RUGIPO Research & Technical Journal; Vol. 3, Issue 1: Issues in Science & Agricultural Technology; Sept, 2024 Edition
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PRODUCTION AND APPLICATION OF COLOURANT FROM SOIL MICROBES ON TEXTILE

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Abstract
Microbial pigments are natural pigments of microbial origin. The research aims to evaluate the extraction of microbial pigments and their potential use for textiles. The following pigmented microbial isolates were isolated from soil samples at Owo local government and identified to be homologous to *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Serratia marcescens* and *Bacillus sphaericus* (bacterial isolates) while *Aspergillus terreus*, *Candidia tropicalis*, *Penicillium ruben*, *Yarrowia lipolytica* and *Metarhizium anisopliae* using morphological and biochemical characteristics. The biomass was biostimulated to improve the growth and corresponding protein yield. Protein content was evaluated using Bradford assay across a temperature range of 25-40ºC. Tin layer chromatography was used to evaluate the pigment separation. The bacterial protein content was highest at 25ºC at 0.2 mg/ml for *P. aeruginosa* and *S. marcescens* while *B. subtilis* and *B. sphaericus* were highest at 30ºC at 0.14 and 0.28 mg/ml respectively. However, a fungal protein was reportedly higher than bacterial protein with Y. lipolytica and *M. anisopliae* recording 1.42 mg/ml followed by 0.8 mg/ml respectively. Percentage pigment exhaustion was highest in the pigment produced by *S. marcescens*, at 66.32 %, followed by *P. aeruginosa* among isolated bacteria while Y. lipolytica and *A. terreus* showed the highest percentage exhaustion among the fungi isolates. Tin layer chromatography showed the separation of different colours with different Rf values expressed by some selected microorganisms. All the produced bio-colorants faded on exposure to sunlight except *Y. lipolytica* and *S. macescens* which seem to be more stable. These pigments thus have the potential to be used as a dyeing agent if optimized for the best condition.

Keywords: Bradford assay, biomass, bio-stimulation, Tin layer chromatography.

Introduction

Pigments are compounds with characteristics of importance to many industries. They can serve as additives, colour intensifiers and antioxidants (Usman *et al*., 2017). In recent years, the use of natural organic dyes from bio-origin has gained global attention. This is because it has generally been regarded as safe since it is of natural source hence, replacement of synthetic ones (Mapari *et al*., 2005). Some textile, cosmetics and food industries are now engaged in the mass production of pigments for large-scale commercial use (Usman *et al*., 2017). These natural colourants help to improve the market values of the products (Singh *et al.*, 2023). Microbial pigments are natural pigments of microbial origin. These are often considered secondary metabolites and are not produced by all microbes. They have been studied to be biodegradable, nontoxic and non-carcinogenic (Cristea & Vilarem, 2006). Some also possess anti-cancer, anti-leishmanial, anti-microbial and anti-oxidant properties (Feng *et al*., 2012). Some of these pigments are helpful in the classification and identification of microorganisms e.g. *P. aeruginosa* produces a greenish pigment (Barreto *et al*.,

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2023). Pigments also help the virulence and survival of pathogens to resist antibodies, and heavy metal resistance (Liu & Nizet, 2009). Industrial application of microbial pigments like paint formulation, textiles, cosmetics and food industries has been reported (Singh *et al.*, 2023).

Microorganisms are associated with the production of many types of pigments; hence the ability to manipulate them in a culture bio fermentor to mass produce makes them a promising source of mass colourants that can be easily scaled up. The negative impacts of synthetic dyes on the consumer have been assessed and this has given rise to a special interest in innate organic dye as a surrogate (Sajda, 2021). In this way, the pigments from microbial sources are a good alternative (Cañizares-Villanueva *et al*., 1998). It should also be noted that this technique may not only be useful for public health alone but also serve as a surrogate for the use of harmful synthetic chemicals released into the environment hence preserving the ecology (Tuli, *et al*., 2015). *Rhodotorula* yeast produces torularodin pigment often yellow in colour and it possesses anticancer properties (Park *et al.*, 2015). *Serratia marcescens* produces prodigiosin with a red-like colour and also processes anticancer properties. *Pseudomonas aeruginosa* often produces green-like colour pyocyanin. The need for the commercial production of microbial colourants as a promising surrogate for the use of synthetic dyes in textile industries cannot be overstated. This research aims to isolate pigments from various microbial cultures of soil origin and apply them to textiles.

Methodology

Sample Collection

Soil samples were collected in sterile polythene bags from agricultural fields, garbage areas and industrial polluted areas in Owo Local Government, Ondo State. The samples were analyzed in the Microbiology laboratory of Science Laboratory Technology Rufus Giwa Polytechnic Owo, Ondo State for microbial isolation. Nutrient broth and Sabouraud Dextrose Broth were used for bacterial and fungi isolates respectively (Baghel & Pandey, 2013).

Biostimulation and Protein Quantification

All the isolates were subjected to fermentation processes by changing one specific parameter, the nutrient composition (yeast extract concentration for Luria Bertani broth and dextrose for Sabouraud Dextrose Broth) for bacterial and fungal growth respectively to define conditions for growth yield and corresponding protein production that leads to the best yield of a high-quality product according to standard procedure. The corresponding total protein content was evaluated using Bradford assay for each isolate according to the method described by Kruger (2009). The absorbance of the tubes was determined at 595 nm within 15 minutes and readings were plotted for quantification of total protein. The reading was done in duplicate ± standard error.

Cultivation, Extraction, and Purification of the Pigment

The selected bacterial isolates were cultivated in Luria Bertani broth. The 250 ml conical flask containing Luria Bertani broth was seeded with 10^6 cells/mL of bacterial isolates grown at 30 °C under static conditions while Sabouraud Dextrose broth was inoculated with freshly cultivated fungi for 4 weeks as described by Anchana (2014). After an incubation period of 3 days for bacterial culture and 4 weeks for fungi culture, the expressed pigments were filtered via sterilized gauze cloth. 95% (*v*/*v*) methanol was introduced to the culture

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broth, and the resulting mixture was kept at 30 °C for 30 min on the rotary shaker at 200× *g*. The methanolic mixture was centrifuged at 5000× *g* for 15 min, and the pellets were dispersed in the remaining volume of methanol and centrifuged again. The supernatant was recovered, and the filtrate was dispersed in the leftover volume of methanol and centrifuged at 5000× *g* for 5 min. Finally, the supernatant was collected and filtered through a Whatman No.1 filter paper and diluted with 95% (*v*/*v*) methanol to a dilution factor of 20. The absorption spectrum was observed at 300–600 nm using Hitachi 3210 spectrometer (Hitachi Ltd., Tokyo, Japan) (De-Santis *et al*., 2005), and the purified pigment was concentrated in a rotary evaporator. The optical density (OD) measured at 535 nm and multiplied by the above dilution factor gives the quantum of the red pigment production (Andreyeva & Ogorodnikova, 2015) and the pigment was concentrated in a rotary evaporator (Partki *et al*., 2021).

Textile Dyeing

Teru was used as the test textile. Dyeing was carried out by textile tad with a 100% concentration of the pigment in 100 mL deionized water for 120 min in an orbital shaker operated at 70 °C, 100 rpm/min, with 1.0 g sodium sulfate (Sivakumar *et al*., 2009) at pH 5. Ammonium sulfate and acetic acid were further added as a fixative. The coloured samples were washed with normal water to eliminate the unfixed pigment in a bath of liquid ratio (L: R = 40:1) using tween 20 for 30 min at 50 °C and then air dried. The dye bath pH was monitored with a pH meter and adjusted with dilute solutions of 1 M sodium carbonate.

Percentage Absorption

The percentage absorption of the dyed fabric was calculated on a UV spectrophotometer at 500-600nm and the percentage absorption was determined for dyed fabric using the following formula:

$$\text{Percentage absorption (\%)} = \frac{\text{(O.D) before dyeing} - \text{O.D after dyeing}}{\text{O.D before dyeing}} \times 100$$

O.D. is optical density. (Anchana, 2014)

Results

Table 1: Morphological and biochemical characteristics of bacterial isolates from the soil sample

No	Colour	Shape	GRM	MOT	SPR	CAP	CAT	OXD	COG	IND	MR	VP	GLC	MAL	SUC	LAC	GAL	FRC	Probable identified organism
BS1	Rhizoid cream on TSA	Rod	+	+	+	-	-	+	-	+	+	-	+	-	+	-	-	+	*Bacillus subtilis*
BS2	Rhizoid cream on TSA	Rod	+	+	+	-	-	+	-	+	+	-	+	-	+	-	-	+	*Bacillus sphaericus*
BS3	Lemon green on PAA	Rod	-	+	-	-	-	+	+	-	+	-	-	+	-	+	-	-	*Pseudomonas aeruginosa*
BS4	Pinkish on NA	Rod	-	+	-	-	+	-	-	-	-	+	+	+	+	-	+	+	*Serratia marcescens*

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Key: BS= Bacterial Species, GRM= Gram staining, MOT=Motility test, SPR= Spore staining, CAP=Capsule staining, CAT= Catalase test OXD= Oxidase test, COG= Coagulase test, IND= Indole test, MR= Methyl red test, VP GLU= Glucose, SUC= Sucrose, LAC= Lactose, GAL= Galactose, MAL= Maltose, FRC= Fructose, NA= Nutrient Agar, TSA= Tryptose Soy Agar, PAA= *Pseudomonas aeruginosa* Agar

Table 1 above shows the various morphological and biochemical characteristics of the isolate from the soil sample. *Bacillus subtilis, B. sphaericus* is gram-positive, motile, oxidase-positive and spore former, Methyl red, VP negative, Galactose fermenter. *Pseudomonas aeruginosa* and *S. marcescens* were gram negative, motile, non-spore formers, non-capsulated, indole negative and had the ability to ferment maltose sugar.

Table 2: Morphological characteristics of fungal isolates from soil samples

Isolates	Morphological features	Microscopic characteristics	Suspected organism
ISF1	Cinnamon brown color	Hyphae are aseptate, Conidiophores bearing conidial heads containing conidia.	*Aspergillus terreus*
ISF2	However butyrous, smooth, White	Conidiophores	*Candidia tropicalis*
ISF3	Redish brown-coloured spore heads	Hyphae are septate, with smooth-walled conidiophores bearing long chains of conidia.	*Penicillium rubens*
ISF4	Ridges, wrinkled yeast –like small circles and mould -like on SDA, cheese like on SDB	Aseptate hyphae	*Yarrowia lipolytica*
ISF5	Greenish powdered like	Aseptate hyphae	*Metarhizium anisopliae*

Keys: C = Contaminated, IS = isolated, F = Fungi

Table 2 showed *A. terreus, P.rubens* and *Candidia tropicalis* form conidiophores. *Aspergillus terreus, P. rubens, M. anisopliae* hyphae were all aseptate.

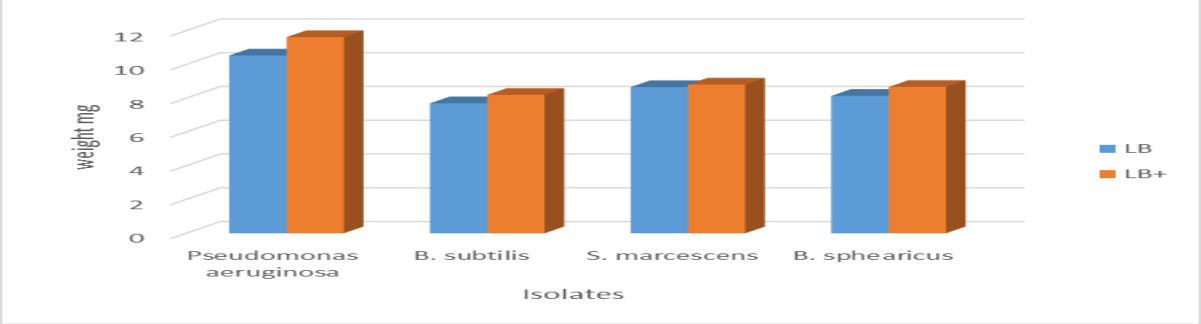


Figure 1 Bacterial biomass determination in mg/l at 35 ^{0}C.
Key: LB= Single strength yeast extract, LB+ = Double strength yeast extract Luria Bertani broth

Figure 1 above shows bacterial biomass isolate at 35ºC. The addition of yeast extract showed an increase in biomass weight for *P. aeruginosa B. subtilis, S.*

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marcescens, *B. sphearicus* from 10 to 11.2 mg/l, 7mg/l to 8 mg/l, 8 to 8.1 mg/l, and 7.8 to 8 mg/l respectively.

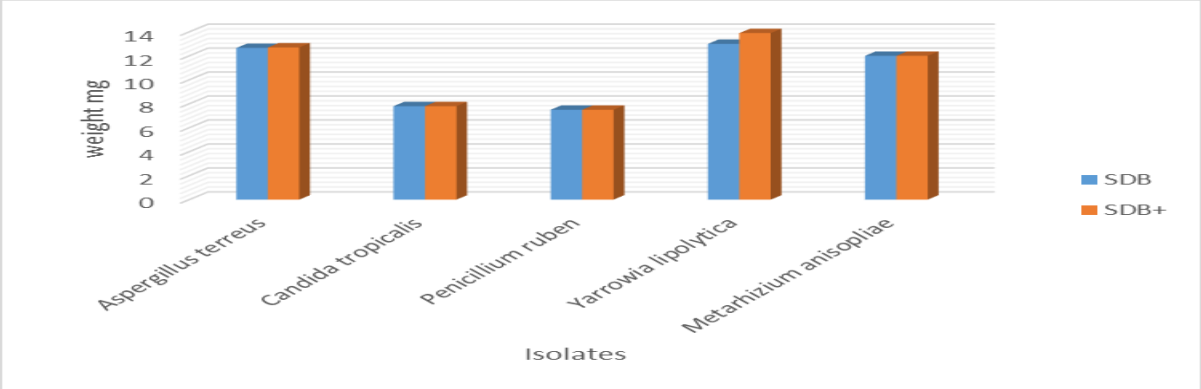


Figure 2: Fungal biomass determination in mg/l at 35 ^{0}C
Key: SDB= Single strength dextrose, SDB+ = Double strength dextrose (saboraud dextrose broth)

The result presented in Figure 2 showed fungal biomass isolate at 35ºC. The addition of dextrose sugar showed no effect on the biomass weight for all the selected isolates except for *Y. lipolytica* from 12.7 mg/l to 13.6 mg/l

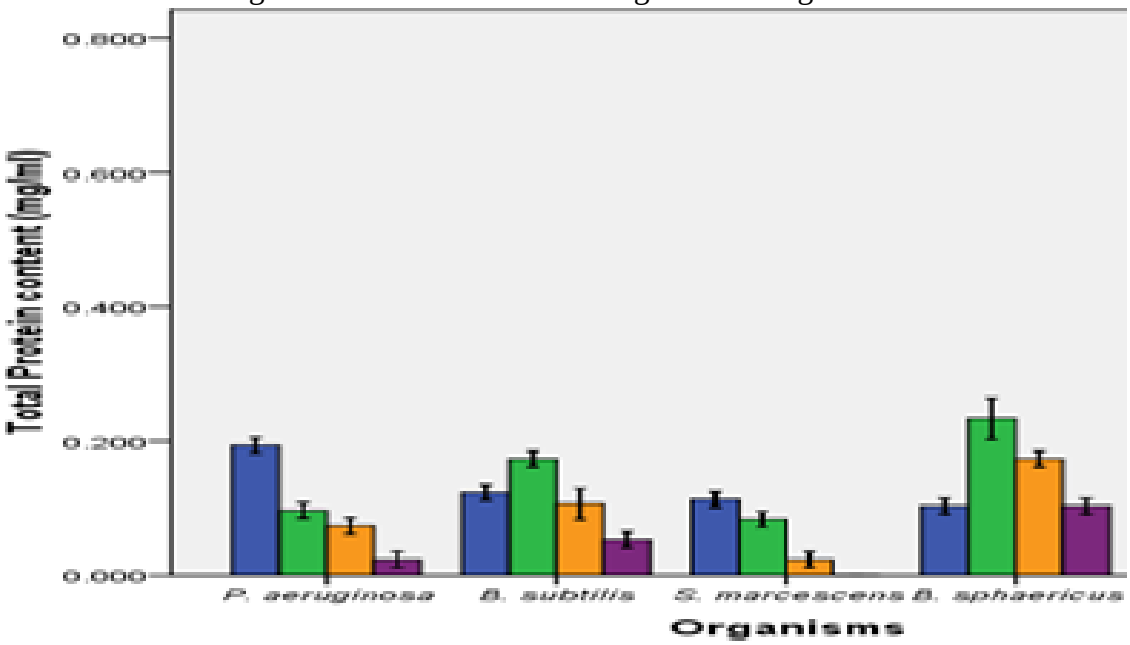


Figure 3: Total protein content of pure bacterial isolates cultivated at different temperatures on LB + medium broth.
The average data of two determinations ± Standard deviation (error bar) are shown.
Key: Blue= 25ºC, Green= 30ºC, Yellow= 35ºC, Purple = 40ºC

Figure 3 shows the total protein content from bacterial biomass isolates cultured on double-strength yeast extract from 25ºC to 40ºC. *Pseudomonas aeruginosa* highest protein content was recorded to be 0.2mg/ml at 25ºC and the stability decreases with an increase in temperature, *B, subtilis* highest protein content was

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recorded to be 0.18mg/ml at 30ºC, S. marcescens total protein content was highest at 25ºC and decline with increase temperature while *B. sphearicus* total protein content was highest at 35ºC and declined with increased temperature

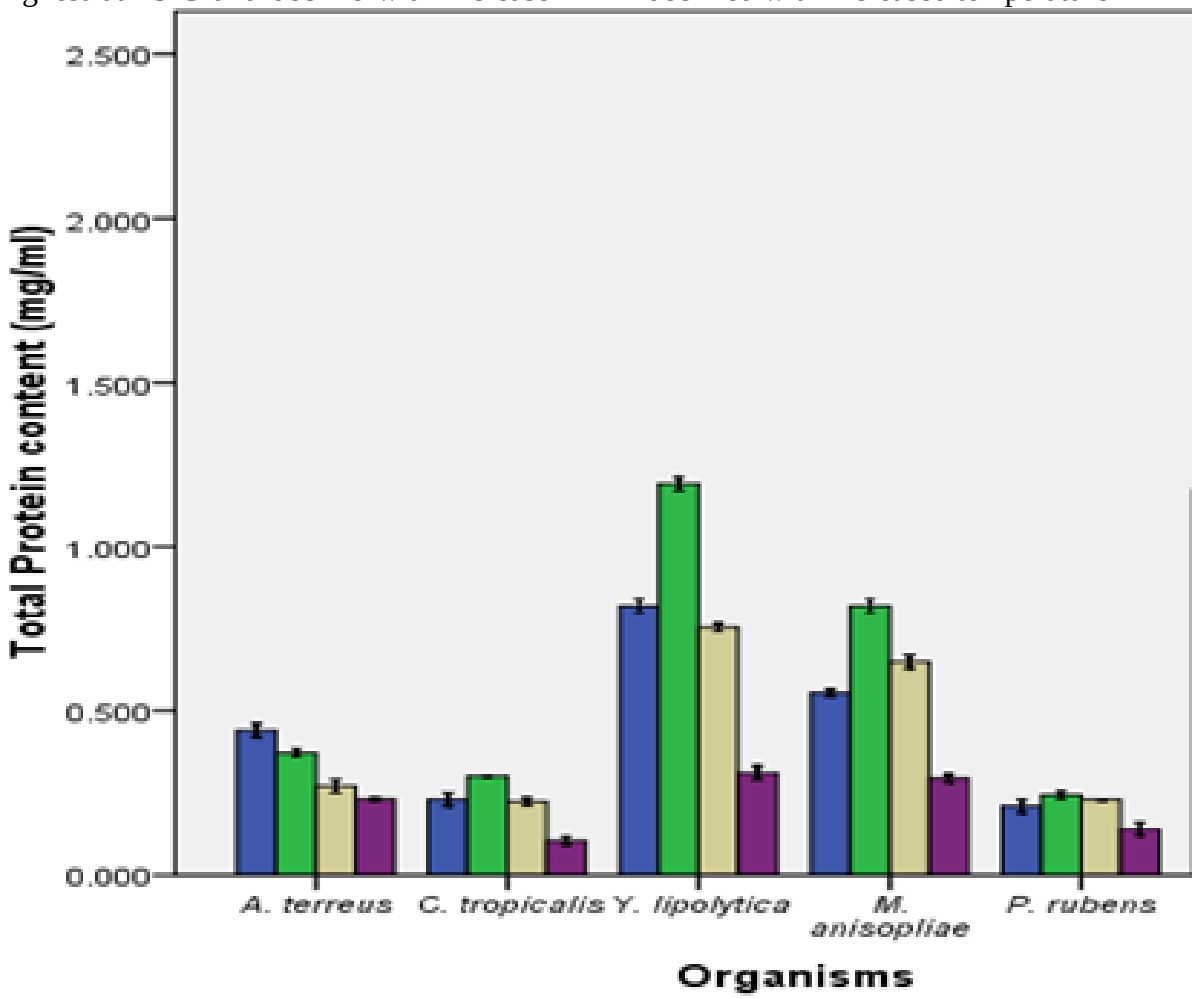


Figure 4: Total protein content of pure fungal isolates cultivated at different temperatures on SDB+
The average data of two determinations ± Standard deviation (error bar) are shown.
Key: Blue= 25ºC, Green= 30ºC, Yellow= 35ºC, Purple = 40ºC

The result in Figure 4 showed the total protein content from fungal biomass isolates cultured on double-strength dextrose sugar from 25ºC to 40ºC. *A. terreus highest* protein content was recorded to be 0.47mg/ml at 25ºC and the stability decreases with an increase in temperature, *C. tropicalis* highest protein content was recorded to be 0.28mg/ml at 30ºC, *Y. lipolytica* and *M. anisopliae* total protein content was highest at 35ºC to be 1.3mg/ml and 0.74mg/ml respectively and decline with further increase temperature, *P. rubens* total protein content was relatively unaffected across all temperature range.

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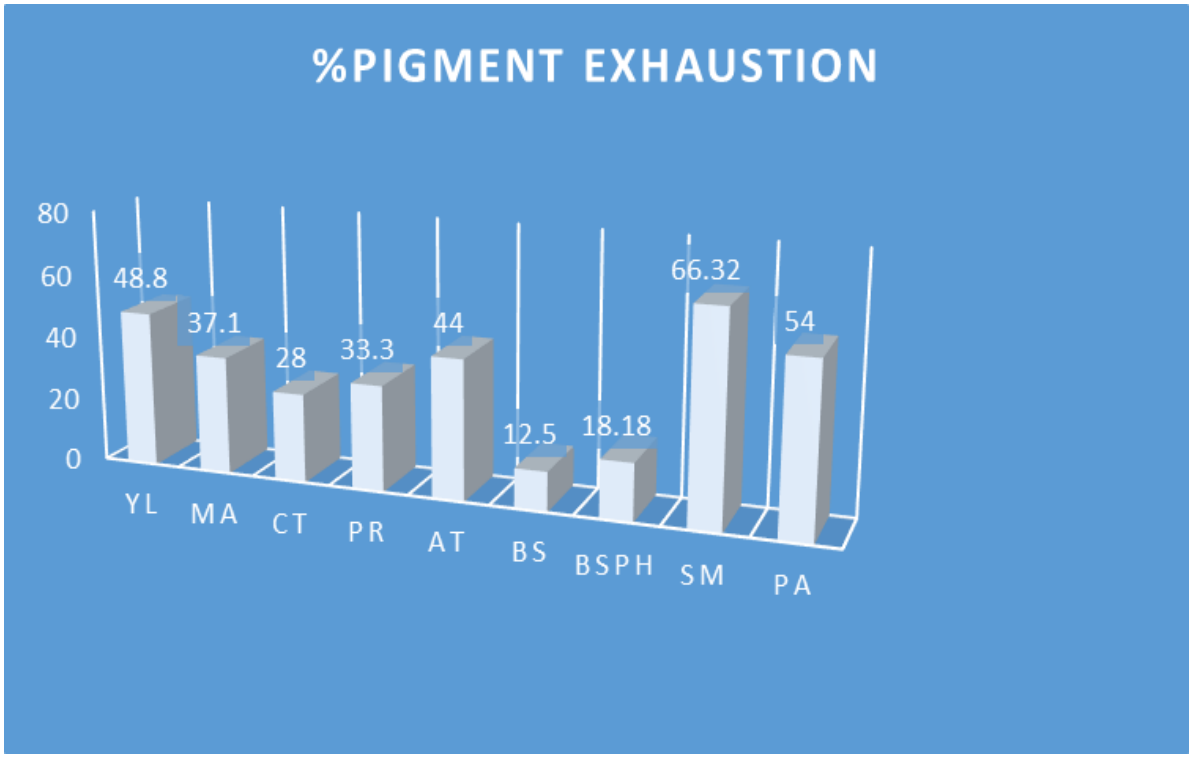


Figure 5: Percentage Pigment exhaustion absorbance at 535nm
Key: YL=*Yarrowia lipolytica, MA=Metarhizium anisopliae, CT=Candida tropicalis, PR=Penicillium ruben, AT=Aspergillus terreus, BS=B. subtilis, BSPH= B. sphearicus, SM=S. marcescens PA=Pseudomonas aeruginosa*

Percentage pigment exhaustion was recorded from the highest value in descending order in Figure 5 above. *S. marcescens*, *P. aeruginosa*, *Y. lipolytica*, *A. terreus*, *M. anisopliae*, *P. ruben*, *C. tropicalis*, *B. sphearicus* and *B. subtilis* at 66.32, 54, 48.8, 44, 37.1, 33.3, 28, 18.18, and 12.5% respectively.

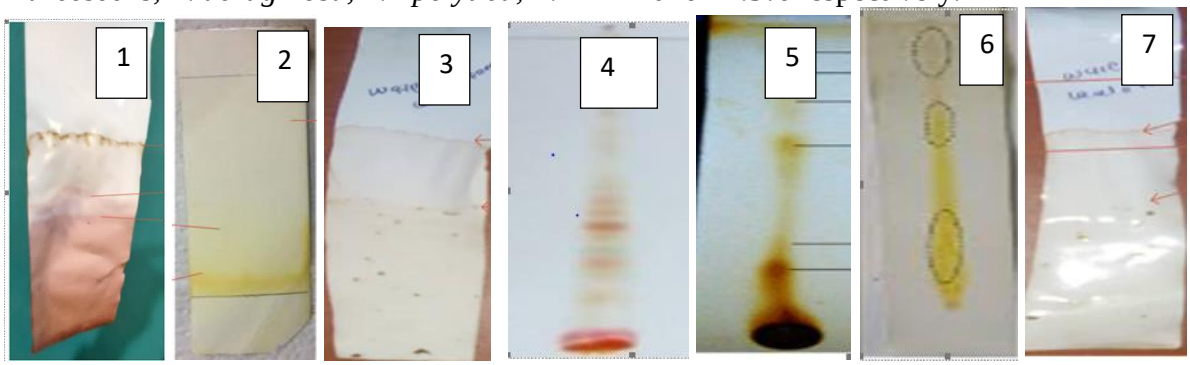


Figure 6: TLC of biocolorants from *Yarrowia lypolitica* (1), *Penicillium rubens* (2), *Candida tropicalis* (3), *Serratia marcescens* (4) *Aspergillus terreus* (5), *Pseudomonas aeruginosa* (6), *Metarhizium anisopliae* (7).

Figure 6 shows the separation of different colours having different Rf values expressed by some selected microorganisms using thin-layer chromatography. These also correspond to the various textiles dyed by the various pigments expressed by the various isolates.

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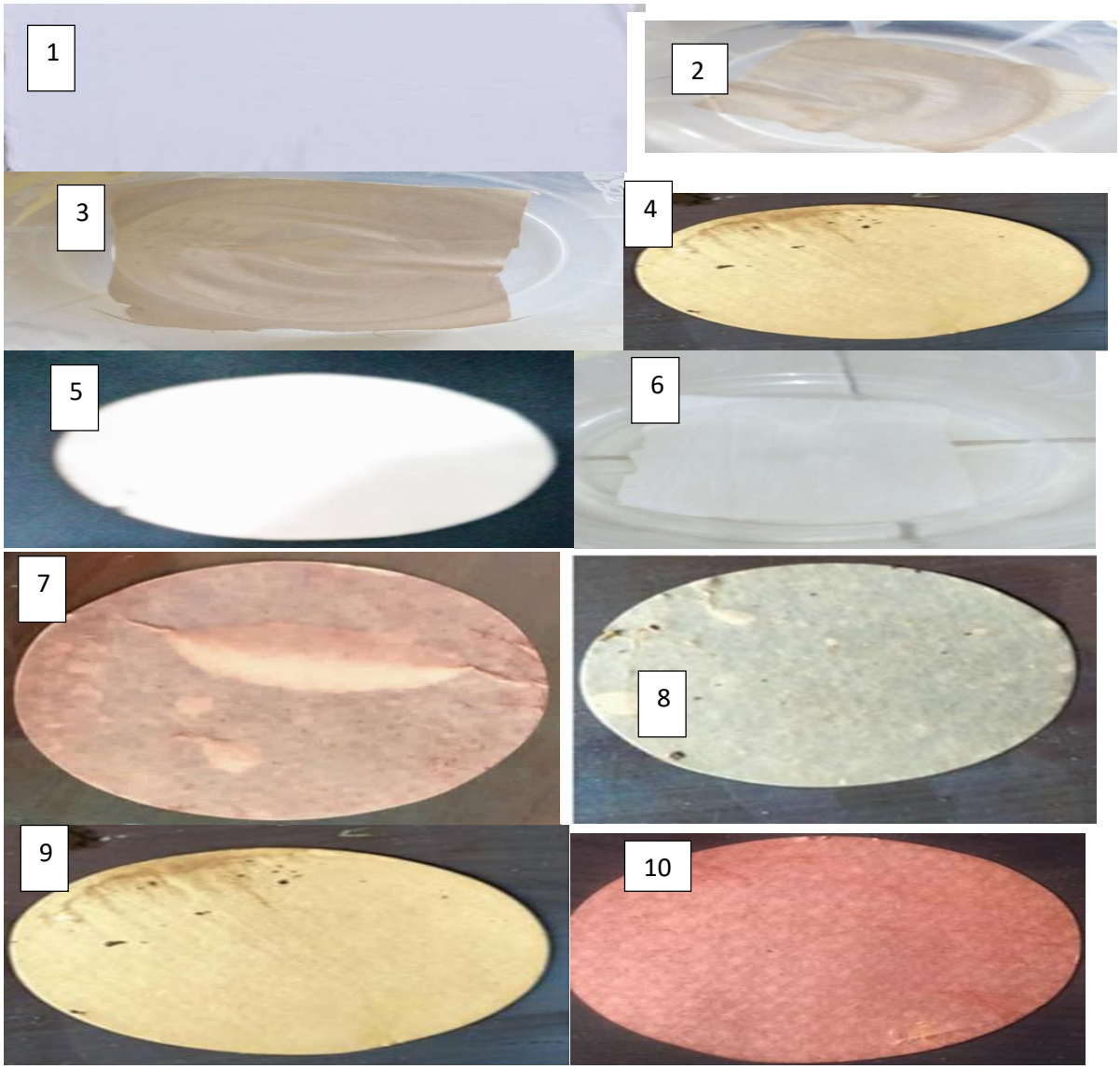


Figure 7: The un-dyed white linen (1) and linen dyed with biocolourants produced from *Pseudomonas aeruginosa* (2), *Aspergillus terreus* (3), *Bacillus sphaericus* (4), *Candida tropicalis* (5), *Bacillus subtilis* (6), *Yarrowia lypolitica* (7), *Metarrhizium anisopliae* (8), *Penicillium ruben* (9), *Serratia marcescens* (10)

Results in Figure 7 showed various colour retention of the pigment on the textile stained after exposure to sunlight with *A. terreus*, *B. sphaericus*, *C. tropicalis*, *Y. lypolitica*, *M. anisopliae*, *P. ruben* and *S. marcescens* showing photo-stability

Discussion

The various isolates in Table 1 and Table 2 are all soil isolates indigenously and not necessarily from contamination. Figure 1 shows an increase in the bacterial biomass with the double strength of yeast extract. The bacterial biomass increases 7mg/l to 8 mg/l for *B. subtilis*, 8 to 8.1 mg/l for *S. marcescens*, 7.8 to 8 mg/l for *B. sphearicus* and 10 to 11.2 mg/l for *P. aeruginosa* when the yeast extract was double strength. However, there was no noticeable increment in the biomass of all the fungi except in *Y. lipolytical* which increased from 12.4 to 13.2 mg/l when the dextrose nutrient was double strength in Figure 2. Rajpurohit and Eiteman (2022) proposed

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that nutrient limitation for biomass may not necessarily mean starvation since the biomass may be in its resting phase. This may explain the reason there was no significant difference in the double strength of yeast extract and dextrose for bacteria and fungi biomass respectively. Bacterial protein content was highest at 25ºC at 0.2 mg/ml for *P. aeruginosa* and *S. marcescens* while *B. subtilis* and *B. sphaericus* were highest at 30ºC at 0.14 and 0.28 mg/ml respectively in Figure 3. However, a fungal protein was reportedly higher than bacterial protein with *Y. lipolytica* and *M. anisopliae* recording 1.42 mg/ml followed by 0.8 mg/ml respectively in Figure 4. Xiaoyun *et al*. (2012) stated that fungi-based systems are more capable of protein production than bacterial-based systems due to the high-level secretion of proteins. This could explain the reason why the total protein content recorded in the various fungal isolates was more in content than that of the selected bacterial isolates. Percentage pigment exhaustion was highest in the pigment produced by *S. marcescens*, at 66.32 %, followed by *P. aeruginosa* among isolated bacteria while *Y. lipolytica* and *A. terrus* showed the highest percentage exhaustion among the fungi isolates in Figure 5. *S. marcescens* and *P. aeruginosa* have been extensively studied to contain prodigiosin a compound found to be rich in spectral properties (Andreyeva and Ogorodnikova, 2015**).** These bacteria are hence viable in pigment production and textile application due to the high percentage exhaustion recorded in this research. This observation is in line with the report of Veni *et al*. (2021) who worked on the extraction and application of pigment from *S. marcescens* SB08 for textile dyeing.

The biosynthesis of colourants for textile applications has attracted increased interest in recent years. Nature produces many biocolourants from various resources including plants, animals, and microorganisms, which are possible alternatives to synthetic dyes and pigments currently employed (Singh *et al*., 2023).

Conclusion and Recommendations

The various colour dyes extracted from all the isolates used as natural dyeing for textile materials showed good fastness properties and high dye uptake. This study showed that *S. marcescens*, at *P. aeruginosa* among isolated bacteria, *Y. lipolytica* and *A. terreus* among fungi isolates are excellent in pigment production and dye application. However, all the biocolorant tends to be photophobic when exposed to sunlight except *Y. lipolytica* and *S. macescens* which seem to be more stable hence the pigment thus has a potential to be used as a dyeing agent. The advantage of the biocolourant of microbial origin is related to its eco-friendly nature, unlike synthetic dye that could alter the biodiversity and ecology balance of nature when released into the environment. The optimized condition for the production of photo-stable biocolourant from each isolate needs to be evaluated to access the varieties of pigments in the cellular biomass for the textile industry.

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