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Isolation and Identification of Yellow Pigment Producing Rod-Shaped Bacterium from Farm Soil in Alimosho Local Government Area, Lagos State, Nigeria

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ABSTRACT: Microbial pigments are generally preferred to other natural pigments due to their easy scaling up, quick pigment extraction methods and simple culturing processes. Hence, the objective of this paper was to isolate and identify yellow pigment producing rod-shaped bacterium from farm soil in Alimosho Local Government Area, Lagos State, Nigeria using appropriate microbial and analytical standard procedures. The identification of the isolate revealed a Gram positive yellow pigment producing rod-shaped bacterium as *Brevibacterium iodinum*. The optimal conditions for pigment production by the *B. iodinum* was achieved in nutrient broth at pH7, 35°C under shaking condition (120rpm) using 5% inoculum of 0.4 $OD_{(600nm)}$. At these optimal conditions, 1.2g/L of biomass produced a total of 0.225g/L of crude pigment. The yellow pigment showed maximum absorption at 455nm. The GC-MS analysis of the crude pigment revealed major compounds such as methenamine; cis-10-Heptadecenoic acid, methyl ester; acetic acid, 2-[bis(methylthio)methylene]-1-phenylhydrazide and 4-Methyl-2-trimethylsilyloxy-acetophenone. This study has shown that *B. iodinum* produces a bacterial pigment which can be explored for different industrial applications.

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Keyword: Biomass; *Brevibacterium iodinum*; GC-MS; Microbial pigment; Optimal conditions

Colour has been an integral part of human existence and also increases the attractiveness of products (Grewal *et al*., 2022; Meng *et al*., 2022). Consequently, colour is an important in product such as paints, food, cosmetics, textiles, etc (Yusuf *et al*., 2017; Meruvu and dos Santos, 2021). Pigments are classified as either natural or manufactured based on where they come from. Some pigments not only impart distinct hues to goods but also include antimicrobial and antioxidant properties, offering a range of health advantages to humans (Tudor *et al*., 2013; Srivastava *et al*., 2022). Though synthetic petro-derived colorants have dominated the sectors due to low cost and high yield, the rising worries about their non-biodegradability, carcinogenicity and environmental toxicity have led both industry and researchers to start conducting researches on natural and safe alternatives. Because natural and eco-friendly biopigments are non-toxic, biodegradable, non-carcinogenic and non-allergic, they have therefore attracted a lot of attention. These qualities make them more acceptable to consumers, eliminate health risks

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at work, and allay environmental worries (Paillié-Jiménez *et al*., 2020; Aman Mohammadi *et al*., 2022). When it comes to production of natural pigments, microorganisms are preferred to plant or animal sources because of their ability to grow fast in culture media, availability throughout the year, low-cost with high yield and ease of controlling microbial cell factories for high production yields (Grewal *et al*., 2022). A large number of bacteria (e.g. *Bacillus* sp. *Brevibacterium* sp. *Corynebacterium michigannise* Pseudomonas sp. *Rhodococcus maris*), molds (e.g. *Aspergillus* sp., *A. glaucus*, *Blakeslea trispora*, *Helminthosporium catenarium*), yeasts (eg *Phaffia rhodozyma*, *Rhodotorula* sp.) (Joshi *et al*., 2003; Usmani *et al*., 2020) and algae produce pigments. Fermentation technique is a promising technology for producing safe pigments. However, the fundamental obstacle to commercial microbial production continues to be financial and marketing challenges (Ramesh *et al*., 2019; Aman Mohammadi *et al*., 2021).

Hence, the objective of this paper was to isolate and identify yellow pigment producing rod-shaped bacterium from farm soil in Alimosho Local Government Area, Lagos State, Nigeria.

MATERIALS AND METHODS

Isolation of Pigment Producing Bacterium: Soil samples were collected under sterile conditions from an agricultural farm in Alimosho Local Government Area in Lagos State. The GP coordinates of the sample sites are 6.538804^{o}N 3.255195^{o}E. The soil samples were mixed together, serially diluted and cultivated on nutrient agar for the isolation of pigmented colonies using standard methods. Yellow pigmented bacterial colonies were selected and maintained as pure cultures on nutrient agar slants at 4^{0}C.

Cultural and Morphological Identification of the Bacterium: The yellow bacterial isolate (31B2) was identified based on cultural and morphological characteristics (Padhan *et al*., 2021). The identity of the pigment producing bacterium was further confirmed with 16S rRNA sequencing for bacterial identification.

16SrRNA Sequencing: Genomic DNA was extracted from the cultures using the Quick-DNA™ Bacterial Miniprep Kit The 16S target region was amplified using OneTaq Quick-Load 2X Master Mix using forward (5'– AGAGTTTGATCMTGGCTCAG-3') and reverse (5'-CGGTTACCTTGTTACGACTT-3') 16S rRNA primers. The PCR products were run on a gel and extracted with the Zymoclean™ Gel DNA Recovery Kit. The extracted fragments were sequenced in the forward and reverse direction and purified. The purified fragments were analysed on the ABI 3500xl Genetic Analyzer (Applied Biosystems, ThermoFisher Scientific) for each reaction for every sample. CLC Bio Main Workbench v7.6 was used to analyse the ABI 3500XL Genetic Analyzer. The results were obtained by a BLAST search (NCBI) (Altschul *et al*., 1997).

Pigment Production by Submerged Fermentation: Inoculum Preparation: The isolate was subcultured on sterile nutrient agar and incubated at 37°C for 24 hours. Thereafter, 5 colonies of the 24-hour culture were inoculated into 20 mL of nutrient broth and incubated overnight at 37ºC.The overnight culture of the bacterial isolate was adjusted to an optical density (OD) of 0.4 at 600 nm wavelength.

Pigment Production and Extraction: This was carried out using static culture technique. Sterile nutrient broth (95 mL in 250 mL Erlenmeyer flasks) was inoculated in duplicate with 5% of the overnight culture (Gerelmaa *et al*., 2018) with an OD of 0.4. This gave a starting inoculum of 7.58 x 10^{8} cells/mL for isolate 31B2. Fermentation was done at 37°C for 5 days (Bhat and Marar, 2015). The cells were separated by centrifuging 5 mL of the culture medium at 4000 rpm for 20 minutes after 5 days incubation. The harvested cells were washed twice with sterile distilled water and centrifuged. The cell pellet was then suspended in methanol and placed in a water bath at 55°C for 15 minutes. Centrifugation was then carried out for 5 minutes at 4000 rpm. The procedure was repeated until the pellet turned white and all the coloured supernatants were gathered. The spectrophotometer (Genesys A10) was used to determine the maximum absorption wavelength (λ max) of the methanolic extract between 300 and 800 nm (Henriques *et al*., 2007; Sasidharan *et al*., 2013; Das *et al*., 2016; Trivedi *et al*., 2017).

Standardization of Culture Conditions for Optimum Pigment Production by isolate 31B2: The effects of various cultural conditions such as different incubation temperatures (28, 35 and 37ºC), pH (5, 6, 7, 8 and 9), shaking (120 rpm) and static conditions, incubation time (1-5 days on growth and pigment production were studied respectively. This was carried out by inoculating bacterial suspension of isolate 31B2 in Erlenmeyer flasks containing sterile nutrient broth (in duplicate). The growth and pigment production were determined separately. The optical density (OD) was determined at 600 nm using a spectrophotometer. For pigment production, pigment was extracted from 10 mL of the culture broth and the absorbance was measured at 455 nm, which is the maximum

absorption wavelength of the yellow pigment that was produced by isolate 31B2.

Effect of Temperature on Pigment Production by Isolate 31B2: The methods of Bhat and Marar (2015) and Padhan *et al*. (2021) were adopted with some modifications. Sterile nutrient broth (in duplicate) was inoculated with overnight culture of the bacterial isolate and incubated at 28°C, 35°C, and 37°C under static condition for 5 days to evaluate the effect of temperature on pigment production by isolate 31B2. Growth was determined by measuring the optical density at 600 nm using the spectrophotometer. Pigment production was determined by measuring the pigment absorbance at 455 nm, after extracting the pigment from 10 mL of the culture broth. The optimum temperature was used for further studies.

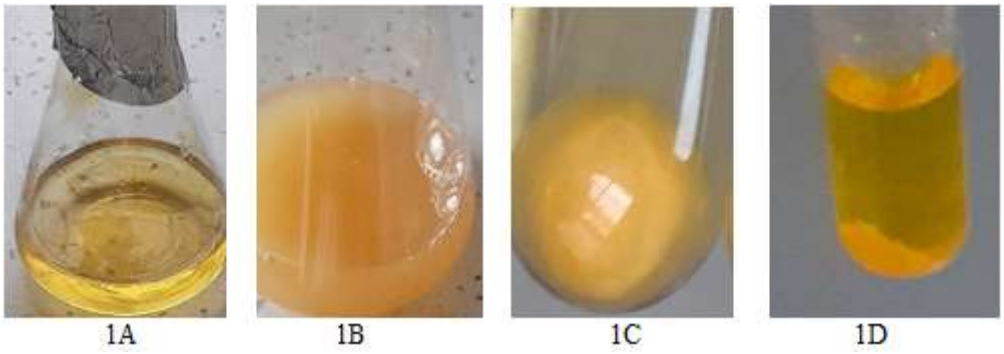

Plate 1: Fermentation broth of isolate 31B2 and the extracted pigment. 1A = Control without the isolate; 1B = Fermentation at 72hours with initial pH of 7 at 35°C; 1C = pellet obtained after centrifugation; 1D = pigment extracted with methanol.

Effect of pH on Pigment Production by Isolate 31B2: The pH was measured with a pH meter (Hanna). The pH meter was always calibrated before measurements were taken. The effect of pH on pigment production was carried at different pH values (5, 6, 7, 8 and 9) using the methods of Bhat and Marar (2015) and Poddar *et al*. (2021). The cultures were grown under static condition for 5 days at 37°C. The growth and pigment production were determined separately. Growth was determined by measuring the optical density at 600 nm using the spectrophotometer. Pigment production was determined by measuring the pigment absorbance at 455 nm, after extracting the pigment from 10 mL of the culture broth. The optimum pH obtained was used for further studies.

Effect of Agitation on Pigment Production by Isolate 31B2: The influence of aeration on pigment production was determined as follows: Sterile nutrient broth (in duplicate) was inoculated with overnight culture with an OD of 0.4 to give starting inoculum of 7.58 x 10^8 cells/mL of the bacterial isolate and incubated under static and shaking conditions at 37°C (Jose *et al*., 2017). The growth and pigment production by isolate 31B2 were determined separately. Growth was determined by measuring the optical density at 600nm using the spectrophotometer. Pigment production was determined by measuring the pigment absorbance at 455 nm, after extracting the pigment from 10 mL of the culture broth.

Effect of Incubation Time by Isolate 31B2: The influence of incubation time on pigment production was determined as follows: Sterile nutrient broth (in duplicate) was inoculated with overnight culture of the bacterial isolate and incubated under shaking condition at 37°C for 24, 48, 72, 96 and 120 hours (Bhat and Marar, 2015; Jose *et al*., 2017). The pH, OD at 600 nm, pigment absorbance at the peak absorption wavelengths (455 nm) and total viable count were determined separately. Optical density was measured at 600nm wavelength while the total viable count (TVC) was done by spread plate method. An aliquot (0.1 mL) of the serially diluted fermentation broth was plated out every 24 hours on nutrient agar and incubated at 37ºC for 48 hours.

Pigment Yield Analysis by Isolate 31B2: The actual pigment yield by isolate 31B2 was determined with 5% v/v inoculum with OD of 1. Sterile nutrient broth (95 mL) was inoculated in duplicate with 5% of overnight culture with an OD of 1.0. The flasks were incubated at 35ºC for 72 hours with shaking at 120 rpm. After 72 hours, pigment was extracted and air dried to a constant weight. Based on the dry weight of yellow pigment obtained, the pigment yield (g/L) was calculated using equation 1 (Ahmad *et al*., 2015).

$$Pigment\ Yield\ \left(\frac{g}{L}\right) = \frac{A1 - A0}{V} \quad (1)$$

Where A_0: Weight of empty vial, A_1: Weight of dried pigment, V: Volume of sample

Pigment Identification: Determination of Maximum Absorption Wavelength of the Pigment: The maximum absorption wavelength (λ max) of the coloured supernatant obtained after extraction was determined with a UV-Vis spectrophotometer (Genesys A10) within a range of 300-800 nm (Henriques *et al*., 2007; Sasidharan *et al*., 2013; Das *et al*., 2016; Trivedi *et al*., 2017).

Gas Chromatography - Mass Spectrometry (GCMS) Analysis of the Pigment: The GC-MS analysis of the pigment was carried out on Agilent chromatography GC (Model 7820A series) coupled to 5975C inert mass spectrometer (with triple axis detector) with electron impact source. The stationary phase of separation of the compounds was HP-5 capillary column coated with 5% phenyl methyl siloxane (30 m length x 0.32 mm diameter x 2.25 µm. The carrier gas was Helium used at a constant flow of 1mL per minute at an initial nominal pressure of 1.4902 psi and average velocity of 44.22 cm per second. 1µL of the crude pigment was injected in splitless mode at an injection temperature of 300°C. Purge flow to split vent was 15 mL per minute at 0.75 minute with a total flow of 16.667 mL per minute. Initially, the oven was set at 40°C for 1 minute, then 12°C per minute to 300°C for 10 minutes, then 5°C per minute and run time was 32.667 minutes with a 3 minute solvent delay. The mass spectrometer was operated in electron-impact ionization mode at 70eV with ion source temperature of 230°C, quadrupole temperature of 150°C and transfer line temperature of 300°C. Acquisition of ion was via Scan mode (scanning from m/z to 50 to 500 amu at 2.0s/scan rate) and selective ion mode (SIM).

Statistical Analysis: Statistical analysis was performed using two-way ANOVA with Graph Pad Prism 9.0. The results are expressed as means ± standard deviation.

RESULTS AND DISCUSSION

Isolation and Identification of Pigment Producing Bacterium: The identification of the yellow pigmented bacterial isolate was based on its cultural and morphological characteristics. The results showed that the isolate was a Gram positive rod-shaped bacterium which produced a circular, raised and smooth colonies on nutrient agar. The 16S rRNA sequencing and BLASTN analysis of the amplified PCR products identified the bacterial isolate as *Brevibacterium iodinum* with a percentage identity of 99.71 with *Brevibacterium iodinum* strain JX393074.1.

Pigment Production by Fermentation: The bacterial strain designated 31B2 which was identified as *B. iodinum* produced an intense yellow pigment as shown in plate 1. The yellow pigment showed maximum absorption at a wavelength of 455 nm.

Standardization of Culture Conditions for Optimum Pigment Production: The results for the standardization of culture conditions for optimum pigment production are represented in Figures 1 to 4. The optimal culture condition for pigment production by *B. iodinum* in nutrient broth was achieved at an initial pH of 7 and incubation temperature at 35°C under shaking condition (120 rpm) using 5% v/v inoculum adjusted to an optical density of 0.4 at 600 nm with a spectrophotometer.

Effect of Incubation Temperature on Biomass and Pigment Production by Brevibacterium iodinum: The effect of incubation temperature on the biomass of *B. iodinum* (measured as OD) and its pigment production are shown in figure 1. The OD value of the fermentation broth at 28°C was significantly higher than the OD value at 37°C ($p=0.0232$). There was no significant difference between the OD at 28°C and 35°C and between 35°C and 37°C. There was also no significant difference among the pigments produced by *B. iodinium* at 28°C, 35°C and 37°C. Therefore, there was positive correlation between the temperature and bacterial growth which is a function of the OD. In line with this finding, Usman *et al*. (2018) reported 35°C as the optimum temperature for pigment production in *Chromobacterium violaceum*. Pathak and Sardar (2012) also reported remarkable production of carotenoid by a halotolerant Gram negative bacterium (SS-12) at temperature 35°C. In another study, Mohana *et al*. (2013) reported that *Micrococcus luteus* exhibited optimum growth and pigment production at 35°C. Similarly, Korumilli and Mishra (2014) reported maximum yield of β-carotenoid using *Bacillus clausii* at 35°C utilizing rice powder as a sole substrate. Parmar *et al*. (2017) also reported that *Streptomyces flavofuscus* ARITM02 isolated from rhizosphere soil also showed optimum pigment production at 35°C. This suggests that the production of microbial pigments is greatly affected by the incubation temperature and this depends on the type of microorganism used (Goswani *et al*., 1998).

The growth of a microorganism can also be expressed as biomass measured as optical density of the culture. An organism may show a higher cell density at one temperature and higher pigment production at another temperature. Pigment production is often not directly proportional to the biomass because pigment production can be affected by metabolic pathways and regulatory mechanisms which are sensitive to the environment. Some microorganism produce pigments

in response to environmental stress. Thus, it can be said that *B. iodinum* grows better at 28°C but produces more pigment at 35°C as a stress response or due to changes in its metabolic pathway. The optimal growth temperatures for Brevibacteria vary from 20°C to 30°C for environmental and food-derived isolates and up to 37°C for the human isolates (Irlinger *et al*., 2017). So it is possible that temperatures beyond or below this temperature range can be seen as stress by this organism because it is an environmental isolate. For instance, when stored in the refrigerator, *B. iodinum* produces more of this yellow pigment, possibly in response to cold shock and from this study, it produces more pigment at 35°C than 28°C.

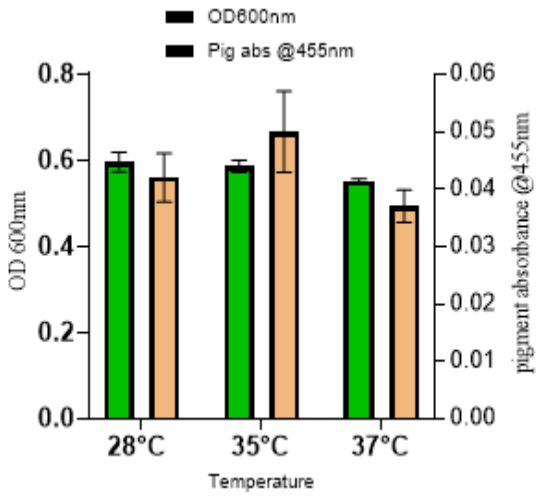

Fig.1: Effect of incubation temperature on growth and pigment production by *Brevibacterium iodinum*.

Effect of Initial pH on Biomass and Pigment Production by Brevibacterium iodinum.

The highest biomass was observed at pH 6 while high pigment production was obtained at pH 7 (Fig.2). Low pH (pH 5) inhibited both biomass and pigment production. Pigment production at pH 5 was significantly lower than the production at pH 7 (p= 0.0045), pH 8 (p = 0.0282) and pH 9 (p =0.0331). Also, pigment production at pH 6 was significantly lower than the production at pH 7 (p =0.0045), pH 8 (p =0.0282) and pH 9 (p=0.0331). The optical density at pH 5 was significantly lower than the OD values at pH 6, pH 7, pH 8 and pH 9 (P<0.0001). The optical density has a positive correlation with the pigment production (r = 0.920) at the different pH values. The results were similar to those of John and Aruna (2019) who reported maximum pigment production by *Kocuria flava* majod at pH 7 while pH 6 gave the highest cell mass. Cruz-Munoz *et al*. (2015) also reported that the maximum pigment synthesis was obtained at pH 7 for *Pycnoporus sanguineus* strain H1 and H2. In another study, Pathak and Sardar (2012) reported remarkable carotenoid production by a halotolerant Gram negative bacterium (SS-12) at pH 7. Mohana *et al*. (2013) reported that *Micrococcus luteus* exhibited optimum growth and pigment production at pH 7.0. Korumilli and Mishra (2014) also reported maximum yield of β-carotenoid using *Bacillus clausii* at pH 7 utilizing rice powder as a sole substrate. In another study, Parmar *et al*. (2017) reported that *Streptomyces flavofuscus* ARITM02 isolated from rhizosphere soil showed optimum pigment production at pH 7.5.

pH affects pigment production by different microorganisms by influencing their metabolism, enzyme activity and stress responses. The result obtained in this study shows that neutral pH favours the production of yellow pigment by *B. iodinum*. Also, an organism may show a higher cell density at one pH and higher pigment production at another pH because pigment production can be affected by metabolic pathways and regulatory mechanisms which are sensitive to the environment. Some microorganism produce pigments in response to environmental stress. Thus, it can be said that *B. iodinum* grows better at pH 6 but produces more pigment at pH 7.

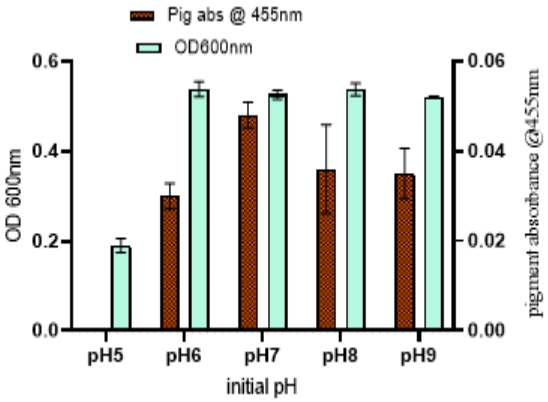

Fig. 2: Effect of Initial pH on growth and pigment production by *Brevibacterium iodinum*

Effects of Static and Shaking Conditions on Biomass and Pigment Production by Brevibacterium iodinum:

The biomass (OD) and pigment production were significantly enhanced by shaking compared to static condition (p<0.0001) and (p= 0.0307) respectively. The OD value at static condition was (0.423±0.006) and that obtained at shaking condition was (1.337 ± 0.076). The pigment production was 0.0185±0.0021 at static condition and 0.187 ± 0.0325 at shaking condition. *B. iodinum* showed increased growth and pigment production at shaking incubation condition compared to static condition (Fig. 3). Agitation improves transfer of substrates and oxygen in aerobic conditions. Similar findings have been reported on pigment production by different microorganisms. For

instance, Valduga *et al*. (2009) obtained maximum total carotenoids of 1019 mg/L when *Sporidiobolus salmonicolor*-CBS 2636 was grown in shaken flasks. John and Aruna (2019) also reported maximum pigment production by *Kocuria flava* majod at shaking condition. Yet in another study, Dag *et al*. (2023) observed that both growth and pigment production by *Fusarium graminearum* occurred only in shaking incubation conditions. This can be attributed to the fact that in shaking conditions, agitation improves the aeration of the culture medium, helps to evenly distribute the nutrients in the medium, prevents clump formation and increases metabolism rate. However, a different observation was reported by Banerjee *et al*. (2011). In their study, they discovered that maximum production of green pigment by *Bacillus cereus* M-16(1) (MTCC 5521) occurred at static condition, which probably indicates that oxygen is inhibitory to the synthesis of this pigment while oxygen promotes the production of pigments by some microorganisms like *B. iodinum*.

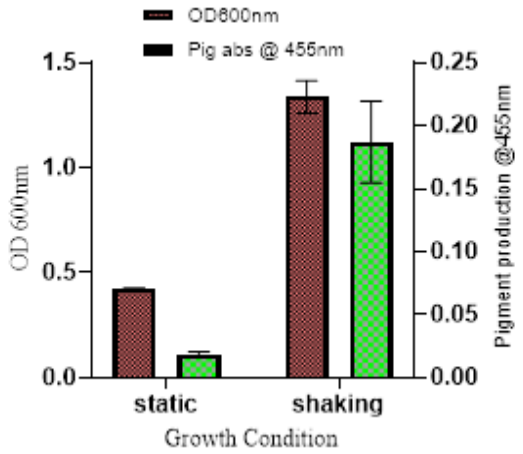

Fig. 3: Effects of static and shaking conditions on biomass and pigment production by isolate *Brevibacterium iodinum.*

Effect of Incubation Time on Biomass and Pigment Production by Brevibacterium iodinum.

As shown in fig. 4, the biomass and pigment production by *B. iodinum* were maximum after 72 h of fermentation. This was also observed by Banerjee *et al*. (2011) in their studies on green pigment from *Bacillus cereus* M1 16 (MTCC 5521). *B. iodinum* started producing the yellow pigment after 24 hours of incubation, which increased till 72 hours of incubation (Fig. 4). The pigment production was optimal at 72 hours and started declining from 96 hours of incubation, while the pH kept increasing till 120 hours of incubation. The total viable count of the *B. iodinum* was highest at 24 hours and started declining after 48 hours incubation. There was no significant difference among the total viable count values at the different incubation times ($p<0.05$). On the other hand, the pH of the fermentation broth generally increased from 24 hours to 120 hours of incubation. The pH of the fermentation broth was more alkaline at 120 hours (8.90 ± 0.283) and neutral at 24 hours (7.5 ± 0.283). The pH at 24 hours was significantly lower than the pH at 72 hours ($p = 0.0027$), the pH at 96 hours ($p = 0.0007$) and pH at 120 hours ($p = 0.0004$). The pH at 48 hours was significantly lower than the pH at 96 hours ($p = 0.022$) and the pH at 120hrs ($p=0.0108$). According to Frengova *et al*. (1994) the biosynthesis of carotenoids causes changes in pH of the medium as a result of growth. Generally, the pH of the fermentation medium falls in the first 72 h of carotenoid production and then rises during the intense phase of carotenogenesis (Zeni *et al*., 2011). This is in line with pH changes observed through the incubation days. Carotenoid production occurs in response to environmental conditions such as growth temperature, pH, light, salt concentration, etc. and studying the regulatory mechanisms behind this process has provided clues on how bacteria adapt to their environments (Sutthiwong *et al*., 2014). The connection between growth and pigment production in *B. iodinum* may be partially attributed to its strict aerobic metabolism (Irlinger *et al*., 2017). This may account for the production of carotenoid pigments for antioxidant protective effect, which suggests that they could be primary metabolites.

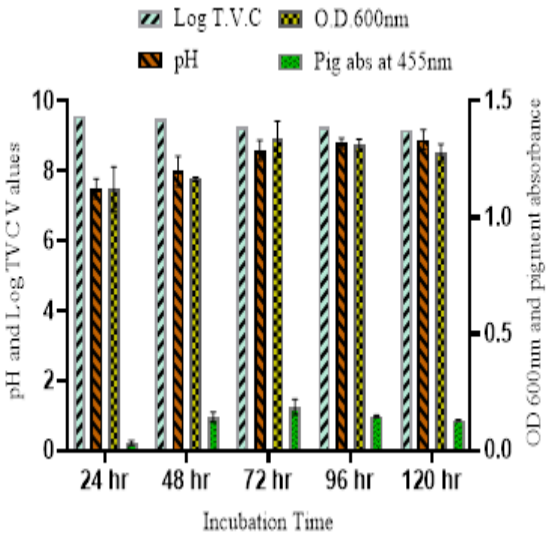

Fig. 4: Effect of incubation days on growth and pigment production by *Brevibacterium iodinum.*

Pigment Yield Analysis: The pigment yield analysis showed that 1 L culture of *B. iodinum* produced a total of 1.2 g of biomass after 72 hours of incubation at the optimal conditions. The solvent extraction process using methanol yielded a total of 0.225 g of crude pigment after evaporation of the methanol from the extraction mixture.

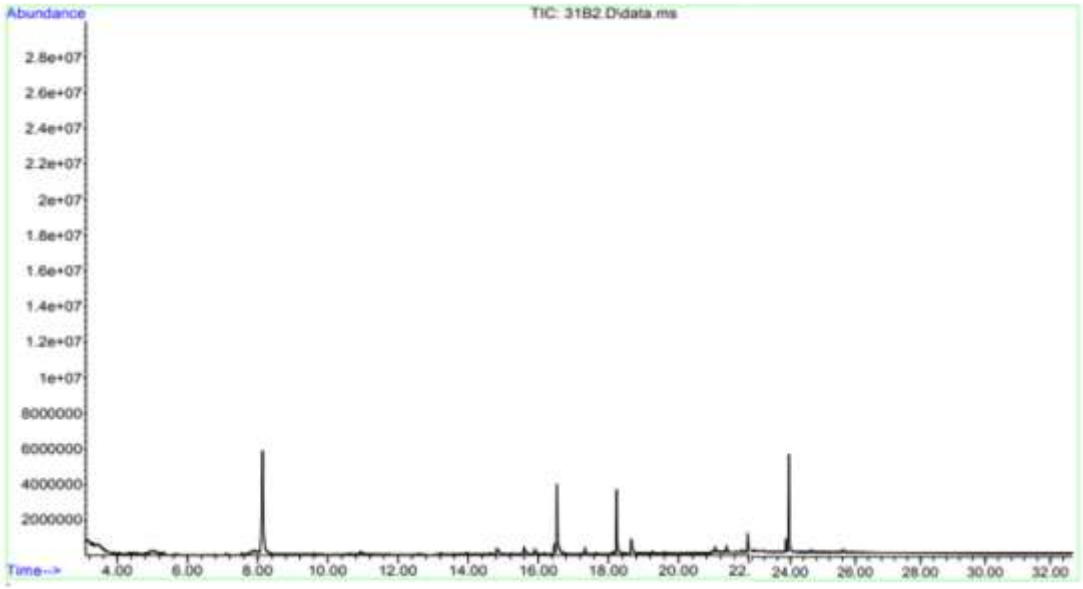

Fig. 6: GC–MS chromatogram of the yellow pigment produced by *Brevibacterium iodinum*

This is almost similar to the yield of pink pigment produced by newly isolated bacterial strain *Enterobacter* sp. PWN1 (Poddar *et al*., 2021) where at optimal conditions, 1 L of cell culture produced 3.77 g of biomass which produced a crude pigment of 0.234 g after solvent extraction and 0.131 g after column chromatography. The yield of the obtained pigment was high enough to draw interest for industrial production, although the application of the pigment is considerable for further study. This is higher than many other reported intracellular bacterial pigments (Poddar *et al*., 2021).

Pigment Identification: Visible Spectrum of Brevibacterium Iodinum Methanolic Extract: The bacterial isolate identified as *B. iodinum* produced an intense yellow pigment with maximum absorption at 455nm (Figure 5). This suggests the possibility of the pigment being a carotenoid since carotenoids typically exhibit absorption in the visible region, around 400 to 500nm. Guyomarc'h *et al*. (2000) in their study on production of carotenoids by *Brevibacterium linens* reported that all the extracts obtained from the strains used in their experiments gave the same carotenoid-like absorption spectrum, with a 454 nm λ max.

Gas Chromatography-Mass Spectrometry (*GC-MS) Profile of the Methanolic Extract:* The GC-MS chromatogram of the crude pigment extract revealed different peaks (Fig. 6). Some of the compounds identified in the chromatogram are methenamine (10.866min), Azulene, 1,2,3,5,6,7,8,8a-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1.alpha.,7.alpha.,8a.beta.)]-(13.968min), cis-10-Heptadecenoic acid, methyl ester (18.38 min), acetic acid, 2-[bis(methylthio)methylene]-1-phenylhydrazide (22.168 min) and 4-Methyl-2-trimethylsilyloxy-acetophenone (24.011 min) with percentage area of 12.03%, 1.62%, 0.09%, 0.70% and 0.54%, respectively.

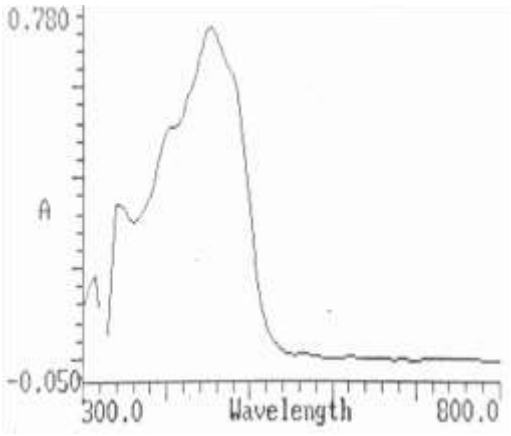

Fig. 5: UV-Visible absorption spectrum of pigment produced by *Brevibacterium iodinum*, showing λ max at 455nm.

Among these identified compounds, methenamine is a heterocyclic organic compound used to prevent or control returning urinary tract infections caused by certain bacteria while cis-10-Heptadecenoic acid, methyl ester is a fatty acid that is found in microalgae and used as a biodiesel. Kohl *et al*. (1983) separated three carotenoid pigments from *B. linens* cells and reported that they all had virtually identical electron spectra, with a 454 nm λ max, which they later identified as isorenieratene, 3-hydroxyisorenieratene and 3,3′-dihydroxy-isorenieratene. Guyomarc'h *et al*.

(2000) in their study on production of carotenoids by *B. linens* also reported that the HPLC analysis revealed three groups of peaks, possibly non-hydroxylated, mono- and di-hydroxylated carotenoids. The peaks showed λ max at 454 nm, 449 nm and 444 nm respectively.

Conclusion: A yellow-colored intracellular pigment was extracted from a pigmented bacterium isolated from soil sample collected from a farm in Alimosho Local Government Area in Lagos State. The isolated strain was found to be Gram-positive and non-endospore forming in nature. The 16S rRNA gene sequencing identified it as *B. iodinum*. To maximize its pigment production, the culture conditions were optimized considering the following parameters: temperature, pH, static and shaking conditions and incubations time. The compounds identified from the GC-MS analysis of the pigment as well as the maximum absorption wavelength of the pigment revealed the probable identity of the pigment as a carotenoid. Thus, this study has shown that isolate 31B2 which was identified as *B. iodinum*, is a source of bacterial pigment with possible diverse industrial applications.

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Declaration of Conflict of Interest: The authors declare no conflict of interest.

Data Availability Statement: Data are available upon request from the corresponding author.

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