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Isolation and Screening for Prodigiosin Producing *Serratia marcences* from Different Sources

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Abstract

Prodigiosin is a red bioactive pigment produced by several bacterial species, particularly *Serratia marcescens*. It has potential applications as a natural colorant and has been reported to exhibit antimicrobial, anticancer, and immunosuppressive properties. This study aimed to isolate and screen *Serratia* isolates from Kano State, Nigeria, for prodigiosin production. Samples were collected from multiple locations across the state. Red-pigmented isolates were screened on agar plates using a pigment-production screening method and identified by Gram staining and biochemical tests. Isolates were further characterized by 16S rRNA gene sequencing. Prodigiosin production was assessed by cultivating each isolate in nutrient broth and determining pigment concentration. Twenty red to red-orange bacterial isolates were recovered from the samples. Of these, three soil-derived isolates, designated DSSK 5, SSK 3, and SSK 7, produced the pigment. Sequence analysis indicated that SSK 3 shared 95.36% similarity with either *Serratia marcescens* strain KNU-AP16 or *Serratia nematodiphila* strain UMAGOD10. DSSK 5 showed 95.88% similarity to *Serratia marcescens* strain ICC-4, whereas SSK 7 showed 98.97% similarity to *Serratia marcescens* strain INS420. Under the tested culture conditions, SSK 3 produced the highest reported prodigiosin concentration (4.9 ± 0.157 mg/L), while DSSK 5 produced the lowest (2.76 ± 0.019 mg/L). These findings indicate that prodigiosin-producing *Serratia* can be recovered from local soils in Kano State and that pigment yield varies among isolates under the same culture conditions.

Keywords
Prodigiosin;
Serratia Marcescens.
16S Rrna Sequencing;
Soil Bacteria;
Pigment Production

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Introduction

Microbial natural products, also referred to as secondary metabolites, are significant sources of possible medicinal compounds. Pigments such as carotenoids, melanins, flavones, quinones and prodigiosin have been produced by bacteria, fungus, and algae and many of them have demonstrated biological functions that are significant to industry and medicine (Palacio-Castaneda et al., 2023). Microorganisms offer a highly efficient pathway for pigment production, outperforming plant and animal sources in both raw yield and residue reduction (Ramesh and Kumar, 2024).

Global interest in microbial pigments has surged because they offer superior biodegradability, environmental compatibility, lower toxicity, and fewer allergic reactions than synthetic alternatives (Venil et al., 2020; Nawaz et al., 2021; Jeong et al., 2022). Consequently, biotechnological manufacturing has grown exponentially to fulfill their vast potential across the food, pharmaceutical, cosmetic, and textile sectors (Sánchez-Muñoz et al., 2020).

Serratia marcescens, a Gram-negative bacterium belongs to the Enterobacteriaceae family, and has been reported for red pigment production. Pigment from Serratia has been characterized as prodigiosin. S. marcescens grows well on ordinary and synthetic media comprising various kinds of carbon sources, under aerobic and anaerobic conditions and temperatures between 20-37°C. Found in diverse environments, including soil, water, plants, insects, animals, and humans, the prodigiosin producing Serratia includes marcescens, plymuthica, and odorifera (Al- Ghanem, 2018).

Prodigiosin is a secondary metabolite that forms during the stationary phase of bacterial growth through the coupling of mono and bipyrrole precursors obtained separately (Han et al., 2021). Many different bacterial species, including Serratia marcescens, Vibrio psychroerythrous, Pseudomonas magneslorubra and others, produce a large amount of this secondary metabolite (Ashlesha and Unnati, 2020). But of all the species, Serratia marcescens is the main producer (Lucas et al., 2022).

The chemical structure of prodigiosin ($C_{20}H_{25}N_3O$; molecular weight 323.44 Da) consists of a tripyrrole ring system which is responsible for its characteristic red hue and lipophilic nature, making it soluble in organic solvents like methanol and chloroform (Kimata et al., 2017). Prodigiosin provides an opportunity for the food industry to meet the safe pigment demand because it is naturally derived, biologically produced, and can be obtained through scalable microbial fermentation systems using inexpensive substrates (Han et al., 2021).

Temperature acts as a critical regulator for Serratia marcescens, as prodigiosin synthesis is heavily inhibited at 37°C (Ma et al., 2025; Zhang et al., 2025). Although this metabolic pathway is functional across both aerobic and anaerobic states, efficient production is highly dependent on specific culture conditions, including strain type, incubation time, pH, salt levels, aeration, and agitation (Rather et al., 2025). Evaluating Serratia metabolic footprints across diverse media reveals that culture matrices dense in proteins and complex carbohydrates profoundly stimulate prodigiosin accumulation (Quintanilla-Villanueva et al., 2025).

Materials and Methods

Collection of Soil Samples

Rhizobial soil samples from Centre for Dry land Agriculture, Plant Science department Garden, Banana Garden close to second gate of Bayero University and Kaura mata irrigated farmlands were collected in sterile container and brought to Microbiology department of Bayero University for analysis

Dump soil samples from dump sites of Bayero University old site, Kofar Naisa, Murtala Muhamed teaching hospital and Yan kaba market were obtained and packed in sterile container, and transported to the laboratory for analysis.

Water samples were also obtained from different ponds at Kofar Naisa, Department of plant Science Garden and sewage from Murtala hospital in a sterile container and were brought to the laboratory for analysis, air samples were obtained at Department of plant Science Garden and Garden near second gate of Bayero University Old Campus.

Isolation and Identification of the Bacteria

Ten grams (10g) of each of the soil samples obtained from the different places was mixed with ninety milliliters (90 ml) of sterile saline. The solution was allowed to settle down for 30 minutes. One millilitre was taken from each of the clear supernatants and was then serially diluted with 9ml of the sterile distilled water. A 0.1ml of the serially diluted samples were spread over the nutrient agar using glass spreader. The plates were incubated at 37°C for 24 to 48 hrs and were then observed for pigmented colonies. Colonies showing red, pink or orange colours were purified on nutrient agar plates (Kavitha et al., 2021). The bacterial isolates were screened for prodigiosin production and the isolates capable of prodigiosin production were identified using Gram staining and biochemical test such as catalase, citrate, oxidase, Voges Proskauer (VP), Methyl Red (MR), urease, TSI (Arijit et al., 2024). Pure cultures were preserved on nutrient agar plates at 4°C in the refrigerator till further use (Elkenawy et al., 2017).

Screening the Bacterial Isolates for Prodigiosin Production on Nutrient Agar Plates

General screening procedure for pigment producing bacteria was used for screening prodigiosin producing bacteria. Nutrient agar plates were spread with 0.1ml of the standardized bacterial cultures of the isolated bacteria and incubated at 300C for 72hrs. The cells were scraped and suspended in 10ml of normal saline and then washed. Twenty millilitres' (10ml) of methanol was added to the cell pellets. The suspensions were vortexed for 5 min. This was followed by centrifuging at agitation speed of 10, 000 rpm for 10 minutes. The supernatants were then collected. The coloured solutions were divided into two portions: one portion was acidified with a drop of concentrated HCl and the other was alkalized with a drop of concentrated NaOH solution (Kamlesh et al., 2020). The red or pink color in acidic condition and the yellow or brown color in alkaline condition confirmed a positive presumptive test for prodigiosin (Sathishkumar and Aparana, 2014; Mansi and Shah, 2015).

Quantification of Prodigiosin produced by the Isolates

For quantification of the pigment produced by the prodigiosin producing isolates, standardized inoculum of the isolates was inoculated in nutrient broth and incubated at 30oC for 3 days. Five milliliters of culture broths were centrifuged at 10000rpm for 10 minutes, then the pellet was suspended and vigorously mixed in 5 ml of acidified methanol. The solution was then centrifuged at 10000rpm for 10 minutes. Resultant solution's optical density was calculated at 535nm. Using the formula below, the total amount of prodigiosin (mg/L) was calculated.

Total prodigiosin (mg/L) = ADV1x323.4x1000x7.07x104V2

A is the absorbance of methanol measured at 535nm, V1 - Volume of methanol added, D the dilution ratio, 7.07X104 is the extinction coefficient of prodigiosin, V2 -Volume of fermentative liquid. 323.4 (Nabaa and Reem, 2025).

All the prodigiosin producing isolates were used for production of prodigiosin using wheat bran as substrate (Kavitha et al., 2021).

Identification of isolates

The isolates were identified following the protocols for microbial identification described by Cheesbrough (2010), the stored isolates were identified based on their cultural and biochemical characteristics. Alongside the Gram reaction, the qualitative biochemical tests conducted included tests for catalase, and oxidase enzyme production; citrate utilization; the ability to release indole in the presence of amino acid (indole test); methyl red; Voges-Proskauer; and the ability to ferment glucose and lactose, and sucrose in Kligler iron agar,

Molecular Characterization of Prodigiosin producing bacteria for 16S rRNA gene

DNA Extraction

The genomic DNA extraction of the bacterial isolate was conducted using the traditional phenol-chloroform method (Prabha et al., 2013). Initially, the bacterium was grown in a nutrient broth for 24 hours after which it was centrifuged at 6000 rpm for 5 minutes to obtain the cells. The pellets were mixed with 200 µL of an extraction buffer, lysozyme, and 20 µL of proteinase K, then left to incubate at 55°C overnight to facilitate cell lysis. Then phenol-chloroform extraction process was then initiated by adding 200 µL of phenol: chloroform: isoamyl alcohol (25:24:1) solution to the sample. The mixture was centrifuged at 12,000 g for 5 minutes, leading to the separation of aqueous and organic phases. The upper aqueous phase, which contained DNA, was meticulously collected using a pipette and transferred to a new 1.5 µL microcentrifuge tube.

The DNA was then precipitated by adding 200 µL of cold 70% ethanol, followed by centrifugation at 12,000 g for 5 minutes, resulting in the formation of a DNA pellet. This pellet was washed with ethanol to eliminate impurities.

After air-drying to remove any remaining ethanol, the DNA pellet was dissolved in distilled water. Finally, the DNA was stored at -20°C for preservation.

PCR Amplification

(a) Amplification of 16s rRNA gene in bacteria

PCR amplification was carried out using the Taq Mix (2x) (Master Mx) and universal primers 27F (forward) and 1426R (reverse), targeting the 16S rRNA gene. The PCR reactions were set up individually for each sample, using the following parameters: Initial denaturation at 95°C for 5 minutes, Denaturation at 95°C for 30 seconds, Annealing at 59 °C for 30 seconds, Extension at 72°C for 30 seconds, Repeated steps 2-4 for 30 cycles and Final extension at 72°C for 5 minutes.

Gel electrophoresis

To verify the success of PCR amplification and check for the presence of specific amplicons, agarose gel electrophoresis was performed. A 1.5% agarose gel was prepared as follows; A 1.5% agarose gel was prepared by dissolving agarose powder in 1X TAE (Tris-Acetate-EDTA) buffer, followed by heating in a Microwave oven. The molten agarose gel was stained with 2 uL ethidium bromide to aid visualization under UV light. The gel was cast in a gel casting tray and allowed to solidify. Comb slots were inserted at one end of the gel for well formation. After gel solidification, the comb was gently removed, creating wells for sample loading. 4 uL of the PCR products were carefully loaded into the wells. Electrophoresis was conducted at 100V until the DNA bands migrated sufficiently.

Sequencing

PCR products of the 16s rRNA sequencing with the expected size were sent for Sanger sequencing using the specific primers used for its amplification at Inqaba Biotech Ibadan, Oyo state, Nigeria.

Bioinformatics: The obtained sequencing data was analyzed using bioinformatics tools. Sequences were compared against public databases to identify the closest species. The results were validated based on sequence similarity and taxonomic classification.

RESULTS

Isolation and screening of prodigiosin producing bacteria

Table 1 shows the results of isolation and screening of prodigiosin producing bacteria from ninety-five different samples. Twenty reddish pigment producing bacteria were isolated and screened for prodigiosin production. Among them only three isolates were prodigiosin producers which were isolate DSSK5 isolated from dump 5 from Kofar Naisa and SSK 3 and SSK 7 isolated from Kauran Mata irrigated farmlands in Madobi Local Government of Kano State.

Identification of the prodigiosin producing Isolates

The prodigiosin producing isolated DSSK 5, SSK 3 and SSK 7 were identified using Gram staining and biochemical test such as catalase, citrate, oxidase, urease, ME-VP, KIA and were found to be Serratia species.

The result of gel electrophoresis of the three prodigiosin producing isolates has shown that all the prodigiosin three isolates have 1500bp 16 rRNA gene as shown in figure 1

416S rRNA Sequencing of the Isolates

Table 4 shows the result of 16S rRNA sequencing of the isolates to identify the closest homologs. The result indicated SSK 3 isolate was 95.36% similar to *Serratia marcescens strain KNU-AP16*, DSSK 5 isolate was 95.88% similar to *Serratia marcescens strain ICC-4* while *SSK 7 isolate as 98.97 similar to Serratia marcescens strain XJalt12.1*

Quantification of the prodigiosin produced by the isolates

The result of the quantification of the prodigiosin produced by the three different isolates in nutrient broth has indicated that SSK 3 isolate produced the highest amount of prodigiosin (4.90±0.157 mg/L) while DSSK 5 isolate produced the lowest amount of prodigiosin (2.76±0.019mg/L) as shown in table 2.

Table 1: Isolation and Screening of different bacterial Isolates for prodigiosin production

S/N	ISOLATES	COLONY MORPHOLOGY	HCL	NAOH	INFERENCE
1.	DSSB6	Red-orange colonies that are circular, convex and smooth	Colourless	Colourless	Negative
2.	DSSB7	Salmon-pink to red-orange that are circular convex and smooth	Light-yellow	Light-yellow	Negative
3.	DSSB9	Vivid orange to reddish-pink colonies that are smooth, moist and opaque	Light red	Dark tomato	Negative
4.	DSSB10	Bright orange to reddish pink colonies that are circular, smooth and glistening	Milky	Milky	Negative
5.	WSK3	Vivid orange to reddish-pink colonies that are smooth and wet	Light pink	Light pink	Negative
6.	DSSK4	Vivid orange to reddish-pink that are smooth, moist and opaque	Baby pink	Baby pink	Negative
7.	DSSK 5	Dark red, circular, raised, Glittery and Butyrous colonies	Pink	Yellow	Positive
8.	DSSM2	Red orange colonies that are circular and smooth	Baby pink	light pink	Negative
9.	DSSM3	Red orange colonies that are smooth, shiny and opaque	Colorless	Baby pink	Negative
10.	WSSM5	Pinkish red colonies that are smooth, moist and glistening	Pinkish red	Pinkish red	Negative
11.	WSPSG 1	Red orange colonies that are circular and smooth	Colorless	Colorless	Negative
12.	SSPSG3	Bright orange to reddish pink colonies that are circular, smooth and glistening	Pinkish red	Colorless	Negative
13.	SSPSG 5	Vivid orange to reddish-pink colonies that are smooth and wet	Light pink	Light pink	Negative
14.	SSBG 3	Pinkish red colonies that are smooth, moist and glistening	Light red	Dark tomatoes	Negative
15.	SSBG 5	Red orange colonies that are circular and smooth	Milky	Milky	Negative
16.	ASPSG 1	Pinkish red colonies that are circular and smooth	Pinkish red	Colorless	Negative
17.	ASPSG 3	Pinkish red colonies that are circular ad smooth	Colorless	Colorless	Negative
18.	DSSY 7	Red orange colonies that are circular, convex and smooth	Pinkish red	Colorless	Negative
19.	SSK 3	Red, circular, raised, Glittery and Butyrous colonies	Pink	Yellow	Positive
20.	SSK 7	Red, circular, raised, Glittery and Butyrous colonies	Pink	Yellow	Positive

Keys: DSSB6: Dump soil sample BUK, WSK: Water sample Kofar Naisa, DSSK: Dump soil sample Kofar Naisa, DSSM: Dump soil sample Murtala hospital, WSSM: Water sample Murtala Hospital, SSBG: Soil sample Banana Garden, ASPSG: Air sample plant science garden, DSSK: Dump soil sample Yankaba, SSK: Soil sample kauran

Table 2: Quantification of the prodigiosin produced by the different bacterial Isolates

S/n	Isolates	Amount of prodigiosin(mg/L)
1.	*Serratia marcescens strain KNU-AP16*	4.90±0.157
2.	*Serratia marcescens strain ICC-4*	2.76±0.019
3.	*Serratia marcescens strain XJalt12.1*	4.30±0.012

Keys: DSSK= Dump soil sample Kofar Naisa SSK= Soil sample Kauran mata

Table 3: Biochemical Properties of the Prodigiosin Producing Isolate

Isolates	Pg	Gram Rxt	Ca	Cit	Ox	Ind	Ur	MR	VP	Slope	T Butt	S GP	I H2S	Inference
DSSK5	Red	- Short rod	+	+	-	+	+	-	+	R	Y	-	-	Serratia spp
SSK 3	Red	- Short rod	+	+	-	-	-	-	+	R	Y	-	-	*Serratia mercences*
SSK 7	Red	- Short rod	+	+	-	-	-	-	+	R	Y	-	-	*Serratia mercences*

Keys: Ca= Catalase, Ox+ oxidase, In= indole, Ur= urease, Pg= Pigmentation, Gf= Glucose fermentation

:SSK= Soil sample kauran mata, DSSK5= Dump soil sample kofar Naisa

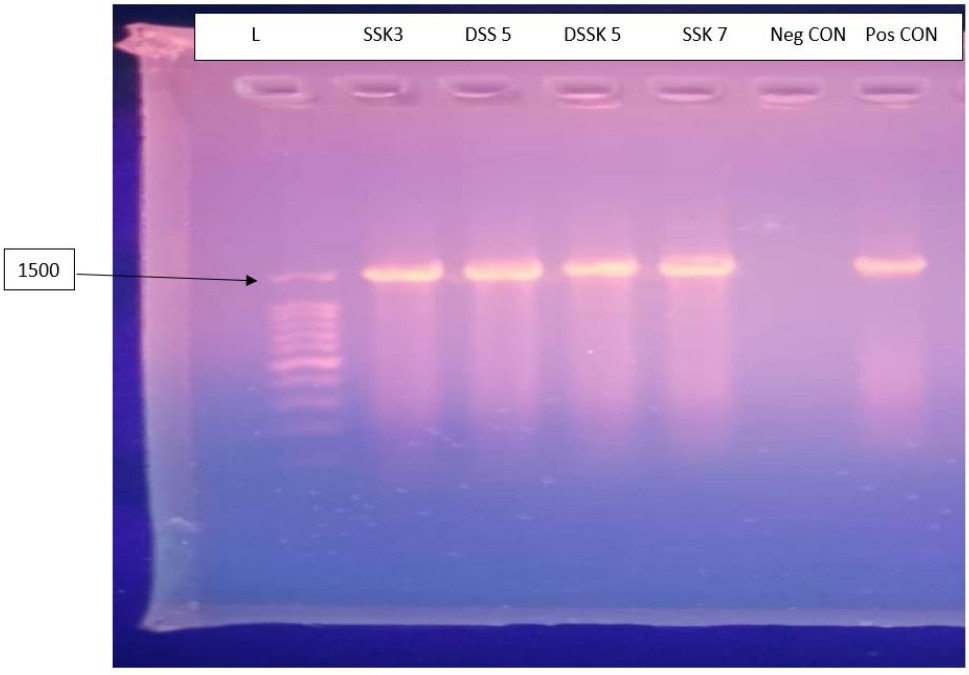


Figure 1: Electropherogram of the amplified 16 rRNA gene

Table 4: Summary of 16S rRNA sequencing results of the isolates to identify the closest homologs

Sample ID	Sequence bp	Identity of the closest homologs of the sequence	% identity	Accession N° of the closest homolog
U03	366	*Serratia marcescens strain KNU-AP16*	95.36%	KT945034.1
		Or		
		Serratia nematodiphila strain UMAGOD10 95.36%	95.36%	MH091061.1
U05	491	*Serratia marcescens strain ICC-4*	95.88%	KJ443715.1
U07	580	*Serratia marcescens strain XJalt12.1*	98.97%	HQ123483.1

DISCUSSION, CONCLUSION AND RECOMMENDATION

The results of isolation and screening of bacteria from different environments for prodigiosin production has indicated the presence of twenty reddish isolates from the different environment, out of the ninety-five samples collected. The result of the screening indicated that only three isolates were found to produce prodigiosin pigment which were DSSK 5 isolated from dumpsite at Kofar Naisa and SSK 3 and SSK 7 isolated from irrigated soil from farmland of Kauran Mata Madobi Local government of Kano state.

The isolation of prodigiosin producing bacteria from the soil may be due to the fact that several strains of *Serratia marcescens* that produce prodigiosin have been discovered in soil (Araujo *et al.*, 2022), While prodigiosin-producing wild strains are increasingly being isolated from diverse ecological niches, including freshwater lakes (Mekael *et al.*, 2023) and marine environments (Harde *et al.*, 2021), a significant majority of high-yield strains continue to be sourced from soil (Giri *et al.*, 2021).

This result is in line with the work of Namratha *et al.*, (2022) who conducted research on extraction and characterization of natural red pigment from a soil isolate *Serratia marcescens*. Red pigmented bacteria were isolated from a soil sample from the agricultural field of Vellore Institute of Technology (VIT), Vellore India. It is also in line with the work of Arijit et al., (2024), (Arivuselvam *et al.*, 2023) and (Anjali Rani *et al.*, 2024) who reported the isolation of *Serratia mercences* for prodigiosin production from the soil.

The result of the positive screening for prodigiosin shown by the three isolates was due to the tripyrrole ring in the pigment which can change color in response to pH variations (Jardak *et al.*, 2022). This outcome validated the identification of the extracted pigment as prodigiosin. This may be due to the fact that *Serratia mercences* are known for prodigiosin production. This is in line with the work of Farhana et al. (2023) and Namrata et al. (2022) who reported change of color from red to pink or red to yellow in the presence of concentrated HCL and concentrated NaOH as a positive preminary test for prodigiosin pigment.

Microscopic and macroscopic observation revealed the isolated bacteria as Gram-negative, rod-shaped, and motile that formed moderate round-shaped red colonies on nutrient agar medium. All the three prodigiosin producing bacteria were identified as *Serratia mercences* species using Gram staining and biochemical test. The result of the biochemical test

of the isolates further confirms the isolates' identities as *S. marcescens*, as they are consistent with standard biochemical descriptions that distinguished them from other closely related Enterobacteriaceae (Garrity, 2025).

The result of molecular characterization has indicated that SSK3 isolate was 95.36% similar to *Serratia mercences* strain KNU-AP16 and SSK 7 isolates was 98.97% similar to *Serratia mercences* strain INS 420 while DSSK 5 isolate was 95.38% similar to *Serratia marcescens strain* ICC-4 after they were blasted in NICB portal. This may due to the fact that the different strains of *Serratia mercences* were known for having the ability to produce prodigiosin pigment.

The result of the determination of prodigiosin produced by the isolates in nutrient broth has indicated that SSK 3 isolate produced the highest amount of prodigiosin (3.53mg/L) among the three isolates. The variation in pigment yield among the isolates is indicative of strain- specific differences in metabolic capacity and pigment biosynthetic efficiency. Such variability occurred in a study during which twelve strains of *Serratia marcescens* isolated from a hospital environment in Cairo produced varying levels of prodigiosin (Elkenawy *et al.*, 2017) and also in a study during which five strains of *Serratia marcescens* isolated from grey water from the discharge points of five different bathing rooms in student hostels at Nnamdi Azikiwe University, Awka Campus, Anambra State, Nigeria, produced varying levels of prodigiosin (Orji *et al.*, 2025). The highest prodigiosin-producing ability of SSK 7 may be attributed to efficient nutrient utilization during its growth (Orji *et al.*, 2025).

CONCLUSION

Twenty different redish colored bacteria were isola ted from different sources and were screened for prodigiosin production. Only three of these isolates were positive for prodigiosin production which were DSSK 5, SSK 3, and SSK 7 obtained from different soils sample. Molecular analysis showed that SSK 3 isolate was 95.36% similar to *Serratia marcescens* strain KNU-AP16 or *Serratia nematodiphila* strain UMAGOD10, DSSK 5 isolate exhibited 95.88% similarity to *Serratia marcescens* strain ICC-4, while SSK 7 isolate demonstrated 98.97% similarity to *Serratia marcescens* strain INS420.

The result of determination of amount of prodigiosin produced by each of the isolates has indicated that *Serratia mercences* strain KNU-AP16 produced the highest amount of prodigiosin (4.90±0.157) while *Serratia marcescens* strain ICC-4 produced the lowest amount of prodigiosin (2.76±0.019) in nutrient broth.

RECOMMENDATION

It is recommended that

1. The prodigiosin from the isolates should be purified and characterize
2. The optimum growth condition for isolate and prodigiosin production should be determined.
3. The antibacterial and antifungal activity of the isolate should be determined

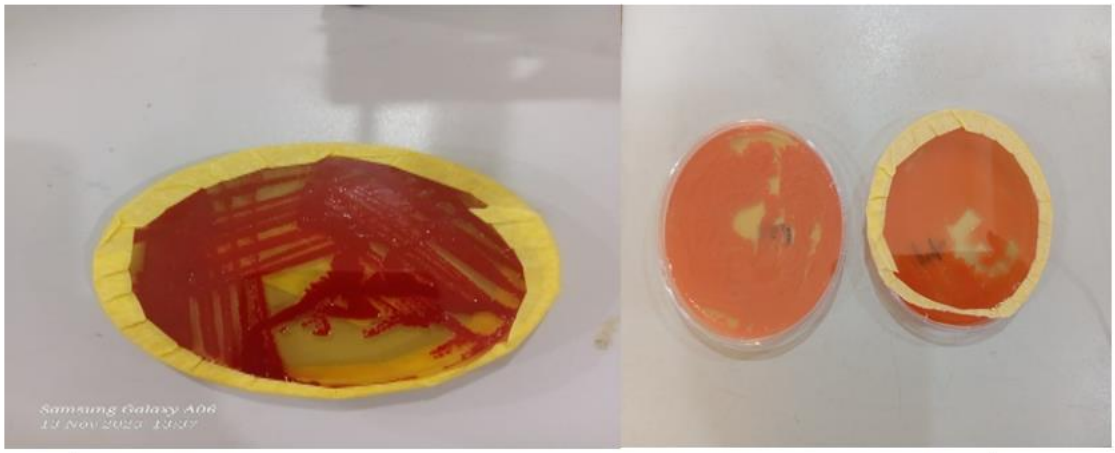


Plate 1: Different *Serratia mercences* isolated from the different soil samples



Plate 2: Screening of the isolates for prodigiosin production indicate change of color from red to pink with the addition of drops of Conc. HCL and from red to yellow with the addition of drops of Conc. NaOH



Plate 3: The different isolated grown in nutrient broth

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