REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 1 (2023)
http://www.veterinaria.org
Article Received: 30 October 2022; Revised: 27 December 2022; Accepted: 24 January 2023

Efficiency of Purified Prodigiosin Pigment from Serratia Marcescens as Bioremediator of Contaminated Soils and Burned Motor Oil with Hydrocarbons

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Abstract

Serratia marcescens red pigment prodigiosin (PG) has been identified as a bioactive secondary metabolite that can be employed as a bioremediator. The prodigiosin red pigment was developed in several *Serratia marcescens* isolates from water. This pigment was extracted at higher quantities using a 1:2 (v/v) mixture of ethanol and methanol, yielding 53.8 mg/ml. The use of 100g/ml prodigiosin on contaminated soil improved the removal of hydrophobic pollutants (48%) from polluted soil more effectively than 150 mg/ml burned motor oil (32%. The proportion of hydrocarbon degradation was also increased by extending the incubation time, reaching 67 percent in polluted soils and 55 percent in burned motor oil after 96 hours, respectively. As a result, bioremediation with prodigiosin pigment has become a normal practice, as biological treatments are more efficient in eliminating waste and protecting natural resources, as well as being more cost-effective.

Keywords: prodigiosin pigment, *Serratia marcescens*, contamination

INTRODUCTION

As a cocluded of human revolution and advanced and industrial growth, large amounts of materils are constantly discharged into the environment. The main pollutants have a global attention in last decads were Petroleum and petrochemical pollution, such as petroleum hydrocarbons (PHCs). Contamination by these chemicals degrades the global ecology and reduces biodiversity significantly (1).

Environmental contamination caused by oil and PHCs is one of the most pressing issues today (2, 3). Bioremediation has been employed in the past to restore PHC-polluted settings (4, 5). Bioremediation has emerged as an alternative approach for cleaning up oil-contaminated environments, in which microbial communities play a crucial part in the process, either by direct pollutant breakdown or through contact with other microorganisms (6, 7).

Bioremediation is a process that uses living organisms' ability to decrease, degrade, and/or eliminate toxins from marine and terrestrial ecosystems, reducing the risk to human health while returning the environment to its original state (7, 8). Reduced solubility, redox

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reactions, and adsorption of pollutants from the damaged environment are the basic principles of bioremediation (9).

Serratia is a member of the Enterobacteriaceae family that is opportunistic to humans, plants, and insects, which is a broad and diverse collection of facultatively anaerobic, non-spore-forming, Gram-negative, rod-shaped bacteria (10, 11). Prodigiosin is a red, non-diffusible pigment produced by some *Serratia* strains (12). Prodigiosins are a class of drugs that have anticancer, antimalarial, antibacterial, antifungal, antiproliferative, and immunosuppressive properties. It also offers a lot of potential in the pharmaceutical, food, and textile industries. Purified, high yield, and cost-effective extraction of prodigiosin are critical from an industrial standpoint (13). Thus this research aimed to the purification of prodigiosin from *Serratia marcescens* and use it as an ecofriendly bioremediator for polluted soils with hydrocarbons and burned motor oil.

MATERIALS AND METHODS

Isolation and identification of *Serratia marcescens*

Water samples from al- Razaza Lake were collected, streaked on MacConkey agar, and incubated at 37°C. After an incubation period, the isolates of *Serratia marcescens* were identified using morphological and cultural properties of the colonies, such as form, texture, color, and edges, as assessed by bacterial growth on MacConkey agar (14).

Primary detection of prodigiosin pigment

Prodigiosin production was measured on modified Luria Bertani (LB) agar plates using peptone instead of tryptone at 37°C (15). To identify pigment-producing strains, pink-red growth was used.

Secondary detection of prodigiosin pigment

Before being chosen for further research, the pigmented isolates were cultivated in Luria Bertani broth supplemented with peptone and cultured at 37°C for 24 hours. After centrifugation, the concentration of prodigiosin was measured using the colorimetric method described by (16), which involved mixing equal volumes of generated cell pellets with methanol. After that, the mixture was stirred for at least three hours. After centrifugation at 10000 rpm for 20 minutes to remove cell debris, the supernatant's absorbance at 530 nm was measured. Then the following equation was applied:

$\{Prodigiosin\frac{g}{L} = \frac{O.D.530x323.4}{7.07x104} x dilluted factor\}$ to calculate the concentration of prodigiosin. The dilution factor is equal to the final volume divided by the sample volume.

Extraction of prodigiosin

To extract crude prodigiosin, different solvent ratios were utilized, such as (1:1, 1:2, 1:3, 1:4 v/v) ethanol: methanol. To summarize, the culture was scraped from Petri plates and distilled water washed. After the cleaned pellet was dissolved in 4 ml of each of the solvents

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provided, an absorbance value of 530 nm was recorded. The pigment extraction was higher when the absorbance value at 530 nm was higher.

Application of biosurfactant in the removal of hydrophobic pollutants in polluted soils and burned motor oil

Polluted soils and burned motor oil were used to test prodigiosin's capability for eliminating hydrophobic contaminants. Separately, 5 g of polluted soil samples and 5 ml of burned motor oil were given the following treatments: (A) 10 mL distilled water (control), (B) 10 mL purified prodigiosin solution at concentrations of 50,100, 200, and 400 mg/mL. The washing solution and sediment were separated by centrifugation at 5000g for 20 minutes after being exposed to 150 rpm for 24, 48, 72, and 96 hours at 30°C. When the same amounts of dirt or heavy precipitant were mixed with toluene, the amount of oil remaining in the treated samples was determined. The hydrocarbon-dissolved toluene was measured at 410 um absorbance after centrifugation (17). The following formula was used to calculate the percentage of hydrocarbon degradation **{ (optical density for control- optical density for test)/ optical density for control}x 100.**

RESULTS AND DISCUSSION

Isolation and primary detection of *Serratia marcescens* producers for prodigiosin

Out of 11 gram-negative bacteria, 8 *Serratia marcescens* isolates were found after cultivating water samples on MacConkey agar. Only three of the eight isolates were able to develop the prodigiosin red pigment, as shown in figure (1), with a diameter ranging from 14 to 23 mm, whereas the remaining isolates appeared as cream-white colonies. *Serratia* was recovered from drinking water systems as Gram-negative cells with motile, non-spore-forming rods of around 1 mm wide and 2 mm long. Anaerobic facultatively, oxidase- and catalase-negative. On nutrient agar, brain heart infusion agar, TSA, R2A agar, and MacConkey agar at 28 C, good growth occurs after 24 hours (18,19)

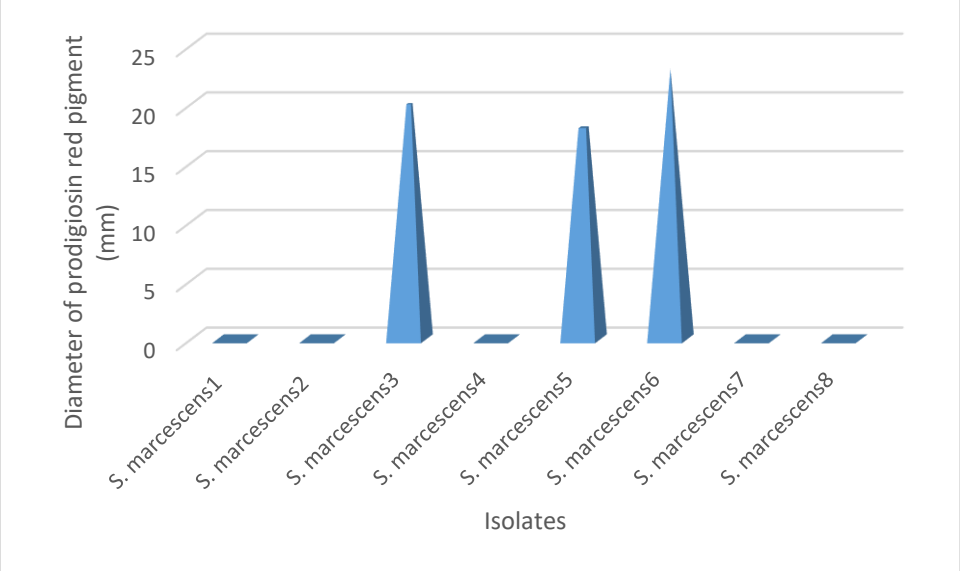

Figure 1: Primary detection of *Serratia marcescens* producers for prodigiosin

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Secondary detection of *Serratia marcescens* producers for prodigiosin

In the secondary analysis, *Serratia marcescens* producers for prodigiosin were used, and the output of prodigiosin pigment appeared to be variable, ranging from 29 to 46 mg/ml (figure-2). A prodigiosin-like pigment was created using *S. marcescens* SS-1. The Luria-Bertani (LB) broth was modified by increasing the amounts of tryptone and yeast extract while removing NaCl from the medium, which is commonly utilized for prodigiosin production with *S. marcescens* strains (20).

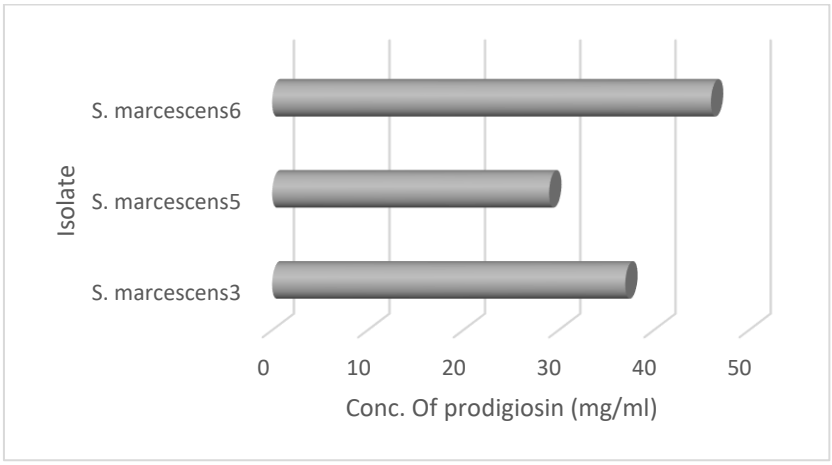

Figure 2: Secondary analysis for determination of *Serratia marcescens* producers for prodigiosin

Extraction of prodigiosin

With a constant volume of ethanol and different volumes of methanol, a mixture of two solvents was used to extract prodigiosin from *Serratia marcescens* 6 isolates, and the results showed that the 1:2 (v/v) combination of ethanol and methanol resulted in extracting this pigment at higher levels, reaching 53.8 mg/ml (figure 3).

To evaluate the efficiency of prodigiosin yield six distinct extraction procedures, like homogenization, ultrasonication, freezing and thawing, heat treatment, organic solvents, and inorganic acids, and found that, ultrasonication followed by organic solvents had the highest extraction rate (13).

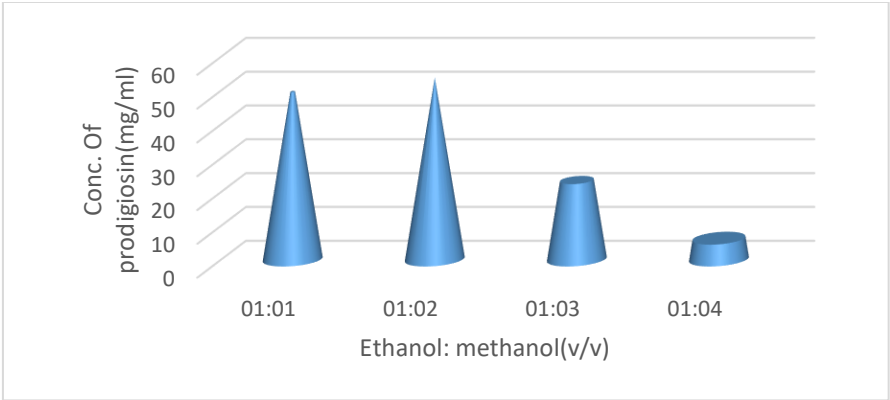

Figure 3: Combination of ethanol and methanol with different volumes to extract prodigiosin pigment from *Serratia marcescens*

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Application of biosurfactant in the removal of hydrophobic pollutants in polluted soils and burned motor oil

Figure (4) shows the results of varying concentrations of prodigiosin generated by *Serratia marcescens* on the rate of hydrophobic pollutant removal. After 24 hours, the data reveals that 100g/ml of prodigiosin increased the removal of hydrophobic pollutants (48%) from polluted soil more efficiently than burned motor oil (32%) at 150 mg/ml.

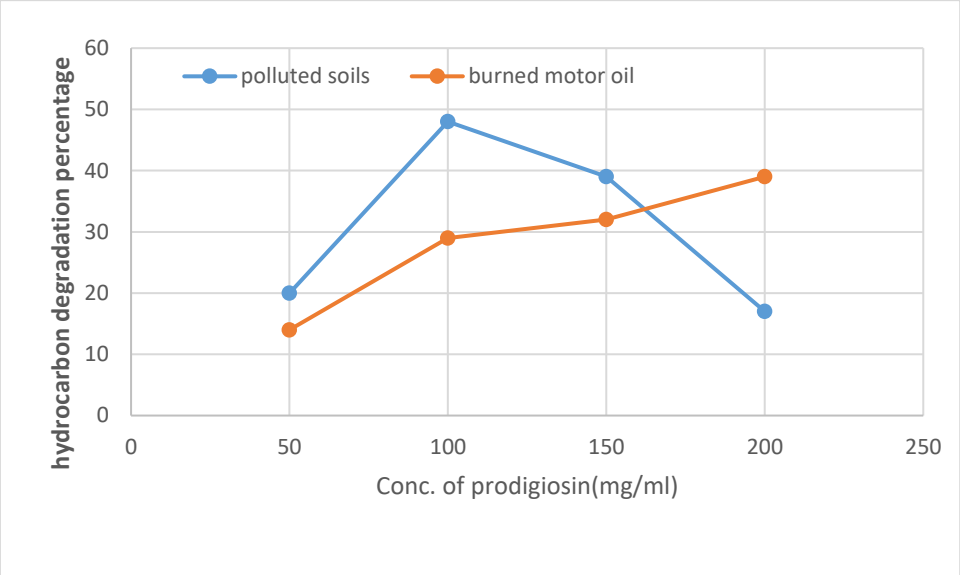

Figure 4: Removal of hydrophobic pollutants in polluted soils and burned motor oil by different concentrations of prodigiosin produced by *Serratia marcescens*

The hydrocarbon degradation percentage was also raised by increasing the incubation duration, reaching 67 % and 55 % after 96 hours in the case of polluted soils and burned motor oil, respectively, as shown in figure (5).

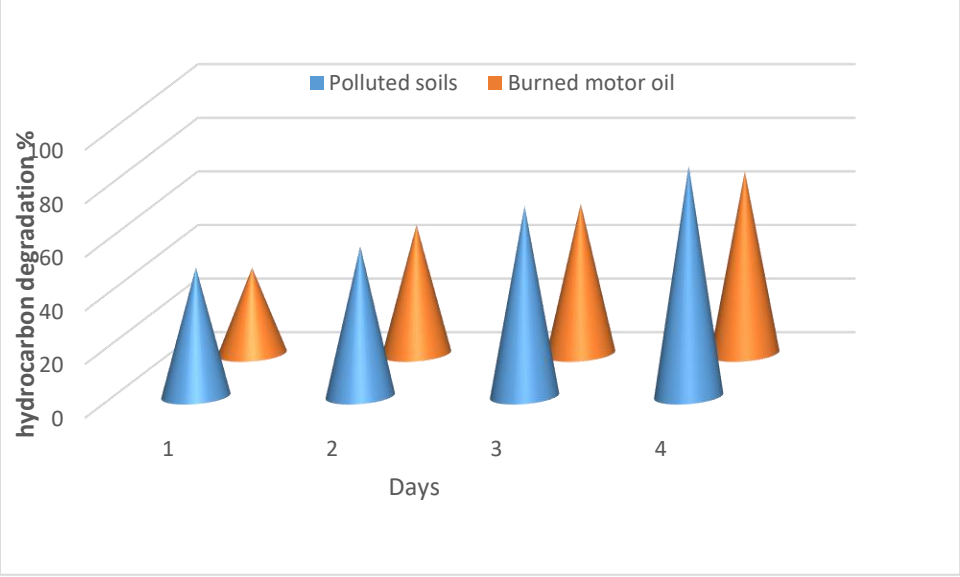

Figure 5: Removal of hydrophobic pollutants in polluted soils and burned motor oil by 100 mg/ml of prodigiosin at different incubation periods

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Due to the fact that prodigiosin binds to heavy metals to both detoxify and remove the lead, previous work by (21) focused on the removal of lead from polluted soil by different concentrations of prodigiosin, and the results suggested that the red pigment could be used as a detoxifier of soil polluted with lead at different concentrations. As a result, bioremediation has arisen as an alternate method for cleaning up oil-contaminated areas.

CONCLUSION

Bioremediation with prodigiosin pigment has become a normal practice, as biological treatments are more efficient in eliminating pollutatns and protecting natural our resouurces..

REFERENCES

[1] Maruthi, Y.M.; Hossain,K. and Sujata Thakre, S.(2013). *Aspergillus flavus*: A potential Bioremediator for oil contaminated soils. European Journal of Sustainable Development 2(1), 57-66.

[2] Gomez, F.; Sartaj,M. (2013). Field scale ex-situ bioremediation of petroleum contaminated soil under cold climate conditions. International biodeterioration & biodegradation.85; 375-382.

[3] Carbonell, G.V.; Colleta, D.; Yano, H.H.M.; Darini, T.; Levy, A.L.C. and Fonseca; B.A.L.(2000). Clinical relevance and virulence factors of pigmented *Serratia marcescens*. FEMS Immunol. Med. Microbiol. ;28 :143–149.

[4] Montaner, B.; Navarro, S.; Piqué, M., Vilaseca, M., Martinell, M. Giralt, E., Gil, J., Peréz-Tómas, R.(2018). Prodigiosin from the supernatant of *Serratia marcescens* apoptosis in haematopoietic cancer cell lines. Br. J. Pharmacol.; 131: 585–593.

[5] Stroud, J. L.; Paton, G. I. and Semple, K. T. (2007). Microbe-aliphatic hydrocarbon interactions in soil: implications for biodegradation and bioremediation. Journal of Applied Microbiology, 102(5):1239-1253.

[6] Xixi Lia, Fuqiang Fana, Baiyu Zhanga, Kedong Zhangb, Bing Chen. (2018). Biosurfactant enhanced soil bioremediation of petroleum hydrocarbons: Design of experiments (DOE) based system optimization and phospholipid fatty acid (PLFA) based microbial community analysis. International Biodeterioration & Biodegradation.132: 216-225.

[7] Fuentes, S.; Barra, B.; Caporaso, J.G. and Seegera, M. (2016). From rare to dominant: a fine-tuned soil bacterial bloom during petroleum hydrocarbon bioremediation. Applied and Environmental Microbiology. 82(3):888-96.

[8] Gkorezis, P.; Daghio, M,; Franzetti, A.; Van Hamme, J.D.; Sillen, W. and Vangronsveld, J. (2016). The interaction between plants and bacteria in the remediation of petroleum hydrocarbons: an environmental perspective. Frontiers in microbiology. 7(1):33-39.

[9] Ojuederie, O.B. and Babalola, O.O. (2017). Microbial and Plant-Assisted Bioremediation of Heavy Metal Polluted Environments: A Review. International journal of environmental research and public health. 14(12). 15-21.

[10] Hardjito, L.; Huq, A. and Colwell, R.R.(2012). The influence of environmental conditions on the production of pigment by *Serratia marcescens*. Biotechnol. Bioprocess Eng.; 7:100–104.

[11] Carbonell, G.V.; Della Colleta; H.H.M., Yano; T., Darini; A.L.C., Levy; C.E., Fonseca; B.A.L.(2000). Clinical relevance and virulence factors of pigmented *Serratia marcescens*. FEMS Immunol. Med. Microbiol. ; 28:143–149.

[12] Montaner, B.; Navarro, S.; Piqué, M., Vilaseca, M., Martinell, M. Giralt, E., Gil, J., Peréz-Tómas, R.(2018). Prodigiosin from the supernatant of *Serratia marcescens* apoptosis in haematopoietic cancer cell lines. Br. J. Pharmacol.;131: 585–593.

[13] Khanam, B. and Chandra, R. (2018). Comparative analysis of prodigiosin isolated from endophyte *Serratia marcescens*. Lett Appl Microbiol.; 66(3):194-201.

[14] McFaddin, J.F. (2000). Biochemical Test for Identification of Medical Bacteria. 3rd. Ed. The Williams and Wilkins. Baltimore, USA.

[15] Hardjito, L.; Huq, A. and Colwell, R.R.(2012). The influence of environmental conditions on the production of pigment by *Serratia marcescens*. Biotechnol. Bioprocess Eng.; 7:100–104.

[16] Venil, C.K. and Lakshmanaperum, P. (2009). An insightful overview on microbial pigment, prodigiosin. Elect J. Biol., 5(3):49-61.

[17] Gohil, N.; Bhattachariee, G. and Singh, V. (2020). Synergistic bactericidal profiling of prodigiosin extracted from *Serratia marcescens* in combination with antibiotics against pathogenic bacteria. Microb Pathog.; 149: 104508

[18] mpfer, P.K. and Glaeser, S.P. (2016).*Serratia aquatilis sp. nov.*, isolated from drinking water systems. International Journal of Systematic and Evolutionary Microbiology 66(1):11-15.

[19] Lin, P.; Shen, J.; Ou, P.; Lin, L.; Chen, Z.; Chu, F.; Wang, J. and Jin, X.(). Prodigiosin isolated from *Serratia marcescens* in the Periplaneta americana gut and its apoptosis-inducing activity in HeLa cells. Oncol Rep.,41(6):3377-3385.

[20] Wei, Y. and Chen, W.(2005). Enhanced production of prodigiosin-like pigment from *Serratia marcescens* SMdelta R by medium improvement and oil-supplementation strategies. J. Biosci.Bioeng.; 99(6):616-22.

[21] Shaba, A.M.; Oyeleke, S.B.; Oyewole, O.A.; Mohammed, S.S.D.; Salahudeen, A.O. and Ikekwem, C.C.(2017). Removal of lead and inhibition of algal growth using prodigiosin produced by *serratia marcescens*. Int. Res. J. Biological. Sci.; 6(8):1-6.