Original Research Article

Bioproduction and Application of Orange and Yellow Pigments Obtained from *Chryseomicrobium palamuruense* and *Micrococcus luteus.*

Abstract

Colour is a fundamental and most likely one of the primary attributes to be observed by the human senses, making it an essential part of human life and culture. This study investigates the bioproduction and potential application of bacterial pigments obtained from abattoir wastewater. Bacteria isolates recovered from abattoir wastewater were screened for pigment production and identified using the 16S rRNA gene sequence. Optimal physicochemical conditions of parameters were investigated and harnessed for bulk pigment production. Pigments produced were characterised using UV-Visible Spectrophotometer and Fourier Transform Infra-Red (FTIR) Analysis. The antibacterial and dyeing ability of the extracted pigments were evaluated. Two bacteria namely, *Chryseomicrobium palamuruense* strain PUI and *Micrococcus luteus* strain 1k produced orange and yellow pigments respectively. The UV-visible spectrum of the orange pigment produced by *Chryseomicrobium palamuruense* showed surface plasmon resonance peaks at 262, 426, and 453 nm while that of the yellow pigment produced by *Micrococcus luteus* showed a single peak at 260 nm. The FTIR analysis of the extracted pigments indicated the presence of some functional groups namely hydroxyl (OH), C-H, primary and secondary C-O, and skeletal C-C vibrations. *Chryseomicrobium palamuruense* and *Micrococcus luteus* showed no antibacterial activity against the selected typed bacterial isolates assessed in this study. The orange pigment extracted from *Chryseomicrobium palamuruense* was retained on cotton and polyester producing different shades of colour on the two fabrics when used as a dyeing agent. The pigment-producing potentials of these organisms suggest their relevance as promising dyes in textile industry.

Keywords: Pigmented bacteria, *Chryseomicrobium palamuruense*, *Micrococcus luteus*, Antibacterial activity, Textile dye

1. Introduction

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The discovery of safe, biodegradable, and sustainable pigments from biological origin became a veritable alternative for synthetic pigments which have been reported to be toxic, posing a huge health risk for humans (Lagashetti *et al.,* 2019; Lyu *et al.,* 2022). These biological pigments have been derived from various sources namely: plants (Azman *et al.,* 2018), animals (Caro *et al.,* 2017), and microorganisms such as microalgae (Yusuf *et al.,* 2017; Arashiro *et al.,* 2020), yeasts and filamentous fungi (Cipolatti *et al.,* 2019; Ramesh *et al.,* 2019), bacteria and actinobacteria (Narsing Rao *et al.,* 2017; Sajjad *et al.,* 2020). Having discovered microorganisms with an abundant source of novel bioactive compounds (Wang *et al.,* 2018), its pigments were most preferred compared to other biological origins such as plants and animals due to their solubility, availability, and simple cultivation technique (Kim *et al.,* 1998), the stability of the pigments produced (Raisainen *et al.,* 2002), rapid growth rate leading to higher productivity without seasonal variation, ease of genetic modification (Venil *et al.,* 2014).

Pigments, like other natural products such as vitamins, steroids, enzymes, and antibiotics, is a secondary metabolite mostly produced within the cytoplasm of microorganisms consist of various chemical components having biological functions often produced in response to normal or harsh ecological conditions (Kim, 2013; Pagano and Dhar, 2015). For example, microorganisms use pigment molecules as safety against ultraviolet irradiation (Becker-Hapak *et al.,* 1997), energy source (Madigan *et al.,* 2012), and confrontational tool against extreme temperature and desiccation (Wada *et al.,* 2013). Pigment-producing bacteria are ubiquitous hence, found in different ecological niches namely, air (Narsing Rao *et al.,* 2017), soil (Zeni *et al.,* 2011; Patki *et al.,*2021), marine (Minal and Rohini, 2018; Metwally *et al.,* 2021; Wahyudi *et al.,* 2022), freshwater and river (Asker *et al.,* 2008; Jayaraman *et al.,* 2020; Lyakhovchenko *et al.,* 2021), halophilic region (Azman *et al.,* 2018), desert (Liu *et al.,* 2009), organic residue (Nakamura *et al.,* 2002), landfills (Siddiqqui, 2017), domestic and industrial effluent (Gowri *et al.,* 2020; Poddar *et al.,* 2021).

Bacteria produce various hues of coloured pigments such as red (*Serratia marcescens, Gordonia jacobae, Deinococcus* sp.), yellow (*Micrococcus luteus, Hymenobacter* sp.), green (*Pseudomonas* sp., *Bacillus cereus*), orange (*Erythrobacter* sp., *Planococcus maritimus*), blue (*Corynebacterium insidiosum, Erwinia chrysanthemi, Vogesella indigofera*), red-yellow (*Kocuria* sp., *Chryseobacterium artocarpi*), and purple (*Chromobacterium violaceum, Janthinobacterium lividum Duganella violaceinigra*) pigments (Chaudhari and Jobanputra, 2013; Choi *et al.,* 2015; Venil *et al.,* 2020). Aside from being used as colourants in textile industries, Bacterial pigments have

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found usefulness in the food industries as colouring agents in yoghurt, milk, carbonated drinks (Namazkar *et al.,* 2013), meat (Giovannucci *et al.,* 2002), poultry feed (Surai, 2012), animal and fish feed (Asker, 2017; Pogorzelska *et al.,* 2018), and as a source of Vitamin A (Kot *et al.,* 2016; Sigurdson *et al.,* 2017). They have also been found relevant in the biomedical industries as antioxidant agents (Durán *et al.,* 2012; Tuli *et al.,* 2015; Jayaraman *et al.,* 2020), anticancer agents (Afra *et al.,* 2017; Rezaeeyan *et al.,* 2017), antiviral agent (Sun *et al.,* 2012) and antimicrobial agent (Zerrad *et al.,* 2014)

Although an array of pigment-producing bacteria has been reported, there is still a need to further discover the potential of untapped bacteria species which could be a suitable replacement for a more secure and low-cost microbial pigment. This study seeks to isolate pigment-producing bacterial species from abattoir wastewater and harness their potential as antibacterial and dyeing agents.

2. Materials and Methods

2.1 Sample Location

Wastewater samples from Ola-Sheu slaughterhouse Ola-Olu (7°29'29.3"N, 4°33'08.9"E) were assessed for this study.

2.2 Isolation and Screening of Pigment-Producing Bacteria

Ten-fold serial dilutions were prepared and 0.1 mL of the diluents were spread-plated on Nutrient agar plates. The inoculated plates were incubated at 37 °C for 24-48 hours, after which the plates were observed for pigmented colonies. The pigmented colonies were inoculated into 5 mL Nutrient broth (HiMedia) and incubated at 37 °C for 24 hrs. Following incubation, 0.2 mL aliquot was inoculated into 2% glycerol-containing Nutrient broth and incubated at room temperature (25 ± 2 °C) for 48 hours under shaking conditions. The isolates with intense colour change in the broth after 48 hours and also with coloured pellets after centrifugation at 3,500 rpm for 20 minutes were selected for identification and further studies (Sinha *et al.,* 2017). The pure colonies were selected and stored in Nutrient agar slants for further use.

2.3 Molecular Characterisation of the Bacterial Isolates

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Bacterial DNA was extracted using the modified method of Yamagishi *et al.* (2016). The Polymerase Chain Reaction (PCR) amplification was performed using a final reaction volume of 25 µL containing master mix (12.5 µL); forward primer 5' – CCAGCAGCCGCGGTAATACG -3' (1 µL); reverse primer 5' - ATCGGCTACCTTGTTACGACTTC -3' (1 µL) (Lu *et al.,* 2000); nuclease-free water (5.5 µL) and DNA template (5 µL). The reaction was performed in an automated thermal cycler (GeneAmp PCR System 9700) under the following optimized cycling program: an initial denaturation step at 94 °C for 4 min; 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 53 °C for 1 min, extension at 68 °C for 2 minutes; and a final extension at 68 °C for 5 minutes. The temperature was maintained at 4 °C till it was subjected to gel electrophoresis (Barghouthi, 2011). The amplified PCR fragments were subjected to electrophoresis using 1.5% agarose gel. The amplified PCR fragments were purified and sequenced. The resulting sequences were analyzed using Basic Local Alignment Search Tool (BLAST) and aligned with the most similar bacterial species found in the GenBank.

2.4. Optimisation of Physicochemical Factors on Pigment Production

For each physicochemical parameter considered, 100 mL Conical flasks containing 25 mL of Nutrient broth were used for the optimization procedures. The optimum incubation period for pigment production was determined using 1% of the standardized inoculum volume equivalent to 1.5×10^8 cfu/mL. Samples were withdrawn at 12 hours intervals for up to 72 h. The agitation effect on pigment production was determined for 48 h both in an incubator and rotary shaker (120 rpm) (Hizbullahi *et al.,* 2018). Optimum inoculum volume supporting bacterial pigment production was conducted using different volumes namely, 0.5%, 1.0%, 1.5%, 2.0%, and 2.5% (Banerjee *et al.,* 2011). Similarly, optimum pH validating adequate pigment production was determined by adjusting Nutrient broth pH with 1N sodium hydroxide (NaOH) or 1N of hydrochloric acid (HCl) from pH 3 to 11 (Hizbullahi *et al.,* 2018). Suitable temperature was determined from varying temperatures namely, 25, 30, 35, 40, 45, and 50 °C (Garg *et al.,* 2017). All parameters excluding temperature were incubated at room temperature (25±2 °C), and shaking condition of 120 rpm. The absorbance readings of samples were determined using a visible spectrophotometer at a fixed wavelength of 590nm.

2.5 Production and Extraction of Bacterial Pigment

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Bulk production of bacterial pigments was carried out using optimal conditions of the screened parameters. Thereafter, bacterial cells were harvested by centrifuging at 3,500 rpm for 20 min, after which it was washed in distilled water and then centrifuged at 3,500 rpm for 20 min. The pellets were re-suspended in Ethanol for pigment extraction. The mixture was vortexed and the suspension was centrifuged at 3,500 rpm for 20 min to collect the supernatant. Centrifugation was repeated till the cell pellets became colourless. After centrifugation, the supernatants containing the diffused pigments were filtered using 0.45 mm Whatman filter paper followed by a 0.22 µm millipore membrane filter (Sinha *et al.,* 2017).

2.6 Characterisation of the Extracted Pigment

Characterisation of the pigments was carried out using UV-Vis Spectrophotometer and Fourier Transform Infra-Red (FTIR) Analysis. The surface plasmon resonance (SPR) of the extracted pigments was observed by a UV-Vis spectrophotometer (UV-1800 Series, Shimadzu, Japan) with a scanning range of 200-600 nm. Absorbance peaks for each pigment produced were observed. Similarly, spectral peaks of the extracted pigments were obtained using Fourier Transform Infra-Red Analyser (Agilent Technologies, USA) (Srinivasan *et al.,* 2017).

2.7 Antibacterial Activity of Bacterial Pigments

Evaluation of the antibacterial activity of the extracted pigments was carried out on some laboratory typed bacterial isolates using the agar well diffusion method. Six Gram-positive bacteria isolate namely, *Clostridium sporogenes* (NCIB 532), *Bacillus polymyxa* (NCIB 4747), *Staphylococcus aureus* (NCIB 8588), *Bacillus stearothermophilus* (NCIB 8222), *Bacillus cereus* (NCIB 6349), *Bacillus subtilis* (NCIB 3610) and four Gram-negative isolates namely, *Proteus vulgaris* (NCIB 67), *Klebsiella pneumoniae* (NCIB 418), *Proteus morganella* (NCIB 10466), *Proteus rettgeri* were used as test isolates. The test isolates were standardized (0.5 McFarland) and seeded on Mueller Hinton agar (MHA) plates. Bored wells in inoculated plates were filled with various volumes of the bacterial pigments solution (25 µL, 50 µL, 75 µL, and 100 µL). The plates were incubated at 37 ^{o}C for 24 h and the plates were observed for a zone of inhibition.

2.8 Evaluation of dyeing properties of Bacterial Pigments

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A 2 cm^2 area of three (3) white pieces of cotton and polyester fabric was soaked in 3 mL of the solvent extract of the pigments and subjected to incubation at room temperature for 48 h at room temperature. After incubation, the pieces of cloth were allowed to dry and each fabric was treated with 0.1 M HCl (an acid solution), 0.1 M NaOH (an alkali solution), and soap solution for 15 minutes. Effects of different mordants were observed relating to colour fixation, colour stickiness, and colour removal (Agha *et al.,* 2019).

2.9 Statistical Analysis

Experiments were conducted in triplicates, and values obtained are expressed as mean ± standard error. Statistical analysis and graphical representation of data obtained were performed using Microsoft Excel software.

3 Results and Discussion

3.1. Isolation and Identification of Isolates

Two pigment-producing bacterial species were isolated (AW6 and AW8) and selected after screening for further studies. The 16S rRNA sequencing and BLASTN analysis of their amplified PCR products identified the bacterial isolates (AW6 and AW8) as *Chryseomicrobium palamuruense* strain PU1 with a percentage identity of 98.55% and *Micrococcus luteus* strain 1k with percentage identity of 99.05% respectively. The pictorial representation of the isolates on Nutrient agar plates is presented in Figure 1. *Chryseomicrobium palamuruense* first reported by Pindi *et al.* (2016) was isolated from a sediment sample collected from drainage. *Micrococcus luteus* isolated from soil, water, and landfills has been established by several studies (Siddiqqui, 2017; Sinha *et al.,* 2017; Minal and Rohini, 2018). The precedence of these pigmented bacteria in the environment suggests their abundance in soil, agro-wastes, and wastewater as their natural ecological niche.

3.2. Optimisation Conditions for Pigment Production of Identified Bacterial Isolates

The result of the effect of the incubation period on pigment production revealed that *Chryseomicrobium palamuruense* showed the highest peak at 36 h with an absorbance of 1.894 at 590 nm while *Micrococcus luteus* showed the highest peak at 60 h with absorbance of 2.677 at 590 nm. Adaptability and varying generation times for these isolates could be attributed to the maximum incubation period as suggested by Hizbullahi *et al.* (2018).

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In this study, it was observed that pigment production was higher under shaking conditions as both *Chryseomicrobium palamuruense* and *Micrococcus luteus* gave an absorbance of 1.546 and 1.234 under shaking conditions as against 0.224 and 0.272 respectively under static conditions (Figure 3). Availability and frequent circulation of oxygen are vital for bacteria growth and pigment production. Laboratory experiment subjected to shaking conditions increases microbial activities. Pigment under different shaking conditions was produced in high volumes as against static conditions as reported by Garg *et al.* (2017) for *Micrococcus luteus* and *Micrococcus varians* and Hizbullahi *et al.* (2018) for *Salinococcus roseus*. This validates the increased pigment production observed for *Cryseomicrobium palamuruens* and *Micrococcus luteus* under shaking as compared to the static condition.

To study the effect of inoculum volume, the absorbance reading for the culture broths inoculated with different volumes of standardized inoculum (0.5-2.5%) was shown in Figure 4. It was observed that pigment production increased with an increase in inoculum volume from 0.5-1% and a decrease from 1.5% for *Chryseomicrobium palamuruense* with maximum absorbance at 1% inoculum volume. For *Micrococcus luteus*, there was an increase in pigment production with an increase in inoculum volume up to 2.5%. Several studies have established the significance of inoculum volume in pigment production. For this study, the maximum and workable inoculum volume was observed to be 1% for *Chryseomicrobium palamuruense.* Banerjee *et al.* (2011) documented that pigment production increases with an increase in inoculum volume by up to 1%, however, a further increase in inoculum volume leads to a decrease in pigment production. An increase in the inoculum volume suggests a rapid exponential phase and utilization of available nutrients with a rapid stationary and decline period. This might have some effect on pigment production as suggested in this study. A different finding was observed for *Micrococcus luteus* as 2.5% inoculum volume still showed a steady and significant increase in pigment production.

The result of the effect of pH on pigment production of *Chryseomicrobium palamuruense* and *Micrococcus luteus* revealed that the rate of pigmentation was higher around neutral pH (Figure 5). Growth was not observed for *Chryseomicrobium palamuruense* at pH 3 to 5 while there was pigmentation at pH 6 to 11 with the maximum pH for pigmentation at pH 8. For *Micrococcus luteus,* there was no growth observed between pH 3 to pH 5 and pH 10 to pH 11 but the maximum pH for pigment production was observed at pH 8 (absorbance 1.875 at 590 nm). In summary, at acidic pH 3-5, both organisms showed no growth and both showed the highest pigmentation at pH 8.

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The acidity and alkalinity of the culture medium guarantee microbial growth, especially pigment-producing bacteria. Roundabout neutral and slightly alkaline pH (7 and 8) was found to support both *Chryseomicrobium palamuruense* and *Micrococcus luteus* growth and pigment. Slight alkaline pH of 8 recorded the highest pigmentation for *Chryseomicrobium palamuruense* and *Micrococcus luteus*. Similar findings reported by Pindi *et al.* (2016) observed *Chryseomicrobium palamuruense* to grow at a pH range of 7-10 with optimum growth at pH 8 corresponds to the findings of this study. Similar pH conditions were reported for other bacteria. Hisbullahi *et al.* (2018) observed that *Salinococcus roseus* and *Pseudomonas aeruginosa* showed the highest pigmentation at pH 7 while *Chromobacterium violaceum* showed the highest pigmentation at pH 8. Also, Garg *et al.* (2017) and Poddar *et al.* (2021) in their reports observed *Micrococcus varians* and *Enterobacter* sp. PWN1 to pigment at pH 7 and 7.2 respectively. It is important to state that at acidic pH of 3-5, both organisms showed no growth while at alkaline pH of 9-11, *Micrococcus luteus* showed no visible growth. A somewhat different report by Minal and Rohini (2018) recorded the growth and survival of *Micrococcus luteus* at pH 2, 4, 7, 8, and 10 with optimum pH of 7. As observed in all reported studies, this implies that the organisms require neutral pH and a slightly alkaline medium for their growth and pigment production as evidenced in the absence of growth in acidic. It could be suggested that acidic (strong and weak) and strong alkaline mediums could repress and stall their metabolic activities.

The result of the effect of temperature on pigment production revealed that the highest pigment production was observed at 35 ^{o}C and 30 ^{o}C in this study for *Chryseomicrobium palamuruense* and *Micrococcus luteus* while both had no growth at 50 ^{o}C (Figure 6). Suitable temperature conditions have been established as an important requirement for bacteria growth. For pigment production by bacteria, the optimum temperature is pivotal for substantial yield. Different resultant effects of temperature on growth and pigment production were reported for this study. The highest pigment production temperature was observed at 35 ^{o}C for *Chryseomicrobium palamuruense*. A similar study by Hizbullahi *et al.* (2018) reported 35 ^{o}C as the optimum temperature for pigment production in *Chromobacterium violaceum.* Interestingly, the first study reporting *Chryseomicrobium palamuruense* documented its optimum temperature as 37 ^{o}C (Pindi *et al.*, 2016). For *Micrococcus luteus* isolated in this study, 30 ^{o}C was observed as its optimum temperature. Earlier reports by Garg *et al.* (2017) and Minal and Rohini (2018) isolated *Micrococcus luteus* as a yellow pigment-producing bacteria from bioenhancer '*Amrit pani*' and marine water respectively, jointly reported an optimum temperature of 30 ^{o}C thus, corroborating the finding of this study. All

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organisms reported in all of these studies have been established as mesophilic bacteria whose optimum growth temperature ranges between 25 – 45 ^{o}C.

3.3 Production and Extraction of the Pigment

The fermentation medium was produced using the best parameters of optimization for each of the isolates which are 36 hours incubation period, 1% inoculum volume, 35 ^{o}C incubating temperature, pH value of 8 under shaking conditions for *Chryseomicrobium palamuruense* and 60 hours incubation period, 2.5% inoculum volume, 30 ^{o}C incubating temperature, pH value of 8 under shaking condition for *Micrococcus luteus*. The pigmented broths were centrifuged at 3000 rpm for 20 minutes, the supernatant was discarded and the pellets were re-suspended in Ethanol. The pictorial representation of the production broth, coloured cell pellets, and ethanoic solvent extract of the pigments are shown in Figure 7. The choice of solvent used for extraction was crucial as it must possess the ability to separate the significant analyte from a mixture of compounds (Zhang *et al.,* 2018). In other words, pigment extraction was based on its affinity to the suitable solvent used. A study conducted by Sasidharan *et al.* (2015), reported the use of ethanol as their organic solvent for the extraction of pigment from *Chromobacterium* sp. NIIST. This finding might imply that the chemical composition of each pigment influences the choice of solvent. Since carotenoids are lipophilic, they are found to be soluble in organic solvents like methanol, ethanol, and acetone.

3.4. UV-Visible Spectroscopy of Extracted Bacterial Pigment

The UV-visible spectrum of the orange pigment produced by *Chryseomicrobium palamuruense* showed surface plasmon resonance peaks at 262, 426, and 453 nm while the UV-visible spectrum of the yellow pigment produced by *Micrococcus luteus* showed a single peak at 260 nm (Figure 8).

3.5. Fourier Transform Infra-Red (FTIR) Analysis of Extracted Bacterial Pigment

The Fourier Transform Infra-Red (FTIR) spectroscopy of the pigments produced by *Chryseomicrobium palamuruense* and *Micrococcus luteus* indicate the presence of some functional groups such as hydroxyl (OH), C-H, primary and secondary C-O, skeletal C-C vibrations for the pigments. Spectral peaks and detailed interpretations of the pigments are presented in Figure 9.

3.6. Antibacterial Activity of Bacterial Pigment

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The extracted pigments (orange and yellow) of both isolates (*Chryseomicrobium palamuruense* and *Micrococcus luteus*) showed no antibacterial activity on the Gram-positive and Gram-negative test organisms obtained from the Department of Microbiology, Obafemi Awolowo University, Ile-Ife. This result was similar to a study conducted by Park *et al.* (2020) where an increase in cell growth of *Escherichia coli* was observed when it was treated with flexirubin (FL) extracted from *Chryseobacterium* specie. Although, several reports had supported the antimicrobial activity of different pigments obtained from pigmented bacteria. The carotenoid pigment produced by *Micrococcus luteus* was found to be effective against *Colletotricum gloeosporioides* and *Fusarium solani* (Garg *et al.*, 2017). As part of control measures, this study and Park *et al.* (2020) tested ethanol and ($NaHCO_3$) respectively against the selected bacterial isolates with both solvents showing antibacterial activity. Park *et al.* (2020) documented that a cell growth inhibition effect should be expected when the solvent with antibacterial activity was used for pigment extraction, as reported in their study against *Escherichia coli*. The antibacterial activity of pigment recorded in most studies might be a result of the bactericidal activity of some solvents used in the extraction process.

3.7. Dyeing Potential of Bacterial Pigment

The cotton absorbed the orange pigment more than the polyester while none of the fabric absorbed the yellow pigment. It was noted that when the orange-stained fabrics were treated with an acid solution, alkaline solution, and soap solution, both fabrics retained their colour in the alkaline solution, the cotton fabric alone retained the pigment in the acid solution while both fabrics lost their pigmentation in soap solution. Figure 10 gives the pictorial representation. The mechanism by which dye were absorbed is influenced by the chemical composition of the textile fabric to be dyed. In this study, it was observed that the orange pigment extracted from *Chryseomicrobium palamuruense* was absorbed by the cotton fabric than the polyester fabric retaining light orange colouration. Alkaline mordant retained the colour in both fabrics however, acidic mordant could only retain the colour on cotton fabrics. The choice of suitable mordant was important for better fixation, adhesion, and sometimes a change in the colour shade of the pigment to the fabric (Yusuf *et al.,* 2017). Cotton fabric, as stated by Banerjee *et al.* (2014), retained significant bacterial pigment while the silk fabric retained little bacterial pigment in their study.

4. Conclusion

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The significant outcome of this study was the isolation of *Chryseomicrobium palamuruense* with little background information on its pigment production prowess. Optimum conditions required for high-yield pigment production for each bacterial isolate were reported in this study. Likewise, contrary to expectations, this study was able to report that the pigments have no antibacterial activity suggesting most organic solvents possess antibacterial activity before the antibacterial assessment.

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Figure 1: Pictorial Representation of *Chryseomicrobium palamuruense* strain PU1 (AW6), *Micrococcus luteus* strain 1k (AW8)

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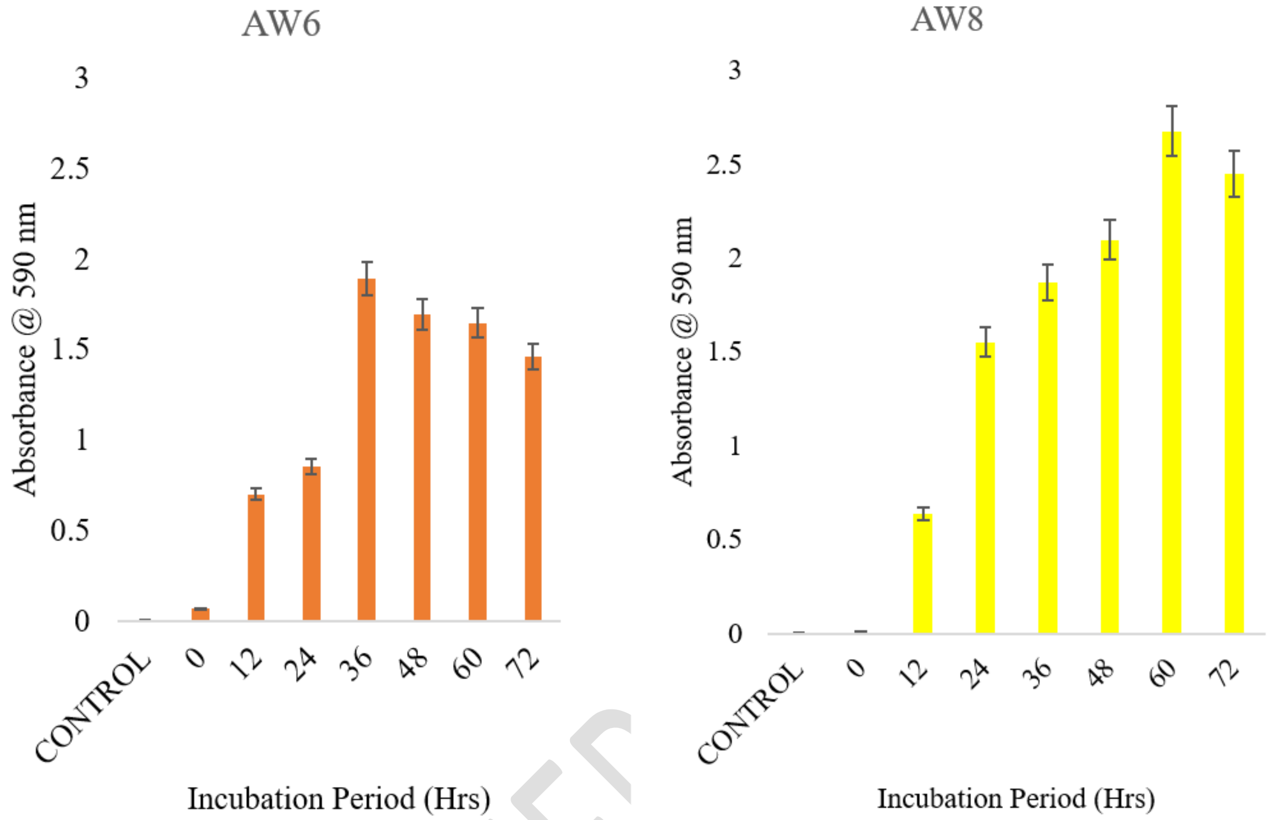

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Figure 2: Effect of Incubation Period on Pigment Production of *Chryseomicrobium palamuruense* (AW6), *Micrococcus luteus* (AW8)

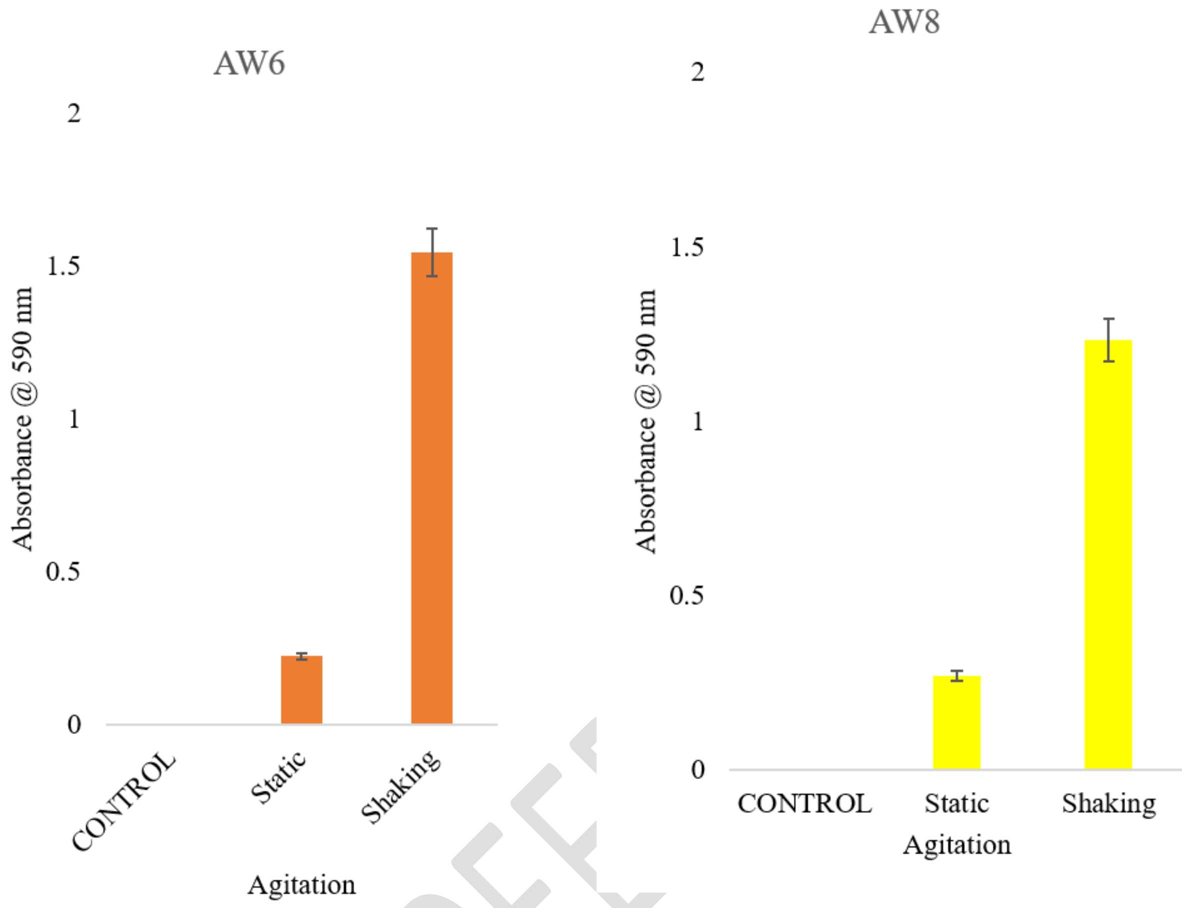

Figure 3: Effect of Agitation on Pigment Production of *Chryseomicrobium palamuruense* (AW6), *Micrococcus luteus* (AW8)

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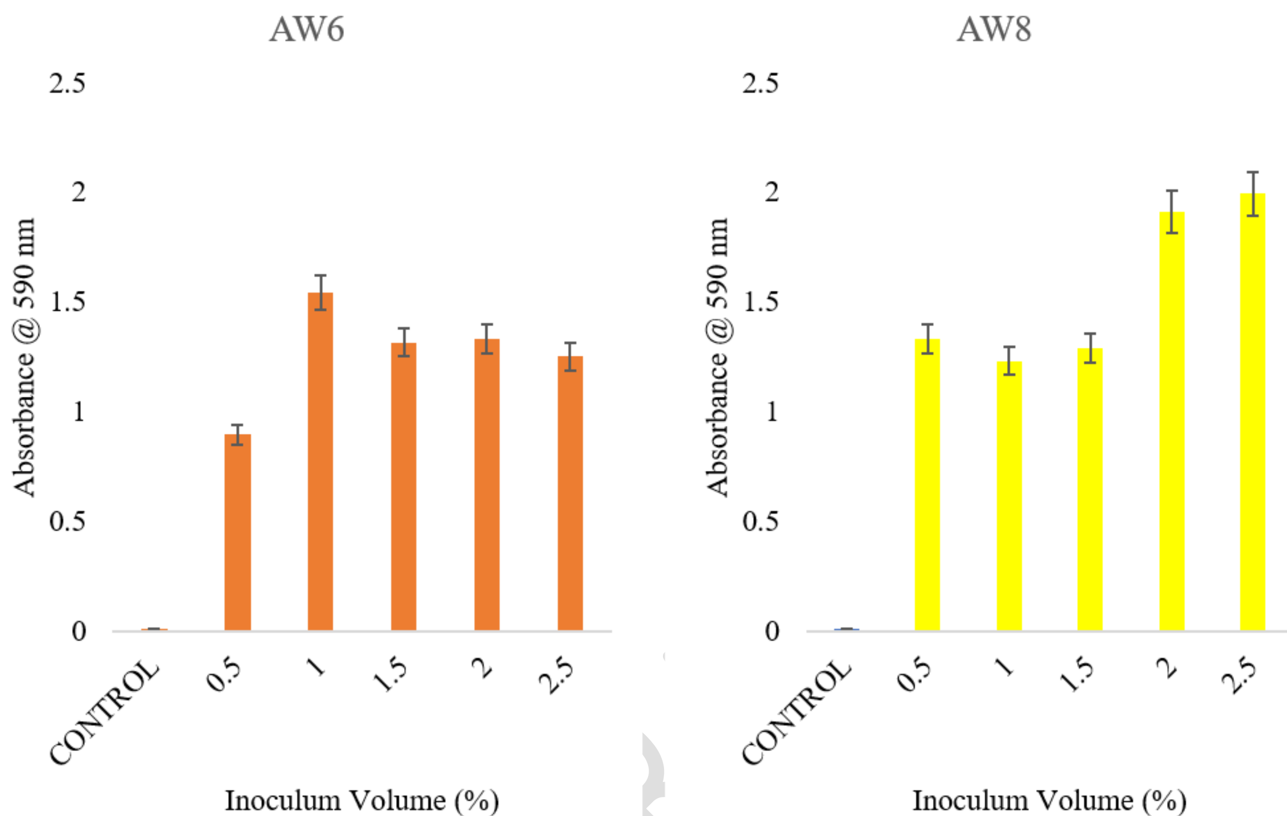

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Figure 4: Effect of Inoculum Volume on Pigment Production of *Chryseomicrobium palamuruense* (AW6), *Micrococcus luteus* (AW8)

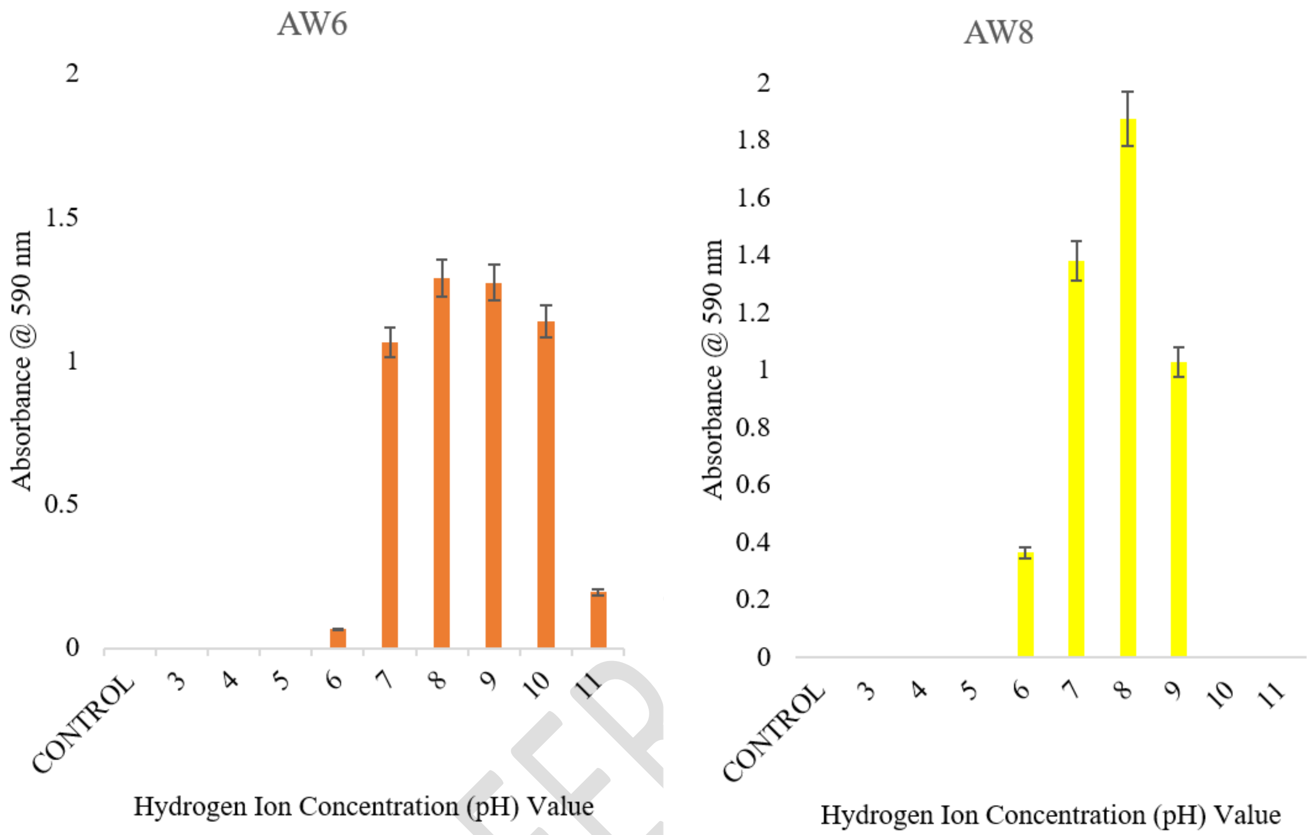

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Figure 5: Effect of pH on Pigment Production of *Chryseomicrobium palamuruense* (AW6), *Micrococcus luteus* (AW8)

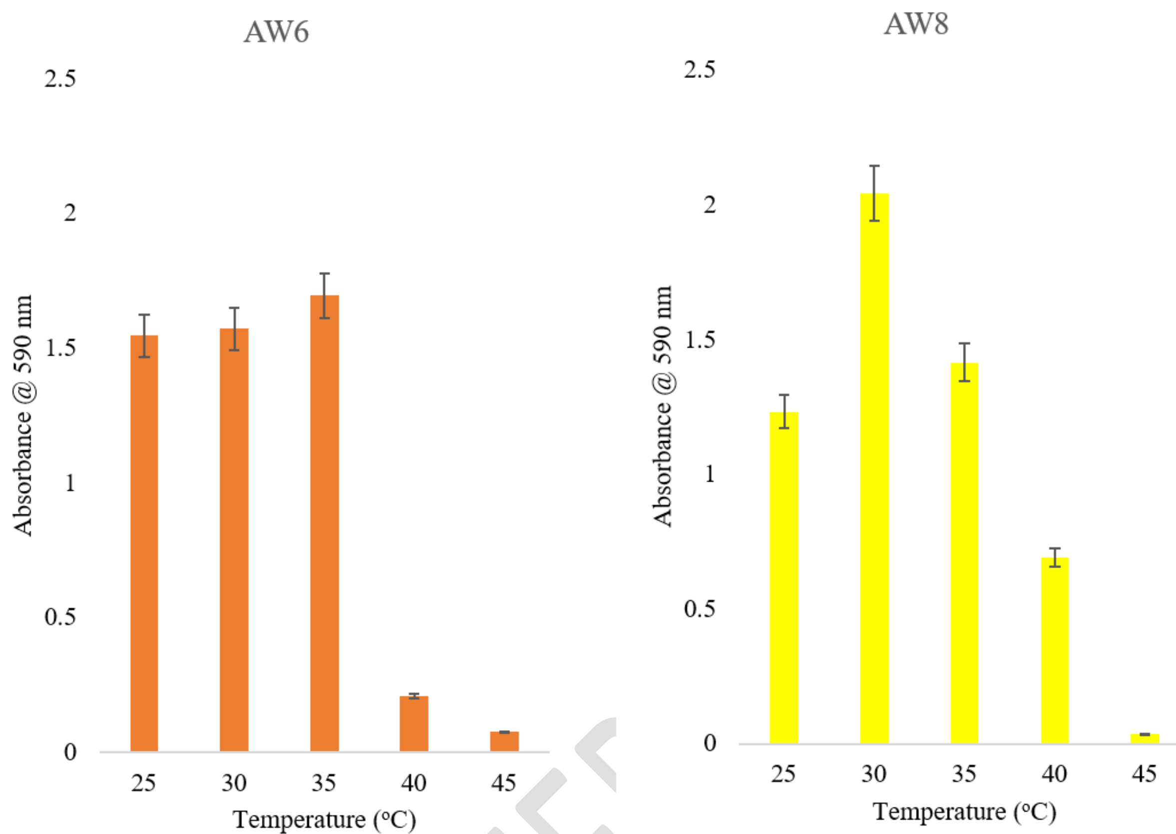

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Figure 6: Effect of Temperature on Pigment Production of *Chryseomicrobium palamuruense* (AW6), *Micrococcus luteus* (AW8)

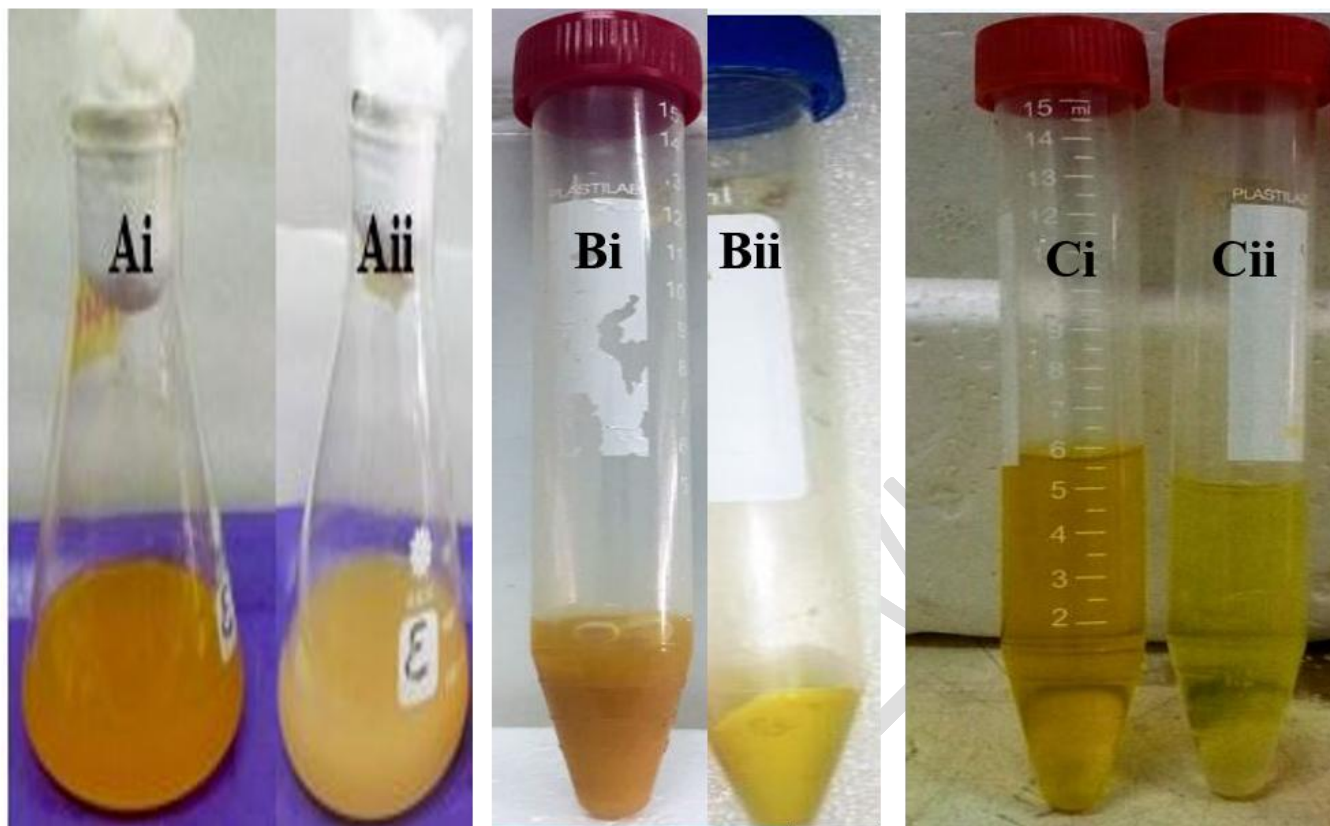

Figure 7: Pigmented Cultured Broth (A), Pigmented Cell Pellets (B) and Ethanoic Solvent Extracts (C) of *Chryseomicrobium palamuruense* (i), *Micrococcus luteus* (ii)

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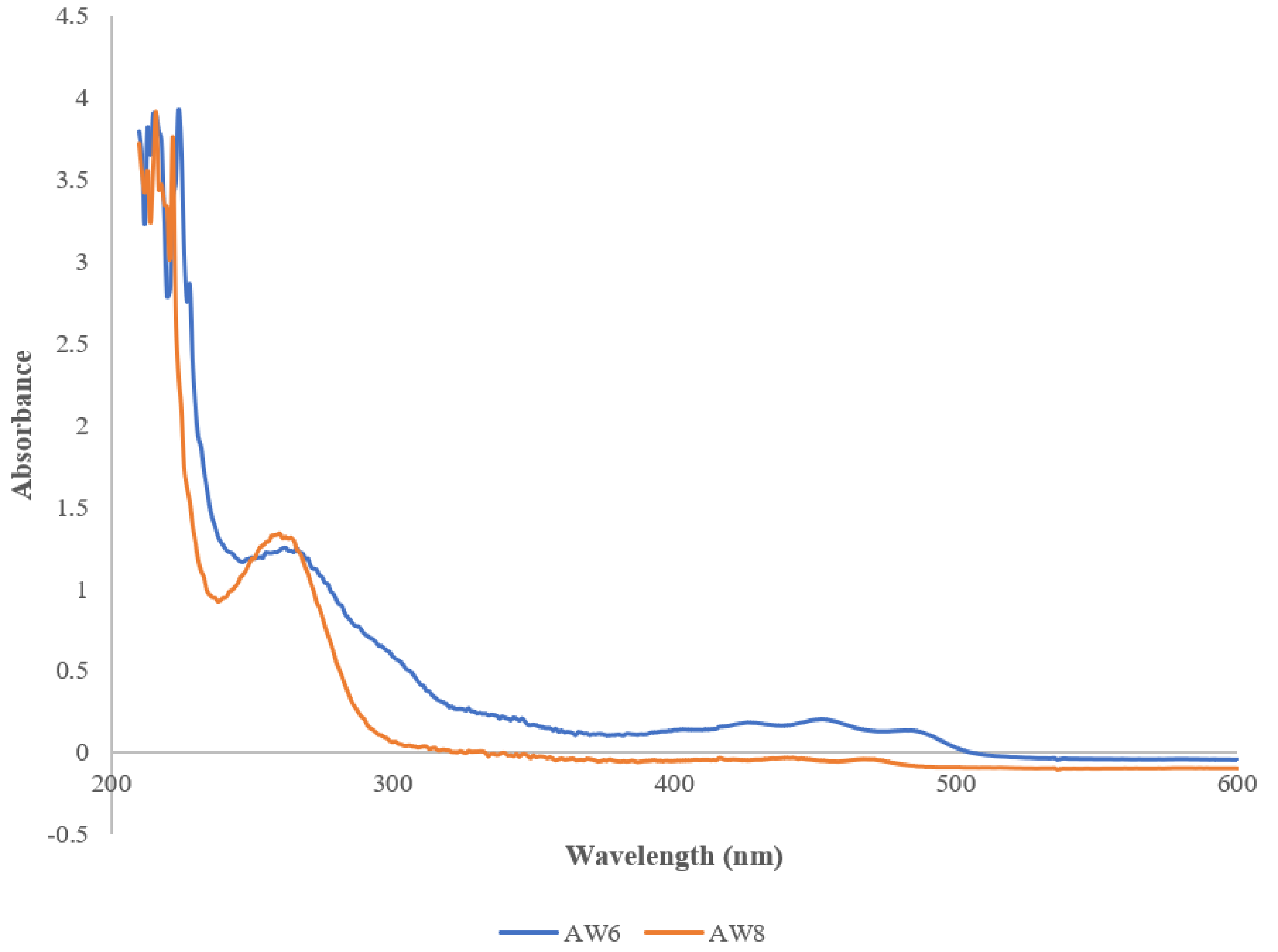

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Figure 8: UV-Vis Spectroscopy of *Chryseomicrobium palamuruense* (AW6), *Micrococcus luteus* (AW8)

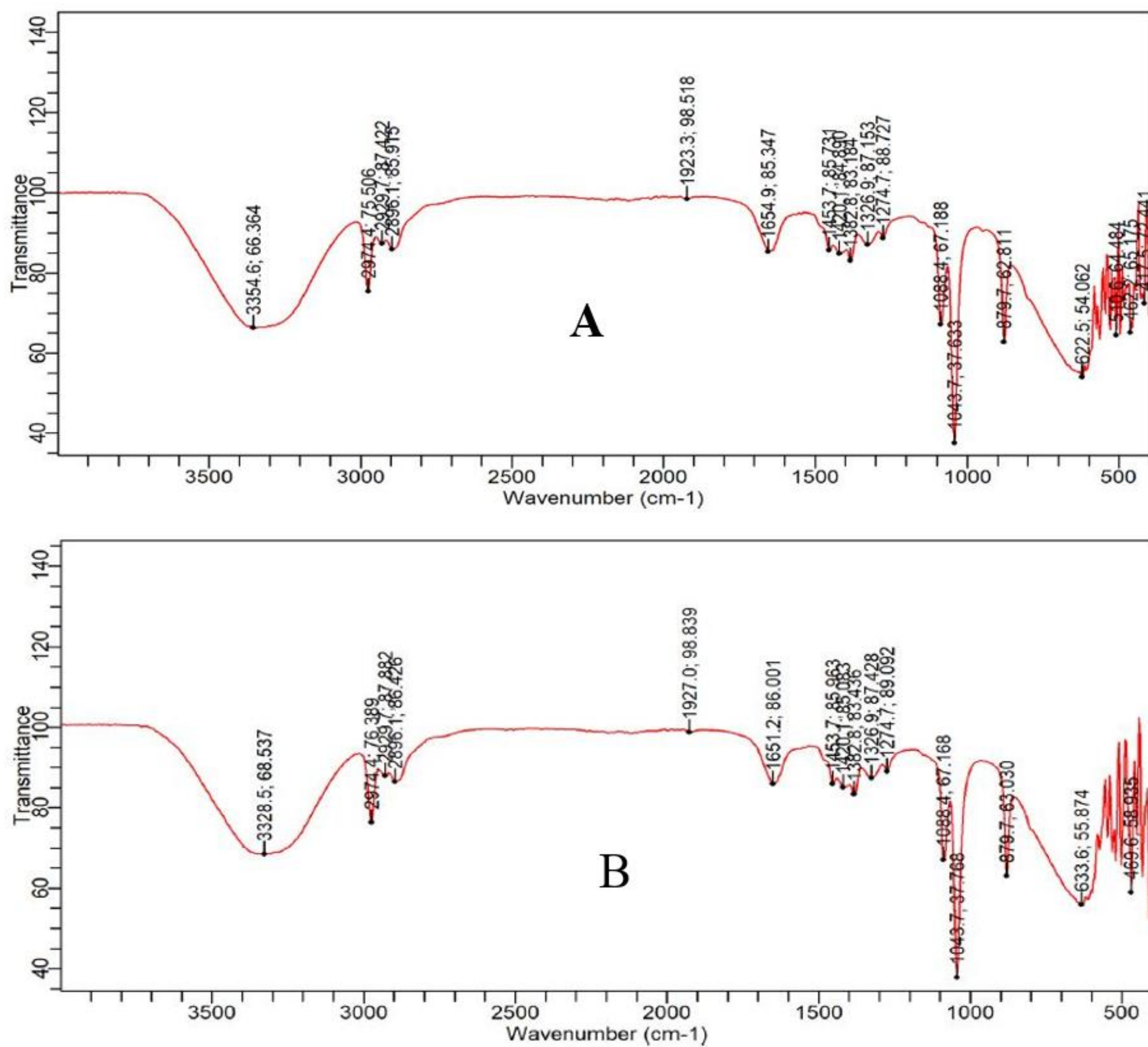

Figure 9: FTIR Spectrum of *Chryseomicrobium palamuruense* (A), *Micrococcus luteus* (B)

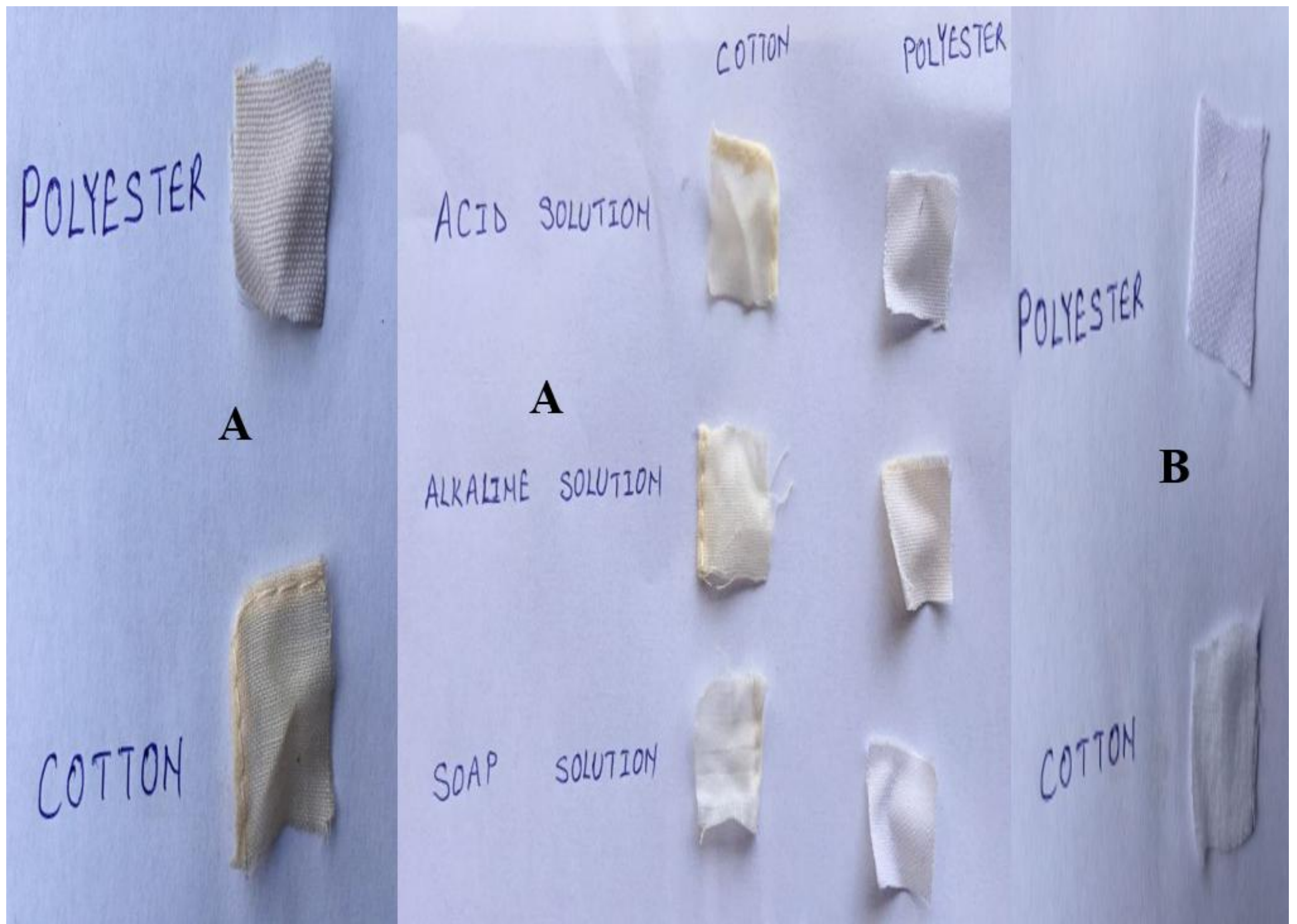

Figure 10: Dyeing Image of Extracted Pigments from *Chryseomicrobium palamuruense* (A), *Micrococcus luteus* (B)

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