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OPTIMIZED PRODIGIOSIN PRODUCTION BY *SERRATIA MARCESCENS* AND ITS ANTIMICROBIAL EFFECT

Chikodili G. Anaukwu [1]*, Vivian, N. Anakwenze [1], Chito C. Ekwealor [1], Chibuike G. Obi [1], Blessing I. Onyebuagu[1], Tobechukwu M.C. Ajogwu[1], Victoria I. Anyaoha[1], Amarachukwu, B. Isiaka[2]

[1]Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Nigeria

[2]Qatar Airways Medical Division, Qatar

Corresponding author: cg.anaukwu@unizik.edu.ng

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ABSTRACT

Serratia marcescens commonly synthesize a red pigment known as prodigiosin. Prodigiosin is considered a promising pharmaceutical due to its documented properties of having antimicrobial, anticancer, and immunosuppressive effects. This investigation involved the isolation of *Serratia marcescens*, a bacterium capable of producing prodigiosin, from grey water samples collected in Nnamdi Azikiwe University Awka campus, Nigeria. The central composite design (CCD) of the experiment was applied to generate a set of 31 experimental combinations to study the optimal conditions for pigment production using nutrient broth supplemented with glucose as a fermentation medium. A regression model that described the relationship between the test variables for optimum prodigiosin yield was developed. The regression coefficient (R^2) value of 72.48% implied adequate model fitness. The optimal conditions identified were 24 g/L glucose concentration, pH 7.2, 2.4 mL inoculum size, and 180 rpm agitation speed. A 4.25-fold increase in prodigiosin yield was recorded in optimized condition than in unoptimized condition. Antimicrobial activity against *E. coli* and *Candida albicans* shows that prodigiosin has significantly higher activity than the conventional antibiotics tested. Our results indicate that prodigiosin production by *Serratia marcescens* can be enhanced using statistical models, and the pigment can be an alternative to conventional antibiotics for treating microbial infections.

Keywords: Prodigiosin, *Serratia marcescens*, Response surface, Optimization, antimicrobial

INTRODUCTION

Prodigiosin, an alkaloid crimson chromophore, is a naturally occurring chemical predominantly produced by bacteria such as *Serratia, Pseudomonas, Streptomyces, Vibrio, Streptoverticillum, Rugamonas, Hahella, Actinomadura,* and

Alteromonas (Islan *et al*., 2022). Its IUPAC name is 4-methoxy-[(2)-(3-methyl-4-pentyl-2,1-pyrrol-2-yl)methylene]-1-pyrrole (NCBI, 2024). This linear tripyrrole red pigment is synthesized in the stationary phase of bacterial cell growth through the coupling of mono and bipyrrole precursors obtained separately (Han *et al*., 2021).

The distinctive tripyrrole chemical composition of prodigiosin is a rich resource for antibacterial agents and bio-colourants that can be used in numerous applications (Araujo *et al*., 2022). The synthesis of prodigiosin occurs by a split process that involves the enzymatic condensation of two crucial precursors. It possesses medical uses such as cancer treatment, antibacterial effects, regulation of the immune system, suppression of immunological responses, ability to kill cells, and ability to prevent oxidation. At high temperatures, it lacks stability and can dissolve in alcohol, chloroform, or ether, depending on the specific bacterial strain.

Serratia marcescens is known for being a significant producer of prodigiosin. The growth of the organism reaches its maximum potential within a time frame of 24 to 72 hours and is characterized by the presence of three chromogenic species: *Serratia marcescens*, *Serratia plymuthica* and *Serratia rubidea* (Araujo *et al*., 2010).

Prodigiosin by *Serratia marcescens* exhibits broad-spectrum antimicrobial activity and can be used against antibiotic-resistant bacteria such as Methicillin-resistant *Staphylococcus aureus*, *Enterococcus faecalis*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* (Yip *et al*., 2021), and *Streptococcus pyogenes*, Oxacillin-resistant *Staphylococcus aureus* (Lapenda *et al*., 2015), *Escherichia coli* (Arivuselvam *et al*., 2023), fungi such as *Candida* species (Rahul *et al*., 2016), and even some protozoa (Choi *et al*., 2021). Prodigiosin has also demonstrated the ability to disrupt biofilms (Ma *et al*., 2024; Yip *et al*., 2021). Several studies suggest that prodigiosin disrupts cellular functions by targeting the cell membrane, interfering with DNA replication, and causing oxidative stress (Choi et al., 2021).

Due to its antimicrobial properties, prodigiosin has garnered interest for potential therapeutic applications. Researchers are exploring its use as a novel antimicrobial agent in pharmaceuticals, particularly in the context of combating multidrug-resistant pathogens (Arivusalem *et al*., 2023). Although prodigiosin exhibits potential as an antimicrobial agent, a thorough evaluation of its safety profile and potential toxicity is necessary before its use in clinical settings. Research is ongoing to assess its efficacy, safety, and potential side effects. Other biomedical effects of prodigiosin include anticancer (Islan *et al.*, 2022) and immunomodulatory and immunosuppressive effects (Anwar *et al*., 2022).

The industrial synthesis of prodigiosin using microbial fermentation is a cost-effective technique that involves a straightforward extraction method and can achieve higher yields by carefully selecting active strains and making genetic improvements (Darshan and Manonmani, 2015). The first step of a fermentation enhancement program involves quantifying the product yield as a response to fermentation factors such as the concentration of the medium components. Nutritional requirements can be altered using either the conventional or statistical method. Conventional techniques entail varying a single independent variable while maintaining the remaining variables at a constant level. Statistical approaches, albeit more contemporary, have numerous advantages over conventional methods due

to their speed and reliability. In addition, these strategies are valuable for identifying critical nutrients and subsequently lowering the number of experiments, resulting in significant time and chemical product savings (Ennouri *et al*., 2016).

In this study, we optimized fermentation conditions for prodigiosin production by *Serratia marcescens* using response surface methodology and determined its antimicrobial impact against some organisms.

MATERIALS AND METHODS

SAMPLE COLLECTION:

Sterile sampling bottles were used to collect a total of three greywater samples from three different restrooms in the students' dorms at Nnamdi Azikiwe University Awka. The Nnamdi Azikiwe University Awka campus offers multiple student dormitories, both within and beyond the school premises. These hostels accommodate both male and female students. Each hostel building is equipped with restrooms on every floor. The Nnamdi Azikiwe University Awka campus is located on Enugu-Onitsha Expressway, Ifite Road, Awka, in the state of Anambra, Nigeria. The University is situated in Southeast Nigeria, specifically between latitude 6.245° to 6.283° N and longitude 7.115° to 7.121°E. The samples were appropriately labelled with the date of collection and promptly delivered to the University's Microbiology laboratory for isolation and analysis.

ISOLATION AND IDENTIFICATION OF *SERRATIA MARCESCENS*

A selective medium, Caprylate thallous agar (CTA) was used for the isolation of *S. marcescens*. One ml of the greywater sample was diluted using 10-fold dilution technique, and 1 ml of the diluted sample was inoculated by pour-plating technique onto sterile CTA plates. The inoculated plates were incubated at a temperature of 25°C for 24 hours. Following the incubation period, the number of developed colonies was enumerated, sub-cultured, and preserved on Nutrient agar slants at a temperature of 4ºC. The isolates obtained from CTA plates were identified using basic biochemical tests and 16S rDNA sequencing. The biochemical tests conducted include Gram's reaction, catalase test, motility test, oxidase test, citrate utilization test, and sugar fermentation test. The various tests were conducted following the method described by Cheesbrough (2006).

16S rDNA SEQUENCING

The molecular analysis was carried out at the Genomics and Microbiome core facility of Rush University Medical Center, USA. The genomic DNA (specifically the 16S ribosomal RNA) was isolated using the ZR bacterial DNA miniprep kit from Zymo Research. The DNA was amplified using the polymerase chain reaction (PCR) with Taq 2X Master Mix from New England Biolabs (M0270). The primers utilized were the forward primer (27F: AGAGTTTGATCMTGGCTCAG) and the reverse primer (1525R: AAGGAGGTGWTCCARCCGCA). The cycling conditions for amplifying the 16S rRNA were as follows: an initial denaturation step at 94˚C for 5 minutes, followed by 36 cycles of denaturation at 94˚C for 30 seconds, annealing at 56˚C for 30 seconds, and elongation at 72˚C for 45 seconds. The last elongation step at a temperature of 72˚C lasted for 7 minutes. The amplified fragments underwent sequencing using a Genetic Analyzer 3130xl sequencer from Applied Biosystems, following the instructions provided by the manufacturer. The sequencing kit utilized was the BigDye Terminator v3.1 cycle sequencing kit. The genetic study was done using Bio-Edit software and NCBI. The

evolutionary history was deduced using the Neighbor-joining method (Saitou and Nei, 1987), and the evolutionary distances were calculated using the maximum Composite Likelihood approach (Tamura *et al*., 2004).

PRELIMINARY SYNTHESIS AND EXTRACTION OF PRODIGIOSIN

Prodigiosin synthesis by *Serratia marcescens* was first carried out using three distinct fermentation media: peptone glycerol broth, nutrition broth with 1% w/v maltose supplementation, nutrient broth with 1% w/v glucose supplementation and nutrient broth without any supplementation, as described by Zara (2016). A volume of 1 ml of a 24-hour culture of *Serratia marcescens*, containing 1.2×10^6 cells/ml, was added to a 100 ml Erlenmeyer flask containing 50 ml of the fermentation media. The flask was then placed in a rotary shaker operating at 150 rpm and kept at a temperature of 25ºC.

Following a 72-hour incubation period, the fermentation broth was subjected to two rounds of centrifugation at a force of 10,000 times the acceleration due to gravity for 15 minutes each. The supernatant was collected using a separation funnel and mixed with chloroform in a proportion of 2 parts supernatant to 1 part chloroform. The combination was left undisturbed for 10 minutes. The pigment-containing solvent layer was collected by using a separating funnel. It was then acidified using 0.1M HCl and subsequently neutralized by adding drops of Tris buffer, resulting in the formation of a reddish prodigiosin pigment. The prodigiosin pigment that was produced was collected in a pre-weighed beaker. The pigment was then concentrated by drying it in a hot air oven until it reached a dry state. The pigment's weight (dry mass in g) was calculated by subtracting the initial weight of the beaker from its final weight.

STATISTICAL OPTIMIZATION OF PRODIGIOSIN PRODUCTION – RESPONSE SURFACE METHOD.

The best medium for prodigiosin synthesis by *Serratia marcescens* in this study was nutrient broth supplemented with glucose. A central composite design was employed to optimize crucial media components for improved prodigiosin yield (Anaukwu *et al*., 2020). A 2^4 complete factorial central composite design (CCD) was used to fit a second-order polynomial model. The design had four test variables, each with five levels, eight-star points, and seven replicates at the centre points. The factors assessed were the concentration of glucose (X_1) ranging from 5 to 25g/L, the pH (X_2) ranging from 6 – 8, inoculum size (X_3) ranging from 1 to 3ml, and agitation (X_4) ranging from 50 to 250 rpm (Table 1). The experiment was designed using Minitab software version 17 which coded for the different experimental levels as -2, -1, 0, 1, 2 (Table 1). A total of 31 trials were conducted, with the weight of prodigiosin crude extract serving as the measured experimental response (Y).

Table 1: Variables and their levels used in the experiment.

Variable	**-2**	**-1**	**0**	**1**	**2**
X_1 (g/100ml)	5	10	15	20	25
X_2	6	6.5	7	7.5	8
X_3 (ml)	1	1.5	2	2.5	3
X_4 (rpm)	50	100	150	200	250

EMPIRICAL VERIFICATION OF THE STATISTICAL MODEL

The method described by Anaukwu *et al*. (2024) was employed. The collected findings were further analysed using Analysis of Variance (ANOVA) to determine the impact of each variable on the production of prodigiosin. The R^2 value

determined the amount of variance that could be accounted for by the model in question. The optimal conditions were verified and documented as the mean value plus or minus the standard deviation.

ASSESSING THE SUSCEPTIBILITY OF PIGMENTS TO ANTIMICROBIAL AGENTS

The Kirby-Bauer disc diffusion method was employed to assess the antibacterial efficacy of the generated prodigiosin against *Escherichia coli* and *Candida albicans*. The strains of *E. coli* and *Candida albicans* used in this study were acquired from the microbial collection of the Microbiology laboratory at Nnamdi Azikiwe University in Awka, Nigeria. The test organisms, which were 24 hours old, were initially standardized using a 0.5 McFarland standard. They were then spread on the surface of a sterile Muller Hinton agar. Sterile paper discs impregnated with the crude prodigiosin microorganisms in various concentrations (ranging from 100mg/ml to 500mg/ml were then placed on the surface of the plate containing the test organisms, ensuring a sterile environment. The plate was thereafter incubated at a temperature of 37^{o}C for 24 hours. The diameter of the zones of inhibition surrounding each pigment disc was measured and recorded in millimetres. A conventional antibacterial (chloramphenicol, 30mg/ml) and antifungal (fluconazole, 30 mg/ml) antibiotic were employed as positive controls (Cheesbrough 2006).

STATISTICAL ANALYSIS OF DATA

The analyses were done in triplicate and data were presented as mean±SD. Microsoft excel 365 was used to calculate the mean and standard deviation. Minitab software version 20 was used to carry out the analysis of the variance (ANOVA) of the responses obtained on CCD.

RESULTS AND DISCUSSION

Several strains of S*erratia marcescens* that produce prodigiosin have been discovered in soil (Lin *et al*., 2019; Darshan and Manonmani, 2016; Araujo *et al*., 2022), freshwater lakes (Manas *et al*., 2020; Vitale *et al*., 2020), and the ocean (Alihosseini *et al*., 2008). In this investigation, prodigiosin-producing *Serratia marcescens* was recovered from grey water collected from restrooms. The basic biochemical characteristics of the isolate carried out as preliminary identification of the *S. marcescens* (Table 2) shows that the organism is motile and positive for catalase enzyme production, and glucose, mannitol, and sucrose fermentation, but negative for production of cytochrome oxidase enzyme and lactose fermentation.

Table 2: Basic biochemical test result for the identification of *Serratia marcescens*

Test	Result
Gram reaction	Gram-negative rod
Catalase	+
Oxidase	-
Motility	+
Sugar fermentation	
Lactose	-
Glucose	+
Mannitol	+
Sucrose	+

The phylogenetic analysis (Figure 1) using the 16S rRNA gene identified the prodigiosin-producing organism as *Serratia marcescens*, with a pairwise similarity of 100% to several strains of *S. marcescens* such as strain JW-CZ2 (NCBI accession number CP055161.1), strain N10A28 (NCBI accession number CP033623.1) and strain BWH-35 (NCBI accession number CP020507.1), and 99.93% similarity with strains JCM:20115 (NCBI accession number LC595307.1) and C110 number CP047691.1).

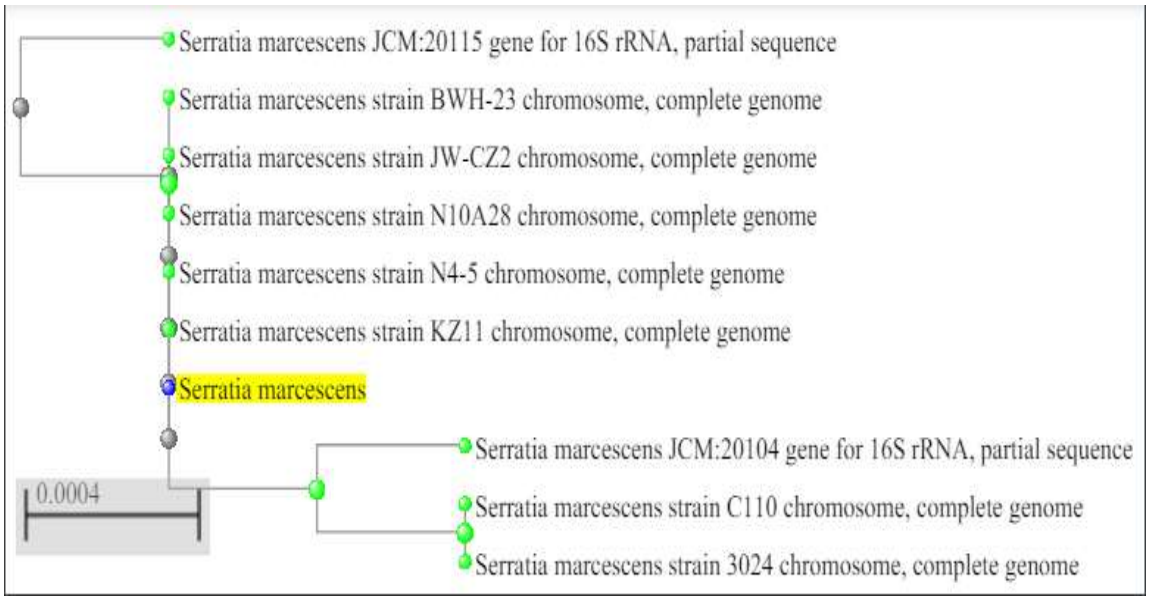


Figure 1: Phylogenetic tree showing evolutionary relatedness of the isolate to other strains of *S. marcescens*.

Serratia marcescens was the first discovered species to synthesize the prodigiosin chemical, (Merlino, 1924). Research in the literature indicates that bacteria produce prodigiosin in varying quantities and fermentation media. Various studies have indicated that peptone glycol medium (Herráez *et al*., 2019; Darshan and Manonmani, 2016; Pore *et al*., 2016), nutrient broth (Lapenda *et al*., 2020; Kamble and Hiwarale, 2012), and lysogeny broth medium (Kimyon *et al*., 2016) are commonly used to produce prodigiosin.

In this study, we experimented with peptone glycerol and nutrient broth supplemented with glucose and maltose to produce prodigiosin in order to identify the best carbon source and media required by the organism. Figure 2 shows that nutrient broth supplemented with glucose gave the highest prodigiosin yield of 1.01g/ml, while nutrient broth without any supplementation gave the least yield. Tao *et al*. (2005) in their work stated that carbon sources are necessary for the production of the prodigiosin compound. Supplementation of fermentation medium with carbon source thus enhanced the production yield recorded in this study. This result corroborates the report of Vijay *et al*. (2022) who utilized glucose as carbon supplementation in nutrient broth for prodigiosin production. They also opined that varying the concentrations of glucose impacted prodigiosin yield. Contrary to the result obtained in this study, Araujo *et al*. (2010) reported the lowest prodigiosin yield in a medium supplemented with glucose. Sundaramoorthy *et al.* (2009) and Shahitha and Poornima (2012) found *Serratia*

marcescens to produce more prodigiosin in maltose-containing medium than glucose-containing medium. Samrot *et al*. (2011) reported no significant difference in the impact of sugar supplementation on prodigiosin yield by *S. marcescens* SU-10.

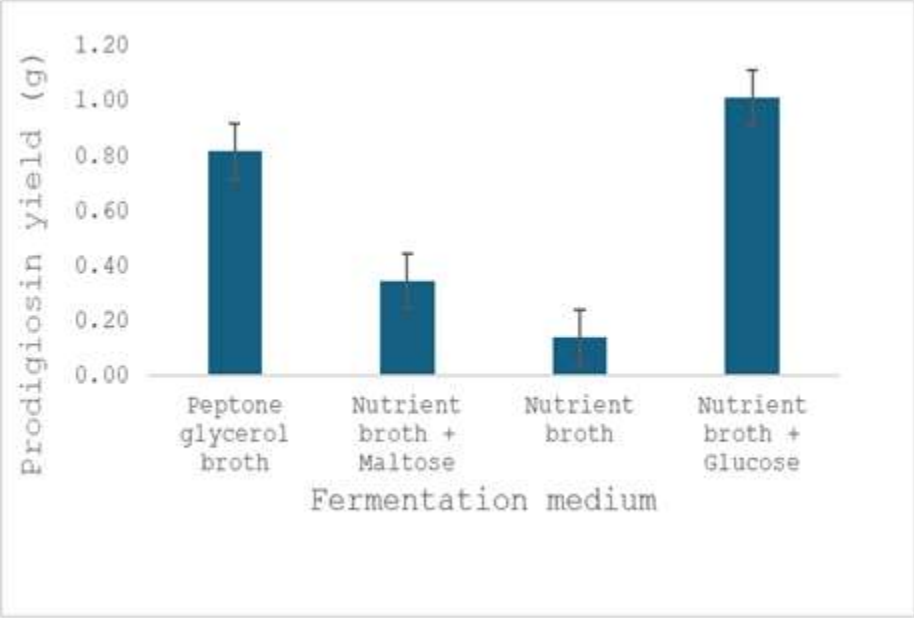


Figure 2: Prodigiosin yield in different fermentation media.

In this study, we showcase the effectiveness of process optimization in quickly and cost-effectively identifying useful waste streams that can be utilized for prodigiosin production. The susceptibility of microorganisms to fermentation conditions makes process conditions crucial in microbial production (Voidarou *et al*., 2021). To produce prodigiosin, it is important to thoroughly analyze the process conditions. This analysis helps identify the key parameters that affect productivity and the best possible yield for a specific organism (Wang *et al*., 2024). Response Surface Methodology (RSM) was employed to create experimental designs that concurrently assess the interactions between process parameters (glucose concentration, pH, inoculum size and agitation speed) and their effects on production yield.

The multiple regression analysis of experimental responses established a regression model to examine the impact of the four selected factors (Nayebi, 2020). Table 3 shows the central composite experimental design runs for optimization of prodigiosin production by the *S. marcescens* and the actual responses obtained.

A total of 31 experiments were conducted, and the prodigiosin yield in g/ml was then recorded as the response. The regression model obtained is a quadratic polynomial equation that represents the empirical interaction between the test variables and the response. It is expressed as

$$Y\ (g/ml) = 1.2198 - 0.0158X_1 - 0.0061X_2 - 0.0514X_3 + 0.0044X_4 - 0.0218X_1^2 - 0.0356X_2^2 - 0.0518X_3^2 - 0.0754X_4^2 - 0.0174X_1X_2 + 0.0294X_1X_3 - 0.0501X_1X_4 + 0.0076X_2X_3 + 0.0210X_2X_4 + 0.0174X_3X_4,$$

Where Y1 represents prodigiosin yield in g/ml, X_1 represents the concentration of glucose, X_2 represents the pH, X_3 represents the inoculum size, and X_4 represents the agitation speed.

The analysis of variance (ANOVA) was conducted to assess the significance and adequacy of the regression model. The results (Table 4) indicated that the regression model was statistically significant ($p = 0.019$) The lack of significance ($P > 0.05$) in the lack of fit test suggests that the model is suitable for achieving optimal prodigiosin synthesis by the organism as shown in Table 4. The impact of the variables on prodigiosin production demonstrated that only the inoculum size had a statistically significant effect on biosurfactant production ($p < 0.05$), while the other variable levels did not have a significant influence on the observed responses (Table 4).

The model's fit was assessed based on the regression coefficient (R^2) value, which was 72.38%. This demonstrates that the regression model has been properly adjusted to the experimental data, and it can account for 72.38% of the variability between the predicted and experimental data. This confirms that the model is suitable for predicting prodigiosin production under the given experimental conditions by *S. marcescens* locally isolated in this study.

The regression model yielded the following optimal process conditions: a concentration of 24g/L for glucose, 7.2 pH, 2.4 ml for inoculum size, and 180 rpm agitation speed. These conditions were coded as -1.7980, 0.4242, -0.8687, and 0.5859 respectively. The RSM optimization in this study resulted in a substantial improvement in prodigiosin production output, with a 4.25-fold increase compared to the initial fermentation conditions. These optimizations are highly valuable and have been proven to be effective in the past. For example, Gohil *et al*. (2021) recorded a 5.19-fold higher prodigiosin yield when fermentation conditions were optimized using a statistical model. Increased prodigiosin pigment production was recorded by Gul *et al*. (2020) using a response surface model of optimization and another study reported an 8.42-fold increased prodigiosin yield using statistical experimental design to optimize medium constituents for production by *S. marcescens* (Su *et al*., 2011).

The antimicrobial activities of the prodigiosin were assessed using the Kirby-Bauer disc diffusion method Table 5 displays the antibiotic susceptibility test result showing the zone of inhibition of growth by the varying concentrations of prodigiosin pigment against *Escherichia coli* and *Candida albicans*, which was compared to the conventional broad-spectrum antibacterial (chloramphenicol) and antifungal (fluconazole) Prodigiosin showed remarkable efficacy against *E. coli*, with a zone of inhibition ranging from 1.2±0.01mm to 9.7±0.05 mm and *Candida albicans*, with zone of inhibition ranging from 0.00±0.00 mm to 4.5±0.12 mm. In comparison, the conventional medicines, chloramphenicol and fluconazole exhibited antimicrobial effects against the test bacterium and fungi with zones of inhibition 0.9±0.23 mm and 2.8±0.01mm respectively. The antibacterial and antifungal effects of the test organisms increased with increasing concentration of the prodigiosin (Table 5) and were significantly higher (p-value < 0.05) than the conventional antibiotics tested. However, prodigiosin inhibited bacterial (*E. coli*) growth more than *Candida albicans* growth based on the zone of inhibition recorded. The antibiotic effect of prodigiosin recorded in this study corroborates reports in other studies. For example, prodigiosin produced by Arivuselvam *et al*. (2023) exhibited high-level antibacterial effects against *E. coli* NCIM 2065 and some other Gram-negative organisms. Several other researchers have recorded a strong antibacterial effect of prodigiosin against *E. coli*, for example, Arivizhivendhan *et al*. (2015; 2019), and Danevčič *et al.* (2016). Suryawanshi *et al*.

(2017) reported 30% inhibition against *Candida albicans* by prodigiosin produced by *Serratia marcescens*. The fundamental mechanisms underlying the microbial activity of prodigiosin involve the fragmentation of genetic material (DNA), disruption of the cell cycle, alteration of pH, destabilization of the plasma membrane through heightened hydrophobic stress, phototoxicity, and generation of reactive oxygen species (Choi *et al*., 2021).

Table 3: Central composite design runs showing actual and coded variables and the responses.

Run	X_1 Glucose (g/L)	X_2 pH	X_3 Inoculum size (ml)	X_4 Agitation (rpm)	Response (Y) Yield(g/100 ml)
1	15(0)	7.0(0)	2.0(0)	250(2)	0.92
2	10(-1)	7.5(1)	1.5(-1)	200(1)	1.20
3	15(0)	7.0(0)	2.0(0)	150(0)	1.22
4	10(-1)	7.5(1)	1.5(-1)	100(-1)	1.10
5	20(1)	6.5(-1)	2.5(1)	200(1)	0.91
6	20(1)	7.5(1)	1.5(-1)	100(-1)	0.95
7	15(0)	6.0(-2)	2.0(0)	150(0)	1.21
8	10(-1)	7.5(1)	1.5(-1)	100(-1)	1.11
9	15(0)	7.0(0)	2.0(0)	150(0)	1.21
10	20(1)	6.5(-1)	1.5(-1)	200(1)	0.90
11	15(0)	7.0(0)	2.0(0)	150(0)	1.22
12	15(0)	8.0(2)	2.0(0)	150(0)	1.02
13	15(0)	7.0(0)	2.0(0)	150(0)	1.23
14	15(0)	7.0(0)	1.0(-2)	150(0)	1.20
15	10(-1)	6.5(-1)	2.5(1)	200(1)	1.15
16	20(1)	7.5(1)	2.5(1)	200(1)	0.99
17	15(0)	7.0(0)	2.0(0)	150(0)	1.21
18	10(-1)	6.5(-1)	2.5(1)	100(-1)	0.90
19	10(-1)	6.5(-1)	2.5(1)	200(1)	0.88
20	25(2)	7.0(0)	2.0(0)	150(0)	1.23
21	10(-1)	7.5(1)	2.5(1)	200(1)	1.12
22	15(0)	7.0(0)	2.0(0)	150(0)	1.22
23	20(1)	7.5(1)	2.5(1)	100(-1)	0.99
24	20(1)	6.5(-1)	2.5(1)	100(-1)	0.98
25	15(0)	7.0(0)	3.0(2)	150(0)	0.90
26	10(-1)	6.5(-1)	1.5(-1)	100(-1)	1.02
27	20(1)	7.5(1)	1.5(-1)	200(1)	0.97
28	15(0)	7.0(0)	2.0(0)	150(0)	1.21
29	5(-2)	7.0(0)	2.0(0)	150(0)	1.11
30	15(0)	7.0(0)	2.0(0)	50(-2)	1.01
31	20(1)	6.5(-1)	1.5(-1)	50(-2)	0.99

Table 4: Analysis of variance (ANOVA) for the quadratic response surface model for prodigiosin production by *Serratia marcescens*

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	14	0.343583	0.024542	2.99	0.019*
Linear	4	0.059075	0.014769	1.80	0.178
X_1	1	0.005419	0.005419	0.66	0.428
X_2	1	0.000791	0.000791	0.10	0.760
X_3	1	0.052199	0.052199	6.37	0.023*
X_4	1	0.000442	0.000442	0.05	0.819
Square	4	0.250571	0.062643	7.64	0.001*
X_1*X_1	1	0.013519	0.013519	1.65	0.217
X_2*X_2	1	0.035928	0.035928	4.38	0.053
X_3*X_3	1	0.076254	0.076254	9.30	0.008*
X_4*X_4	1	0.177640	0.177640	21.68	0.000*
2-Way Interaction	6	0.072439	0.012073	1.47	0.249
X_1*X_2	1	0.004083	0.004083	0.50	0.490
X_1*X_3	1	0.010207	0.010207	1.25	0.281
X_1*X_4	1	0.037076	0.037076	4.52	0.049*
X_2*X_3	1	0.000753	0.000753	0.09	0.766
X_2*X_4	1	0.006864	0.006864	0.84	0.374
X_3*X_4	1	0.004563	0.004563	0.56	0.466
Error	16	0.131126	0.008195		
Lack-of-Fit	8	0.094283	0.011785	2.56	0.103
Pure Error	8	0.036843	0.004605		
Total	30	0.474710			

Key: DF = Degree of freedom, SS = Sum of squares, MS = Mean square, P-value ≤ 0.05 is significant at 95% confidence level. Model Summary: S = 0.0905284, R-sq = 72.38%, R-sq (adj) = 48.21%, R-sq (pred) = 0.00%

Table 5: Antimicrobial sensitivity testing of pigments on test organisms in mm

Test Organism	Prodigiosin Concentration (mg/ml)					Chloramphenicol (30 mg/ml)	Fluconazole (30 mg/ml)
	100	200	300	400	500		
E. coli	1.2±0.01	2.5±0.10	2.7 ±0.02	7.5±0.01	9.7±0.05	0.9±0.23	-
C. albicans	0.0±0.00	0.9±0.02	1.9±0.00	3.2±0.01	4.5±0.12	-	2.8±0.01

CONCLUSION

In this study, *Serratia marcescens* with the potential for prodigiosin production was isolated from grey water. The response surface method of statistical optimization identified the fermentation condition suitable for production by the isolate and improved prodigiosin yield by 4.25-fold. Prodigiosin produced showed a significantly higher antibacterial and antifungal effect against *E. coli* and *C. albicans* respectively than the conventional antibiotics tested. Prodigiosin is thus an excellent alternative to conventional antibiotics. However, the genomic study of the isolate is recommended for enhanced prodigiosin yield.

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