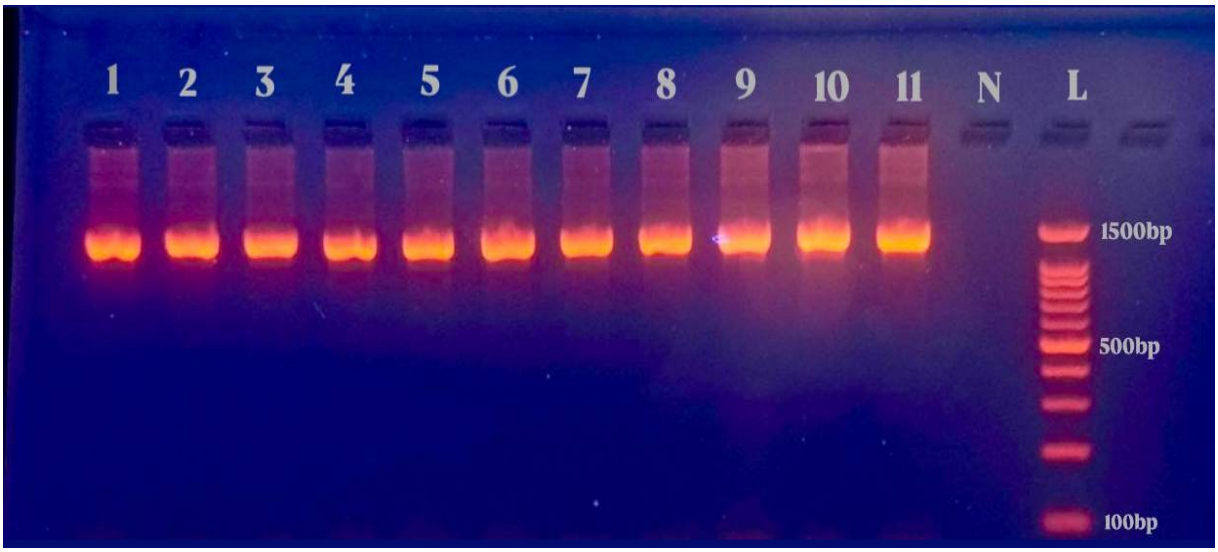

Figure 2. ***16S rRNA*** **gene, the size of the PCR product is 1500bp, L: marker DNA ladder 100bp, N: negative control. Lines (1,2,3,4,5,6,7,8,9,10,11) represent samples.**

Sequencing of the PCR products: Sequencing of the PCR products and subsequent BLAST analysis with the NCBI database verified that the isolates are classified as Serratia marcescens.

Screening of *Serratia marcescens* isolates for prodigiosin production: In order to evaluate the isolates' capacity for producing prodigiosin pigment and the effect of incubation time on its production, the ability of these local isolates to produce pigment was evaluated using NB Medium with different incubation times. This was done by determining the pigment activity (U/cell) in the culture, following the pigment assay procedure described in section (2.2). Eleven Serratia marcescens isolates showed the ability to produce the prodigiosin pigment; the maximum production was observed at 72h as shown in (Table 1) and (Figures 3, 4). Figure 5 shows the standard color of broth media in Comparison with prodigiosin-producing *Serratia marcescens* and non-prodigiosin Serratia *marcescens*

Screening of *Serratia marcescens* isolates for biofilm production: All *Serratia marcescens* isolates utilized in this study exhibited varying rates of biofilm production. Our observation revealed that the (6) isolate had a higher rate of biofilm production than the other isolates (Figure 6). Optical density results of the studied bacteria are presented in (Figure 7).

Table.1 Production of Prodigiosin by *Serratia marcescens* at different incubation times in nutrient broth.

Isolates	24h	48h	72h
1	80.53	130.11	154.71
2	30.76	106.17	264.83
3	47.57	131.82	214.74
4	76.14	80.53	241.22
5	105.48	202.33	514.83
6	73.14	223.65	236.02
7	87.75	133.28	301.92
8	51.43	131.82	285.66
9	161.85	409.69	441.22
10	60.71	119	255.36
11	171.63	219	423.34

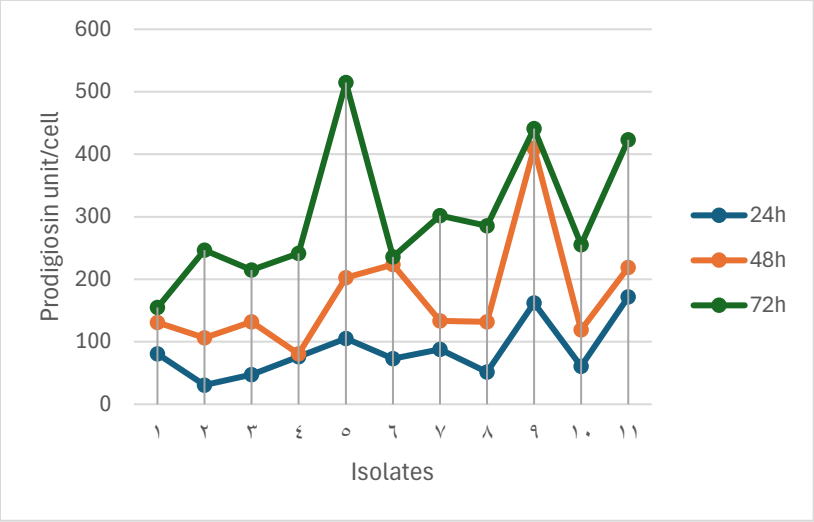

Figure.3 Production of Prodigiosin by *Serratia marcescens* at different incubation time in nutrient broth.

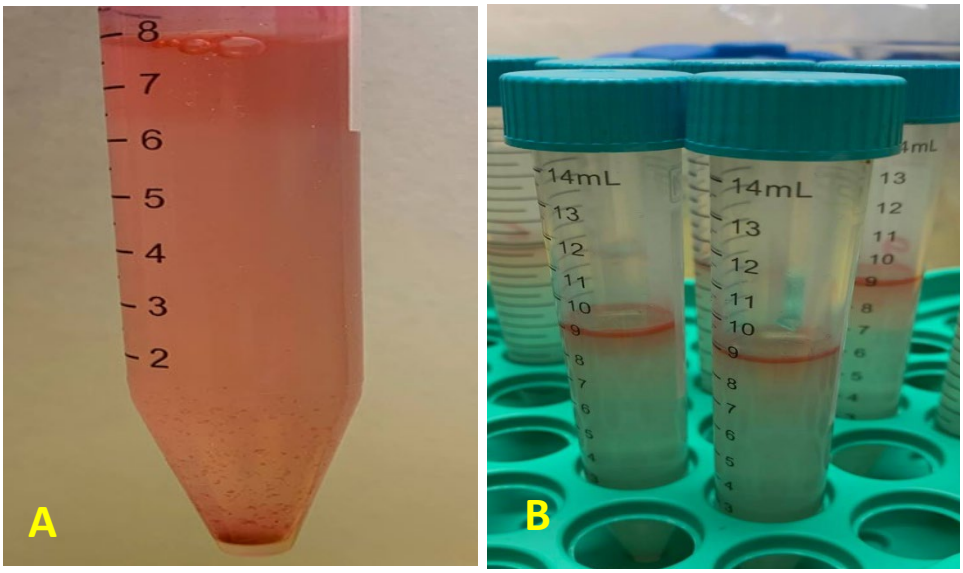

Figure 4. Prodigiosin pigment production by *Serratia marcescens* in nutrient broth at different incubation time: (A) at 72 h. (B) at 24h

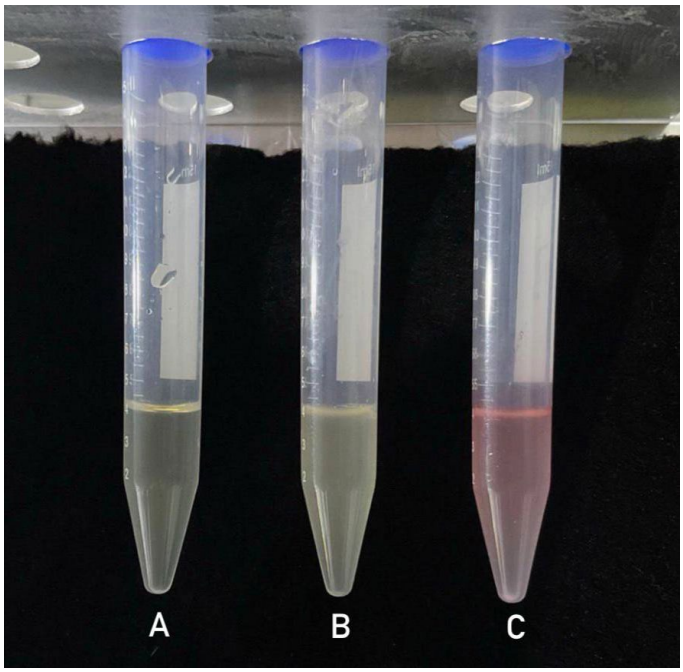

Figure 5. A. control nutrient broth, B. *Serratia marcescens* isolate unproduced prodigiosin pigment, C. *Serratia marcescens* isolate produced prodigiosin pigment

Figure.6 Biofilm production by *Serratia marcescens*

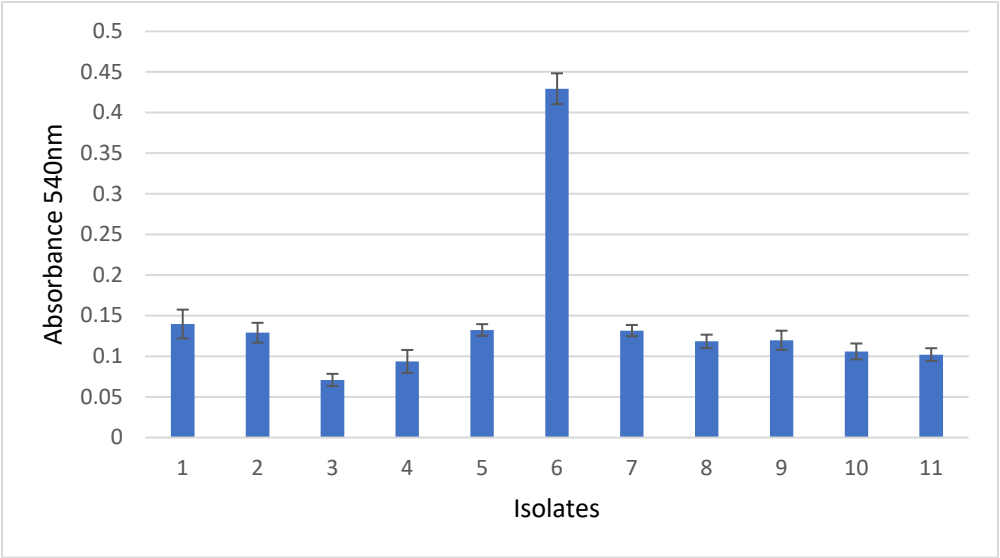

Figure.7 Biofilm production ability (OD average) of bacterial isolates

Discussion

Morphological characteristics of Serratia marcescens were observed on chrome agar (Himedia/India) after 24 hours at 30°C: colonies appeared pink with a dark center. The colonies were red on nutrient agar due to prodigiosin pigment at 28°C for 24h. These findings are consistent with the research conducted by (13). The 16S rRNA was amplified, sequenced, and identified as *Serratia marcescens*.

Microorganisms that create and release secondary metabolites are very interesting. Undoubtedly, these metabolites provide

many pharmacological qualities that will enhance human health and nutrition while also contributing to economic value. Biopigments, which microbes can synthesize, are among the most plentiful secondary metabolites, and Prodigiosin are one of these microbial pigments (16). Prodigiosin is regarded as a significant molecule due to its application in several fields and its potential as a promising study area. Prodigiprodiginine family member, consistingprodiginine family member, consisting of natural red pigments with a low molecular weight of 323.4 Daltons. These pigments are only present in the later stages of bacterial growth, specifically during idiophase. Numerous strains of Serratia spp synthesize Prodigiosin (C20H25N3O). (16).

Prodigiosin is attracting significant interest due to its wide variety of biological functions. It exhibits greater antibacterial efficacy against Gram-positive bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *and* Gram-negative bacteria such *as Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* (17, 12). Prodigiosin has also been effective against *Borrelia burgdorferi*, the causative agent of Lyme disease (18).

Prodigiosin pigment is also well known for having antifungal properties against many fungi (19–21). In order to ascertain the ideal pigment synthesis, we assessed the capacity of Serratia marcescens isolates to create the prodigiosin pigment at various incubation durations. Additionally, it exhibits antimalarial (22) and antiparasitic (23) properties. We discovered that the bacterial isolate produced more pigment at 28 °C and that as the temperature rose, the production rate fell. Furthermore, after 72 hours of incubation, pigment synthesis increased but dropped after 96 hours, indicating that the bacteria employed the protein prodigiosin as a source of nourishment while under stress. Furthermore, according to research, cultures cultured at 37°C do not develop any pigment. (24).

Also, the -forming assay of the isolates of Serratia marcescens from cow milk in the 96-well microtiter was the central study in our research. The formation of the Biofilm by each isolate at various rates was not the same. The can be rather dangerous. , which may affect its raw form, consequently affecting the safety and quality of cow milk and byproducts. Dairy biofilms included protein, calcium phosphate, milk remnants, and bacterial extracellular polymeric molecules. Single cells of the planktonic

bacteria category are less resistant to antimicrobial agents than bacteria formed in a biofilm. (25).

Psychotropic bacteria can produce extracellular enzymes, such as proteases and lipases, which can lead to milk spoilage. In addition, most psychotropic bacteria can create biofilms on different types of milk storage and processing equipment (26, 27).

Serratia has been isolated from several cases of bovine mastitis. Also, it can produce biofilms on the inner surfaces of the milk processing lines. This action spoils the line and product at different points (1). *Serratia marcescens* because of adherence to the inside stainless-steel surfaces of milk processing pipes and pasteurization equipment, can contaminate and spoil milk and milk products (28).

Conclusion

Serratia marcescens is a member of the family *Yersiniaceae*. This bacterium can produce prodigiosin pigment and form biofilms. In this study, *Serratia marcescens* was isolated from milk. Then, it is identified by cultural characteristics and conventional methods. The study involved determining the prodigiosin production of isolates. More prodigiosin pigment production was observed at 28°C, and this decreased with the increase in temperature. The optimum pigment production rate peaks at 72 hours of incubation but decreases after 96 hours. The study concludes that all isolates produce, exhibiting varying rates of biofilm production.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical Clearance

This work is approved by The Research Ethical Committee.

Reference

1. Friman M. J, Eklund M. H, Pitkala A. H, Pitkälä A. H, Rajala-Schultz P. J, Rantala M. H. J. (2019) . Description of two Serratia marcescensassociated mastitis outbreaks in Finnish dairy farms and a review of literature.Friman et al. *Acta Vet Scan*d, *61*:54 DOI: 10.1186/s13028-019-0488-7

2. Chen, S., Blom, J., & Walker, E. D. (2017, August 10). Genomic, Physiologic, and Symbiotic Characterization of Serratia marcescens Strains Isolated from the Mosquito Anopheles stephensi. *Frontiers in Microbiology*, *8*:1483. DOI:10.3389/fmicb.2017.01483

3. Kiran, F., Karaca, B., & Erdoğan, A. F. (2022). Microbial Biofilms in Veterinary Medicine. *Ankara ÜNiversitesi Veteriner FakülLtesi Dergisi*, *70*(1), 107–114. DOI:10.33988/auvfd.1097786
4. Bryers, J. D. (2008b). Medical biofilms. *Biotechnology and Bioengineering*, *100*(1), 1–18. DOI:10.1002/bit.21838
5. Khatoon, Z., McTiernan, C. D., Suuronen, E. J., Mah, T. F., & Alarcon, E. I. (2018). Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. *Heliyon, 4*(12), e01067. DOI:10.1016/j.heliyon.2018.e01067
6. Sharma, A., & Johri, B. (2003). Growth promoting influence of siderophore-producing Pseudomonas strains GRP3A and PRS9 in maize (Zea mays L.) under iron limiting conditions. *Microbiological Research, 158*(3), 243–248. DOI:10.1078/0944-5013-00197
7. Gristwood, T., Fineran, P. C., Everson, L., Williamson, N. R., & Salmond, G. P. (2009). The PhoBR two-component system regulates antibiotic biosynthesis in Serratia in response to phosphate. *BMC Microbiology*, *9*(1). DOI:10.1186/1471-2180-9-112
8. Radzki, W., Mañero, F. J. G., Algar, E., García, J. a. L., García-Villaraco, A., & Solano, B. R. (2013). Bacterial siderophores efficiently provide iron to iron-starved tomato plants in hydroponics culture. *Antonie Van Leeuwenhoek*, *104*(3), 321–330. DOI:10.1007/s10482-013-9954-9
9. De Araújo, H. W. C., Fukushima, K., & Takaki, G. M. C. (2010). Prodigiosin Production by Serratia marcescens UCP 1549 Using Renewable-Resources as a Low Cost Substrate. *Molecules/Molecules Online/Molecules Annual*, *15*(10), 6931–6940. DOI:10.3390/molecules15106931
10. Zang, C. Z., Yeh, C. W., Chang, W. F., Lin, C. C., Kan, S. C., Shieh, C. J., & Liu, Y. C. (2014). Identification and enhanced production of prodigiosin isoform pigment from Serratia marcescens N10612. *Journal of the Taiwan Institute of Chemical Engineers*, *45*(4), 1133–1139. DOI:10.1016/j.jtice.2013.12.016
11. Hasan, M. F., Islam, M. A., & Sikdar, B. (2022). First report of Serratia marcescens associated with black rot of Citrus sinensis fruit, and evaluation of its biological control measures in Bangladesh. F1000Research, 9, 1371. DOI:10.12688/f1000research.27657.2
12. Yip, C. H., Mahalingam, S., Wan, K. L., & Nathan, S. (2021). Prodigiosin inhibits bacterial growth and virulence factors as a potential physiological response to interspecies competition. *PloS One*, *16*(6), e0253445. DOI:10.1371/journal.pone.0253445
13. Samrot, A. V., Pachiyappan, S., Chandana, K., & Gopakumaran, N.

(2011). Optimization of prodigiosin production by Serratia marcescens SU-10 and evaluation of its bioactivity. *Int. Res. J. Biotechnol.14*:47–53. DOI: 10.1016/j.btre.2017.04.001

14. Lutfi, Z., Usup, G., & Ahmad, A. (2014). Inhibition of Serratia marcescens Smj-11 biofilm formation by Alcaligenes faecalis STN17 crude extract. *AIP Conference Proceedings*. DOI:10.1063/1.4895260
15. Khayyat, A. N., Hegazy, W. a. H., Shaldam, M. A., Mosbah, R., Almalki, A. J., Ibrahim, T. S., Khayat, M. T., Khafagy, E. S., Soliman, W. E., & Abbas, H. A. (2021). Xylitol Inhibits Growth and Blocks Virulence in Serratia marcescens. *Microorganisms, 9*(5), 1083. DOI:10.3390/microorganisms9051083
16. Gulani, C., Bhattacharya, S., & Das, A. (2012). Assessment of process parameters influencing the enhanced production of Prodigiosin from Serratia marcescens and evaluation of its antimicrobial, antioxidant and dyeing potentials. *Malays J Microbiol* *8*(2):116-122. DOI: 10.21161/mjm.03612
17. Darshan, N., & Manonmani, H. K. (2016). Prodigiosin inhibits motility and activates bacterial cell death revealing molecular biomarkers of programmed cell death. *AMB Express, 6*:50. DOI:10.1186/s13568-016-0222-z
18. Feng, J., Shi, W., Zhang, S., & Zhang, Y. (2015). Identification of new compounds with high activity against stationary phase Borrelia burgdorferi from the NCI compound collection. *Emerging Microbes & Infections*, *4*(1), 1–15. DOI:10.1038/emi.2015.31
19. Suryawanshi, R. K., Patil, C. D., Koli, S. H., Hallsworth, J. E., & Patil, S. V. (2016). Antimicrobial activity of Prodigiosin is attributable to plasma-membrane damage. *Natural Product Research*, *31*(5), 572–577. DOI:10.1080/14786419.2016.1195380
20. El-Bondkly, A. M. A., El-Gendy, M. M. A., & Bassyouni, R. H. (2012). Overproduction and biological activity of prodigiosin-like pigments from recombinant fusant of endophytic marine Streptomyces species. *Antonie Van Leeuwenhoek, 102*(4), 719–734. DOI:10.1007/s10482-012-9772-5
21. Duzhak, A. B., Panfilova, Z. I., Duzhak, T. G., Vasyunina, E. A., & Shternshis, M. V. (2012). Role of Prodigiosin and chitinases in antagonistic activity of the bacterium Serratia marcescens against the fungus *Didymella applanata*. *Biochemistry (Moscow), 77*(8), 910–916. DOI:10.1134/s0006297912080123

22. Papireddy, K., Smilkstein, M., Kelly, J. X., Shweta, N., Salem, S. M., Alhamadsheh, M., Haynes, S. W., Challis, G. L., & Reynolds, K. A. (2011b). Antimalarial Activity of Natural and Synthetic Prodiginines. *Journal of Medicinal Chemistry, 54*(15), 5296–5306. DOI:10.1021/jm200543y

23. Ehrenkaufer, G., Li, P., Stebbins, E. E., Kangussu-Marcolino, M. M., Debnath, A., White, C. V., Moser, M. S., DeRisi, J., Gisselberg, J., Yeh, E., Wang, S. C., Company, A. H., Monti, L., Caffrey, C. R., Huston, C. D., Wang, B., & Singh, U. (2020). Identification of anisomycin, prodigiosin and obatoclax as compounds with broad-spectrum anti-parasitic activity. *PLoS Neglected Tropical Diseases, 14*(3), e0008150. DOI:10.1371/journal.pntd.0008150

24. Qadri, S. M. H., & Williams, R. P. (1972). Induction of Prodigiosin Biosynthesis after Shift-Down in Temperature of Nonproliferating Cells of Serratia marcescens. *Applied Microbiology, 23*(4), 704–709. DOI:10.1128/am.23.4.704-709.1972

25. Weber, M., Liedtke, J., Plattes, S., & Lipski, A. (2019). Bacterial community composition of biofilms in milking machines of two dairy farms assessed by a combination of culture-dependent and –independent methods. *PloS One, 14*(9), e0222238. DOI:10.1371/journal.pone.0222238

26. Ngo, T. P., Bui, H. L., Pham, T. B. N., Huynh, V. T., & Bui, V. (2023). Identifying biofilm forming bacteria in cow milk in Mekong Delta, Viet Nam. *CTU Journal of Innovation and Sustainable Development, 15*(1), 84–90. DOI:10.22144/ctu.jen.2023.011

27. Yuan, L., Sadiq, F. A., Burmølle, M., Wang, N., & He, G. (2019). Insights into Psychrotrophic Bacteria in Raw Milk: A Review. *Journal of Food Protection, 82*(7), 1148–1159. DOI:10.4315/0362-028x.jfp-19-032

28. Cherif-Antar, A., Moussa–Boudjemâa, B., Didouh, N., Medjahdi, K., Mayo, B., & Flórez, A. B. (2015). Diversity and biofilm-forming capability of bacteria recovered from stainless steel pipes of a milk-processing dairy plant. *Dairy Science and Technology, 96*(1), 27–38. DOI:10.1007/s13594-015-0235-4

توصيف السريشيا مارسينس : انتاج صبغة البرودجيوسين تحت ظروف بيئية مختلفة وتكوين الاغشية الحيوية

لبنى مسلم عبد الكريم , علي بلبول الديوان

فرع الاحياء المجهرية, كلية الطب البيطري ، جامعة البصرة .

الخلاصة

سيريشيا مارسينس معروفة بإنتاجها العالي للبرودجيوسين ، وهي صبغه تعمل كعامل ضراوة وتتميز بخصائص بيولوجية ومضادة للبكتيريا والفطريات ومضادة للملاريا. عامل ضراوة اخر لبكتريا السريشيا هو قدرتها على إنتاج الأغشية الحيوية، وهي تجمعات من الكائنات الدقيقة التي تلتصق بالأسطح محاطة بمصفوفة ذاتية الإنتاج. تشكل هذه الأغشية الحيوية مخاطر صحية عديدة في إنتاج ومعالجة الحليب، مثل فساد الحليب ومنتجاته ذات الجودة الرديئة، ومخاطر صحية أخرى. لوحظ بعد حضن هذه البكتيريا لمدة 24 ساعة وبدرجة 30 مئوية، ظهرت المستعمرات على أجار الكروم باللون الوردي مع مركز داكن، بينما ظهرت المستعمرات على أجار المغذيات باللون الأحمر عند 28 درجة مئوية بسبب قدرة السريشيا على إنتاج الصبغة. تم تقييم انتاج هذه العزلات باستخدام اكار المغذيات في اوقات حضن مختلفة. اظهرت 11 عزلة من عينات الحليب القدرة على إنتاج تركيز عالٍ من البرودجيوسين عند حضنها لمدة 72 ساعة. ايضا وجد أن جميع العزلات تنتج الأغشية الحيوية بمعدلات مختلفة ولاحظنا اعلى انتاج في العزلة (6).

الكلمات المفتاحية: سريشيا مارسينس، برودجيوسين ، صبغة ، غشاء حيوي ، مضاد بكتيري.