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MOLECULAR CHARACTERIZATION OF BACTERIAL AND FUNGAL ISOLATES OF TIE AND DYE WASTEWATER FROM LOCAL TEXTILE MILLS AT ITOKU, ABEOKUTA, OGUN STATE, NIGERIA

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ABSTRACT: *Molecular techniques and phenotypic assessments were employed for identification and characterization of indigenous microbes in tie and dye textile wastewater. Genera isolated include Bacillus, Enterobacter, Escherichia, Staphylococcus, Klebsiella, Proteus, Saccharomyces, Penicillium and Aspergillus. Strains isolated have been kept in NCBI GenBank database under accession numbers OR485162, OR492431, OR492366, OR485290, OR492383, OR477460, OR485247, OR492410, OR485289, OR492362, OR485251, OR492370, OR492436, OR492467, OR492478, OR512432, OR492438 and OR492479. Bacterial counts, including textile wastewater-utilizing bacterial counts (TWUBC) and textile wastewater-utilizing fungal counts (TWUFC), were highest in brown-coloured effluent, with mean values of 5.66 ± 4.01 log_{10} CFU/ml and 1.48 ± 0.28 log_{10} CFU/ml, respectively. The lowest TWUBC in green-coloured effluent (1.27 ± 0.47 log_{10} CFU/ml), while the lowest TWUFC was in blue-coloured effluent (1.08 ± 0.51 log_{10} CFU/ml). Mann-Whitney U test indicated that TWUBC readings in brown-coloured effluent exhibited a statistically significant difference ($p < 0.05$) when compared to other coloured effluents. So, no significant difference ($p > 0.05$) was observed in TWUFC among different effluent colours. Statistical test also showed that TWUBC significantly differed ($p < 0.05$) from TWUFC obtained from the textile wastewater. This research emphasizes the presence of a diverse array of bacteria and fungi in textile wastewater, revealing their potential for bioremediation applications.*

KEYWORDS: Tie and Dye, Textile wastewater-utilizing bacteria, Textile wastewater-utilizing fungi.

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INTRODUCTION

Worldwide technological advancement, economic growth and urbanization since the industrial revolution accompanied by over-exploitation of raw water have led to a tremendous increase in environmental degradation due to indiscriminate disposal of wastes (Kamat and Kamati, 2015; Benkhaya and El Harfi, 2019). Industrial effluents account for several non-point and point sources water pollution, while developed nations have adopted stringent water quality regulations to control surface water pollution from point and non-point sources, the situation is different in most developing countries like Nigeria. Wastewater or effluent is the water extracted from the whole textile production process. The wastewater contains a mixture of different dyes, additives such as dispersants, levelling agents, acids and alkalis that are released into water bodies without adequate or effective treatment, resulting in serious environmental concerns (Agarry and Ajayi, 2011). Based on reports, many microorganisms have been found to decolorize and detoxify dyes and additives in textile wastewater and the role of some of the microbial species in the bioremediation of textile dyes has also been reported.

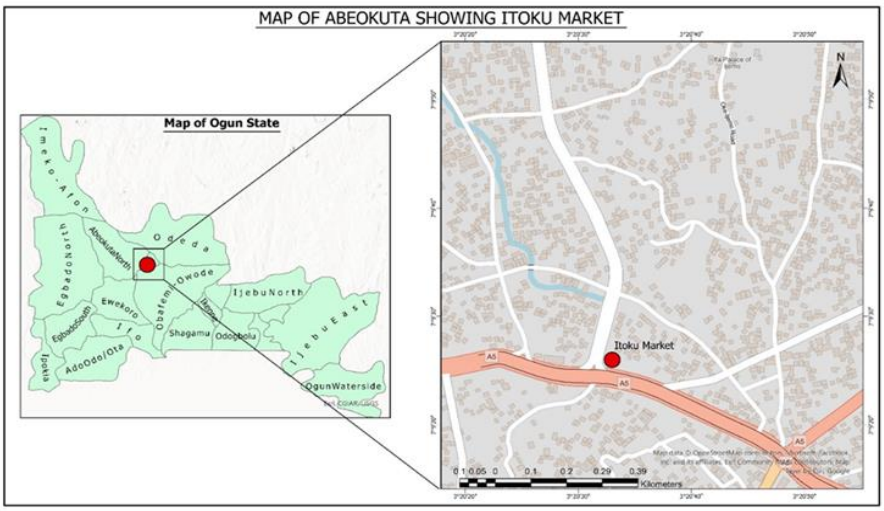
Hence, the need to isolate and identify bacteria and fungi strains with potentials to degrade dyes especially in developing countries where there is little or no efficient treatment for the large volume of wastewaters generated in the daily production processes. Salah & Mohamed, (2016), reported isolation of about 37 different bacterial strains, including *Salmonella sp*., *Aeromonas sp*., *Klebsiella sp*., *Pseudomonas sp*., and *Bacillus sp* from textile effluents from Cairo. Reports on the molecular identification of the microorganisms isolated from the tie and dye textile effluents are scarce or non-existence. This study is targeted to identify bacterial and fungal species present in the wastewater to determine their potential decolourization and degradation abilities to promote sustainable practices by the local textile outlets.

METHODOLOGY

Study Area

Effluent samples were collected from 2 local Tie and Dye mills behind Itoku market (Lat.7º10’.5’’N 3º18’.8” E) Abeokuta South Local Government Area, Ogun State. This is a neighborhood renown for Adire tie and dye work in the region.

Figure 1: Map of Ogun State showing Itoku in Abeokuta



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Collection of samples

Coloured effluents (Brown, Green, Blue and Pink) were collected in triplicates from effluent tanks at each mill into sterile containers at a depth of 15cm in 4 different 2.5L containers. The samples were kept in ice packs and transported to the laboratory for analysis.

Isolation and Enumeration of Textile Wastewater-utilizing and Heterotrophic Microbes

Textile wastewater-utilizing bacteria count (TWUBC), Textile wastewater-utilizing fungi count (TWUFC), total viable bacteria count (TVBC), total coliform count (TCC) and total viable fungi count (TVFC) were performed according to previously described methods (Public Health England, 2014). The TWUBC, TWUFC, TVBC, TCF and TVFC were determined with the spread plate technique. One hundred micro liters (100 µl) of each dilution of the effluents was carefully spread onto Tryptic Soy agar (TSA) and MacConkey agar (MA) plates in duplicate, aimed at isolating and counting the total viable bacteria and total coliforms. Besides, equal volumes were inoculated onto Bushnell Haas mineral salt agar (BMSA) plates, also in duplicate, which were supplemented with 1% of the respective sterile textile wastewater effluents to assist the isolation and enumeration of bacteria capable of using effluents (TWUBC) in the textile wastewater samples. For TWUFC, 100 µl of each dilution was carefully spread onto BMSA medium, which was supplemented with 1% sterile textile wastewater effluent, 4% glucose, and 0.005% chloramphenicol. The TVFC analysis involved spreading 100 µl of each sample dilution on Sabouraud Dextrose Agar (SDA) plates, amended with chloramphenicol to effectively inhibit bacterial growth. The TSA and BMSA plates were incubated for 48 hours at 35°C to promote bacterial proliferation, while the SDA and BMSA plates supplemented with chloramphenicol were incubated at room temperature for an extended period of up to 7 days to support fungal development. Enumeration of the colonies were expressed as bacterial (TWUBC, TVBC and TCC) or fungal (TWUFC and TVFC) colony-forming units per milliliter (CFU/ml) of the wastewater sample.

Characterization of the Microbial Isolates

Genus-level and species-level identification were performed using standard phenotypic and molecular techniques respectively (Krieg and Holt 1984; Zafar *et al*., 2017; Sanger *et al*., 1977; Lane, 1991; Schuurman *et al*., 2004; Schoch *et al*., 2012; Tamura *et al*., 2013).

Phenotypic Identification of the Microbial Isolates

The phenotypic tests used in the identification of the bacterial isolates included Gram-staining, coagulase, catalase, oxidase, citrate, indole, Voges Proskauer, methyl red tests, as well as sugar (lactose and mannitol) fermentation tests (Krieg and Holt, 1984). Mycological examination of the fungal isolates was based on colonial morphology and microscopic characterization by Zafar *et al*., (2017).

Molecular Identification of the Microbial Isolates

Molecular identification employed techniques that involved polymerase chain reaction (PCR) of template DNA extracted from the bacterial and fungal isolates. This was followed by sequencing of the partial 16S and ITS rRNA gene amplicons obtained from the bacterial and fungal isolates, respectively. Universal 16S rRNA bacterial primers [27F-AGAGTTTGATCMTGGCTCAG; 1492R GGTTACCTTGTTACGACTT] often employed

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for bacterial taxonomy were used to determine the presence of 16S rRNA gene, while ITS rRNA gene primers (ITS1-F- CTT GGT CAT TTA GAG GAA GTA A; ITS4- TCC TCC GCT TAT TGA TAT GC) were used to detect the presence of ITS rRNA gene. Amplification of 16S rRNA gene was done in a GeneAmp PCR system 9700 (Applied Biosystems, United States) with the following cycling conditions: initial denaturation at 95°C for 2 minutes, followed by 40 cycles, with each cycle consisting of denaturation at 94°C for 45 seconds, annealing at 55ºC for 60 seconds, extension at 72ºC for 120 seconds, and a final extension at 72°C for 300 seconds. Amplification ITS rRNA gene was performed in a GeneAmp PCR system 9700 (Applied Biosystems, United States) with the following cycling conditions: initial denaturation at 95ºC for 5 minutes followed by 30 cycles of denaturation at 95ºC for 1 minute, annealing for 30 seconds at 55°C, extension for 1 min at 72 °C, and a final extension at 72 °C for 7 minutes. DNA sequencing of the amplicons was performed with the dideoxy-chain termination method (Sanger *et al*., 1977). Taxonomic classification of the microbial isolates was confirmed by a comparison of the experimentally determined nucleotide sequence against the sequence database (rRNA type strains/prokaryotic_16S_ribosomal_RNA). The sequence comparison was performed with the BLASTN 2.8.0+ program [National Center for Biotechnology Information (NCBI)]. The sequences of the identified microbial species were subsequently deposited in the NCBI GenBank database under specific accession numbers.

Phylogenetic Analysis

Multiple sequence alignments were implemented with the MUSCLE algorithm in MEGA software, version 6 (Tamura *et al*., 2013). Phylogenetic trees were constructed using the neighbour-joining algorithm in MEGA software. The statistical significance of the clusters in the trees was estimated by bootstrap iterations (1000 replications).

RESULTS

Textile wastewater-utilizing microbial counts

The textile wastewater-utilizing bacterial and fungal counts are presented in Table 1. The overall mean TWUBC was highest in the brown-coloured effluent (5.66 ± 4.01 $\log_{10}$ CFU/ml) and lowest in the green-coloured effluent (1.27 ± 0.47 $\log_{10}$ CFU/ml) while overall mean TWUFC was highest in the brown-coloured (1.48 ± 0.28 $\log_{10}$ CFU/ml) and lowest in the blue-coloured effluent (1.08 ± 0.51 $\log_{10}$ CFU/ml). The TWUBC recorded in brown-coloured effluent significantly differed ($p < 0.05$) from those recorded in the blue-, green- and pink-coloured textile wastewater effluents. However, no significant differences were seen in TWUFC among effluents ($p > 0.05$). Mann Whitney U (Wilcoxon rank-sum) test also showed that TWUBC significantly differed ($p < 0.05$) from TWUFC obtained from the textile wastewater.

Heterotrophic microbial counts

Table 2 shows the heterotrophic bacterial and fungal counts obtained from the different textile wastewater. The TVC and TCC were highest in the brown-coloured textile wastewaters (overall mean TVC = 6.06 ± 5.43 $\log_{10}$ CFU/ml and overall mean TCC = 6.94 ± 5.43 $\log_{10}$ CFU/ml). The TVC was lowest in the green-coloured textile wastewater (overall mean TCC =

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1.66 ± 1.01 log_{10} CFU/ml) while the TCC was lowest in the blue-coloured effluent (overall mean TCC = 2.05 ± 1.83 log_{10} CFU/ml). The TVC and TCC recorded in brown-coloured effluent significantly differed ($p < 0.05$) from those recorded in the blue-, green- and pink-coloured textile wastewaters. Shapiro-Wilk test indicated that the TVC and TCC datasets of the different textile wastewaters were not normally distributed ($p < 0.05$) but their variances were equal ($p > 0.05$) as indicated by the Levene's homogeneity of variance test. Mann Whitney U (Wilcoxon rank-sum) test showed that there were no significant differences ($p > 0.05$) between TVC and TCC values of the different textile effluents.

The fungal count was highest in the brown-coloured textile wastewater (overall mean TFC = 1.85 ± 0.42 log_{10} CFU/ml) and lowest in the blue-coloured textile wastewater (1.41 ± 0.46 log_{10} CFU/ml). The overall mean fungal counts in the green- and pink-coloured textile wastewater were 1.56 ± 0.55 log_{10} CFU/ml and 1.56 ± 0.48 log_{10} CFU/ml, respectively. There was no significant difference ($p > 0.05$) between the fungal counts in the green-coloured wastewater and pink-coloured wastewater. However, total fungal counts recorded in brown-coloured and blue-coloured effluents significantly differed ($p < 0.05$) from those counts recorded in the green- and pink-coloured textile wastewater. Shapiro-Wilk test revealed that the TFC datasets of the different textile wastewater were normally distributed ($p > 0.05$) with equal variances ($p > 0.05$) as indicated by the Levene's homogeneity of variance test. Fisher one-way ANOVA test showed that there were no significant differences ($p > 0.05$) amongst TFC values of the different textile wastewater.

Table 1: Textile wastewater-utilizing bacterial and fungal counts obtained from the different textile wastewater effluents

		TWUBC		TWUFC	
Effluent sample colour	Factories sampled	Mean count Log_{10} CFU/ml N = 6	Overall mean count Log_{10} CFU/ml F = 12	Mean count Log_{10} CFU/ml N = 6	Overall mean count Log_{10} CFU/ml F = 12
Pink	1	1.05 ± 1.07	1.53 ± 1.31	1.33 ± 0.60	1.27 ± 0.41
	2	2.01 ± 1.93		1.21 ± 0.44	
Blue	1	2.13 ± 2.01	2.28 ± 1.83	1.05 ± 0.81	1.08 ± 0.51
	2	2.43 ± 1.14		1.11 ± 0.61	
Green	1	1.24 ± 0.43	1.27 ± 0.47	1.29 ± 0.32	1.25 ± 0.31
	2	1.31 ± 0.81		1.21 ± 0.34	
Brown	1	5.36 ± 4.17	5.66 ± 4.01	1.43 ± 0.11	1.48 ± 0.28
	2	5.95 ± 4.21		1.52 ± 0.72	

N: number of samples from each factory; F: total number of samples from all factories examined; TWUBC: textile wastewater-utilizing bacterial count; TWUFC: textile wastewater-utilizing fungal count

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Table 2: Heterotrophic bacterial and fungal counts obtained from the different textile wastewater effluents

Effluent sample colour	Factories sampled	Total Viable Counts (TVC)		Total Coliform Counts (TCC)		Total Fungal Counts (TFC)	
		Mean count Log_{10} CFU/ml N = 6	Overall mean count Log_{10} CFU/ml F = 12	Mean count Log_{10} CFU/ml N = 6	Overall mean count Log_{10} CFU/ml F = 12	Mean count Log_{10} CFU/ml N = 6	Overall mean count Log_{10} CFU/ml F = 12
Pink	1	1.13 ± 1.02	2.24 ± 2.07	2.32 ± 1.75	2.42 ± 1.97	1.57 ± 0.80	1.56 ± 0.55
	2	2.52 ± 2.31		2.50 ± 2.29		1.55 ± 0.59	
Blue	1	2.58 ± 2.31	2.74 ± 2.12	2.04 ± 1.22	2.05 ± 1.83	1.39 ± 0.73	1.41 ± 0.46
	2	2.86 ± 1.65		2.06 ± 1.48		1.43 ± 0.45	
Green	1	1.58 ± 1.09	1.66 ± 1.01	2.06 ± 1.44	2.09 ± 1.30	1.57 ± 0.78	1.56 ± 0.48
	2	1.73 ± 1.24		2.11 ± 1.53		1.55 ± 0.26	
Brown	1	5.99 ± 5.73	6.06 ± 5.43	6.89 ± 6.07	6.94 ± 5.43	1.87 ± 0.47	1.85 ± 0.42
	2	6.12 ± 5.23		6.98 ± 6.36		1.84 ± 0.66	

N: number of samples from each factory; F: total number of samples from all factories examined; TWUBC: textile wastewater-utilizing bacterial count; TWUFC: textile wastewater-utilizing fungal count

Identified bacteria and fungi

The phenotypic investigations carried out to identify microbial isolates from the different textile wastewater effluents are presented in Tables 3 to 6. The main bacterial genera (Table 3) isolated included *Klebsiella, Enterobacter, Escherichia, Proteus*, *Bacillus* and *Staphylococcus*. The *Bacillus* genus had the highest frequency of occurrence amongst the bacteria that were isolated from the textile wastewater effluents. The fungal genera that were isolated include *Saccharomyces*, *Penicillium* and *Aspergillus*; with the most frequently occurring fungi being the *Aspergillus* species (Table 4). Representative bacterial strains (Table 5) inclusive of *B. licheniformis* strain SOLANKE&OKORIE 102, *E. cloacae* strain SOLANKE&OKORIE 113, *E. coli* strain SOLANKE&OKORIE 109, *S. aureus* strain SOLANKE&OKORIE 106, *K. aeruginosa* strain SOLANKE&OKORIE 111, *B. licheniformis* strain SOLANKE&OKORIE 101, *B. cereus* strain SOLANKE&OKORIE 103, *P. vulgaris* strain SOLANKE&OKORIE 112, *S. epidermidis* strain SOLANKE&OKORIE 105, *E. coli* strain SOLANKE&OKORIE 108, *B. cereus* strain SOLANKE&OKORIE 104 and *K. aerogenes* strain SOLANKE&OKORIE 110 were deposited in the United States National Center for Biotechnological Information (NCBI) GenBank database under accession numbers OR485162, OR492431, OR492366, OR485290, OR492383, OR477460, OR485247, OR492410, OR485289, OR492362, OR490764, OR485251 and OR492370, respectively. As shown in Table 4.6, representative fungal strains consisting of *A. flavus* SOLANKE&OKORIE 114, *A. niger* SOLANKE&OKORIE 116, *S. cerevisiae* SOLANKE&OKORIE 117, *P. rubens* SOLANKE&OKORIE 119, *A. flavus* SOLANKE&OKORIE 115 and *S. cerevisiae* SOLANKE&OKORIE 118 were also deposited at the NCBI GenBank under accession

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numbers OR492436, OR492467, OR492478, OR512432, OR492438 and OR492479, respectively. Gel electrophoresis of the representative bacterial and fungal strains is presented in Figures 2 and 3.

Table 3: Phenotypic identification of bacterial isolates obtained from the wastewater effluent samples

Effluent sample colour	Representative bacterial colonies	Colonial and morphological characteristics		Biochemical characterizations									Identified bacterial genera
		Growth on the Petri plates	Gram staining	CO	CA	OX	IN	MR	VP	CI	LA	MA	
Pink	1	Mucoid growth	Negative rods	NP	+	-	-	-	+	+	+	V	*Klebsiella*
	2	Mucoid growth	Negative rods	NP	+	-	-	-	+	+	+	V	*Enterobacter*
	3	Mucoid growth	Negative rods	NP	+	-	+	+	-	-	+	+	*Escherichia*
	4	Dry colony	Positive rods	NP	+	V	NP	NP	NP	+	V	+	*Bacillus*
Blue	1	Yellow colony	Positive cocci	-	+	V	NP	NP	NP	V	V	V	*Staphylococcus*
	2	Yellow colony	Positive cocci	+	+	-	NP	NP	NP	+	V	V	*Staphylococcus*
	3	Mucoid growth	Negative rods	NP	+	-	-	-	+	+	+	V	*Klebsiella*
	4	Mucoid colony	Positive rods	NP	+	V	NP	NP	NP	+	V	V	*Bacillus*
Green	1	Mucoid growth	Negative rods	NP	+	-	-	-	+	+	+	+	*Klebsiella*
	2	Mucoid growth	Negative rods	NP	+	-	-	-	+	+	+	+	*Enterobacter*
	3	Dry colony	Positive rods	NP	+	V	NP	NP	NP	+	V	+	*Bacillus*
Brown	1	Mucoid growth	Negative rods	NP	+	-	-	-	+	+	+	+	*Enterobacter*
	2	Mucoid growth	Negative rods	NP	+	-	+	+	-	-	+	V	*Escherichia*
	3	Mucoid colony	Positive rods	NP	+	V	NP	NP	NP	+	V	V	*Bacillus*
	4	Yellow colony	Positive cocci	+	+	-	NP	NP	NP	+	V	V	*Staphylococcus*
	5	Mucoid colony	Positive cocci	-	+	+	-	-	-	-	V	V	*Micrococcus*

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CO: Coagulase test. CA: Catalase test. OX: Oxidase test. CI: Citrate test. IN: Indole test. MR: Methyl red test. VP: Voges Proskauer test. LA: Lactose fermentation test. MA: Mannitol fermentation test. +: Positive results. -: Negative results. F: Fractional prevalence. %: Percentage prevalence. V: Variable reaction. NP: Not performed.

Table 4: Molecular characterization of bacterial isolates from the wastewater effluent samples

Effluent colour	Rep. Bacterial Colonies	Molecular analysis		Identified bacterial species		
		16S gene Homology	16S gene identity		Strain	Accession number
Pink	1	99%	100%	*Bacillus licheniformis*	SOLANKE&OKORIE 102	OR485162
	2	100%	100%	*Enterobacter cloacae*	SOLANKE&OKORIE 113	OR492431
	3	99%	99%	*Escherichia coli*	SOLANKE&OKORIE 109	OR492366
Blue	1	97%	99%	*Staphylococcus aureus*	SOLANKE&OKORIE 106	OR485290
	3	99%	99%	*Klebsiella aerogenes*	SOLANKE&OKORIE 111	OR492383
	4	99%	100%	*Bacillus licheniformis*	SOLANKE&OKORIE 101	OR477460
Green	3	99%	99%	*Bacillus cereus*	SOLANKE&OKORIE 103	OR485247
	4	99%	99%	*Proteus vulgaris*	SOLANKE&OKORIE 112	OR492410
Brown	1	100%	99%	*Staphylococcus epidermidis*	SOLANKE&OKORIE 105	OR485289
	2	99%	99%	*Escherichia coli*	SOLANKE&OKORIE 108	OR492362

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Table 5: Phenotypic identification of fungal isolates obtained from the textile wastewater effluents

Effluent Colour	Representative isolates	Morphology on Petri plates	Microscopy	Identified fungi genera
Pink	1	White velvety colonies on surface of agar plates that turn yellowish-green	Conidiophores arising from septate hyphae with central vesicles that were completely filled with conidia.	*Aspergillus*
	2	White colonies on the surface of agar plates	Unicellular oval cells that appeared purple upon gram staining with some cells exhibiting budding	*Saccharomyces*
Blue	1	White velvety colonies on surface of agar plates that turn yellowish-green	Conidiophores arising from septate hyphae with central vesicles that were completely filled with conidia.	*Aspergillus*
	2	White colonies on the surface of agar plates	Unicellular oval cells that appeared purple upon gram staining with some cells exhibiting budding	*Saccharomyces*
Green	1	Greenish colonies on the surface of agar plates	Multiple phialides arising from a single conidiophore carrying chains of conidia	*Penicillium*
	2	White colonies on the surface of agar plates	Unicellular oval cells that appeared purple upon gram staining with some cells exhibiting budding	*Saccharomyces*
Brown	1	Conidiophores arising from surface of agar plates that turn yellowish-green	septate hyphae with central vesicles that were completely filled with conidia.	
	2	White colonies on the surface of agar plates	Unicellular oval cells that appeared purple upon gram staining with some cells	*Saccharomyces*

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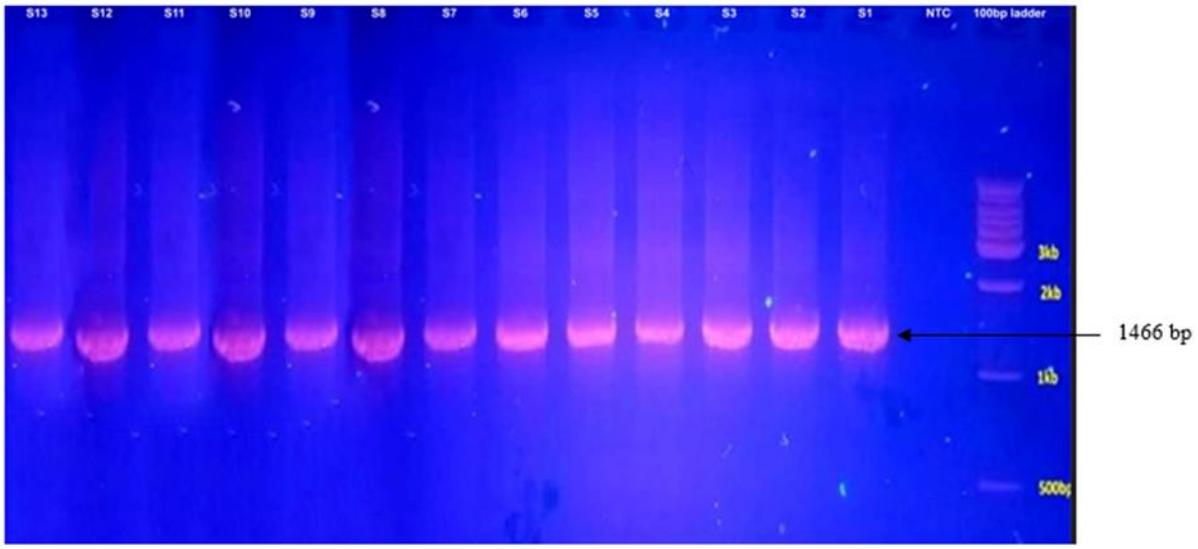
Table 6: Molecular characterization of fungal isolates from the wastewater effluent samples

Effluent colour	Representative bacterial colonies	Molecular analysis: 16S gene Homology	Molecular analysis: 16S gene identity	Identified Fungal species	Strain	Accession number
Pink	1	99%	99%	*Aspergillus flavus*	SOLANKE& OKORIE 114	OR492436
Blue	1	99%	99%	*Aspergillus niger*	SOLANKE& OKORIE 116	OR492467
	2	99%	99%	*Saccharomyces cerevisiae*	SOLANKE& OKORIE 117	OR492478
Green	1	99%	99%	*Penicillium rubens*	SOLANKE& OKORIE 119	OR512432
Brown	1	100%	100%	*Aspergillus flavus*	SOLANKE& OKORIE 115	OR492438

Figure 2: Gel electrophoresis of amplified 16S rRNA genes from bacterial isolates present in the textile wastewater effluents



Lanes S1 to S13 were positive samples for 16S rRNA genes obtained from bacterial isolates present in the textile wastewater effluents (S1 is *B. licheniformis* SOLANKE&OKORIE 101, S2 is *B. licheniformis* SOLANKE&OKORIE 102, S3 is *B. cereus* SOLANKE&OKORIE 103, S4 is *B. cereus* SOLANKE&OKORIE 104, S5 is *S. epidermidis* SOLANKE&OKORIE 105, S6 is *S. aureus* SOLANKE&OKORIE 106, S7 is *M. luteus* SOLANKE&OKORIE 107, S8 is *E. coli* SOLANKE&OKORIE 108, S9 is *E. coli* SOLANKE&OKORIE 109, S10 is *K. aerogenes* SOLANKE&OKORIE 110, S11 is *K. aerogenes* SOLANKE&OKORIE 111, S12

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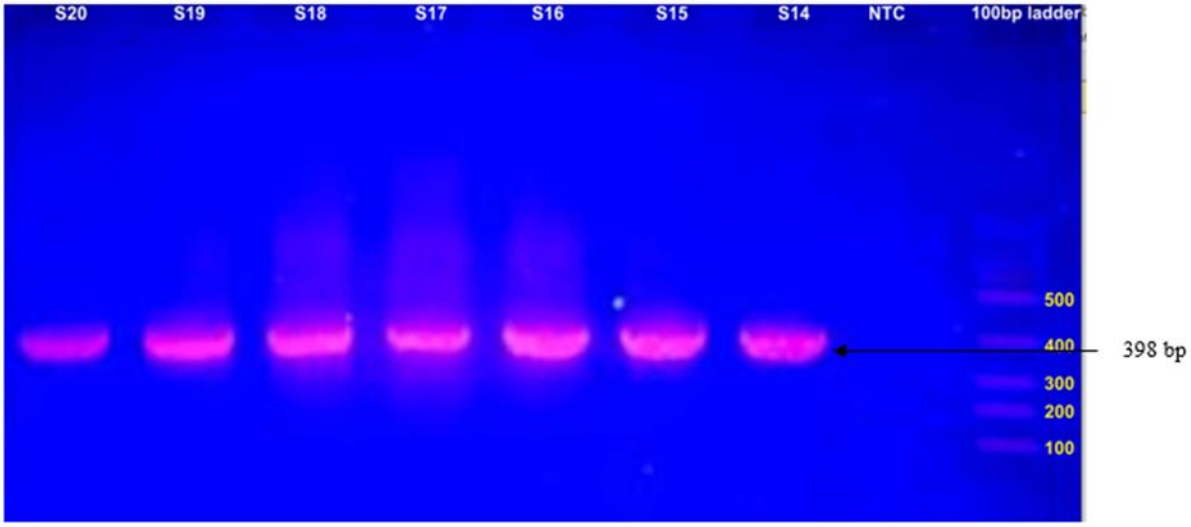
is *P. vulgaris* SOLANKE&OKORIE 112 and S13 is *E. cloacae* SOLANKE&OKORIE 113). Lane NC is the negative control (distilled water). Lane L is the molecular ladder (100 base ladder). Forward primer is 27F (AGAGTTTGATCMTGGCTCAG) and Reverse primer is 1492R (GGTTACCTTGTTACGACTT). Gel electrophoresis was performed with 2% agarose.

Figure 3: Gel electrophoresis of amplified ITS rRNA genes from fugal isolates present in the textile wastewater effluents Phylogeny



Lanes S14 to S20 were positive samples for ITS rRNA genes obtained from bacterial isolates present in the textile wastewater effluents (S14 is *A. flavus* SOLANKE&OKORIE 114, S15 is *A. flavus* SOLANKE&OKORIE 115, S16 is *A. niger* SOLANKE&OKORIE 116, S17 is *S. cerevisiae* SOLANKE&OKORIE 117, S18 is *S. cerevisiae* SOLANKE&OKORIE 118, S19 is *P. rubens* SOLANKE&OKORIE 119, S20 is *F. oxysporum* SOLANKE&OKORIE 120). Lane NC is the negative control (distilled water). Lane L is the molecular ladder (100 base ladder). ITS rRNA fungal primers (ITS1-F- CTT GGT CAT TTA GAG GAA GTA A; ITS4- TCC TCC GCT TAT TGA TAT GC. Gel electrophoresis was performed with 2% agarose.

Phylogenetic trees highlighting the evolutionary relatedness of microbial strains, belonging to the phyla Proteobacteria, Firmicutes and Ascomycota that were isolated from the textile wastewater effluents and reference microbial strains which were isolated from other environmental sources in the world are shown in Figures 4 to 6. *P. vulgaris* strain SOLANKE&OKORIE 112 (OR42410) shared a common ancestry with a cluster of bacterial strains such as *P. mirabilis* strain ATCC 29906 (NR 11441) and *P. vulgaris* strain ATCC 29905 (NR 115878), with a 62% likelihood. There was 99% likelihood that *K. aerogenes* SOLANKE&OKORIE 110 (OR42370) and *K. aerogenes* SOLANKE&OKORIE 111 evolved from a common ancestry with *K. aerogenes* strain ATCC 13048 (NR 118556) and *K. aerogenes* KCTC 2190 (NR 102493). *P. rubens* strain SOLANKE&OKORIE 119 (OR512432) shared a common ancestry with *P. rubens* strain CBS 129667 (NR 111815) and a cluster of other fungal strains, with 99% likelihood.

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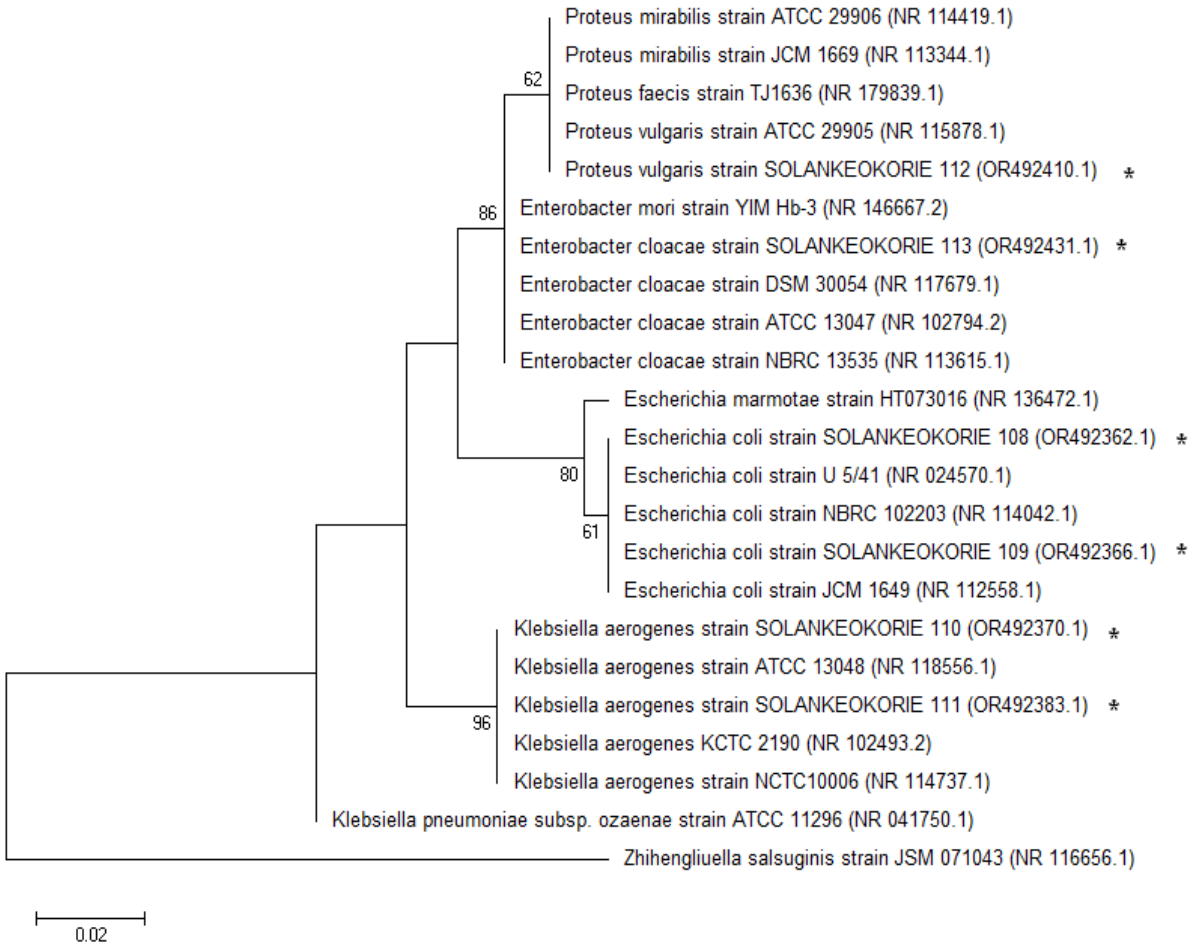
Figure 4: Phylogenetic tree highlighting bacterial strains of the Phylum Proteobacteria constructed with the neighbour-joining method



*is used to indicate a bacterial strain of the Phylum Proteobacteria isolated from the textile effluents. GenBank accession numbers of all the strains used to implement the phylogenetic tree are indicated in parenthesis. The tree was rooted on midpoint and only bootstrap values that were above 50 % are displayed on branches. *Zhihengliuella salsuginis* strain JSM 071043 served as the out-group.

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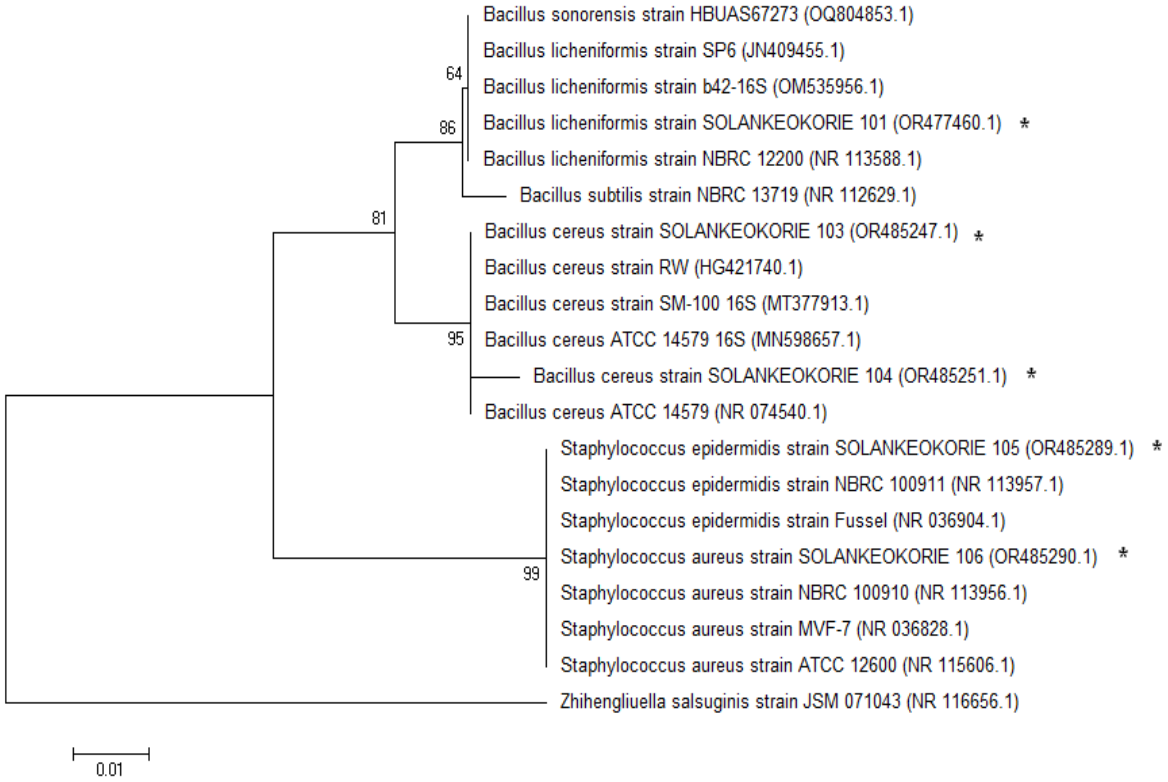
Figure 5: Phylogenetic tree highlighting bacterial strains of the Phylum Proteobacteria constructed with the neighbour-joining method



* is used to indicate a bacterial strain of the Phylum Firmicutes isolated from the textile effluents. GenBank accession numbers of all the strains used to implement the phylogenetic tree are indicated in parenthesis. The tree was rooted on midpoint and only bootstrap values that were above 50% are displayed on branches. *Zhihengliuella salsuginis* strain JSM 071043 served as the out-group.

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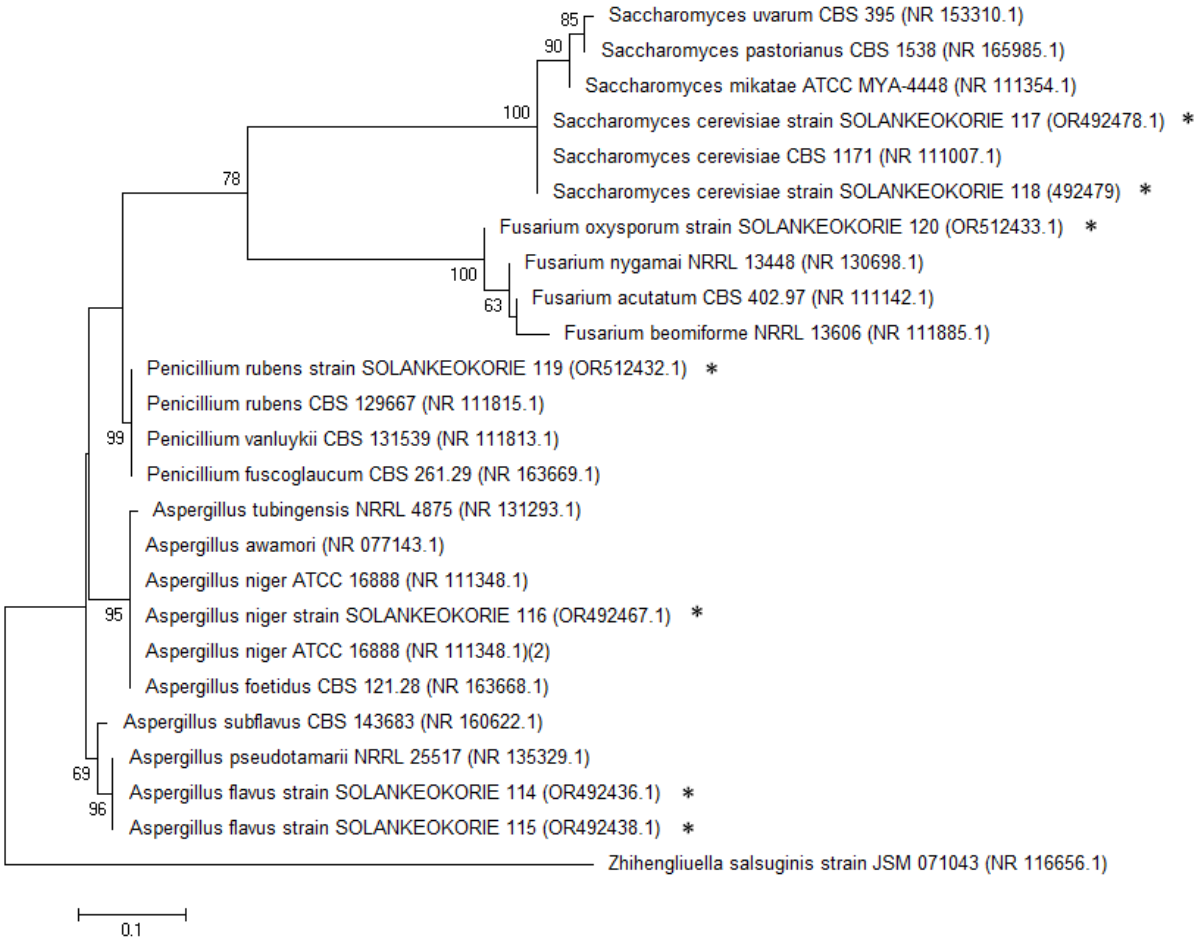
Figure 6: Phylogenetic tree highlighting some fungal strains of the Phylum Ascomycota constructed with the neighbour-joining method



* is used to indicate a fungal strain of the Phylum Ascomycota isolated from the textile effluents. GenBank accession numbers of all the strains used to implement the phylogenetic tree are indicated in parenthesis. The tree was rooted on midpoint and only bootstrap values that were above 50 % are displayed on branches. *Zhihengliuella salsuginis* strain JSM 071043 served as the out-group.

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DISCUSSIONS

The capabilities of microbes to degrade recalcitrant dye and pigment pollutants present in textile wastewater effluents have been widely studied. The potential application of isolated dye and pigment-utilizing microbe in the bioremediation of textile wastewater effluents provides a cost-effective means of sustaining the environment. In the present research, an array of bacteria was isolated from the textile wastewater effluents which could be used as potential consortia for bioremediation of the textile wastewater effluents. The isolated bacteria in the present research were similar to the genus *Bacillus megaterium* isolated by Galadima *et al*., (2018) and Yahaya *et al*., (2022) also reported *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Lactobacillus* spp. and *Escherichia coli* in textile effluents collected from kano and Lagos respectively in Nigeria. Ajao *et al*., (2011) isolated and immobilized *Pseudomonas aeruginosa* and *Bacillus subtilis* from textile wastewater, Saranraj *et al*., (2010) isolated *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella pneumoniae*, and *Escherichia coli*.

Furthermore, the high levels of bacteria in the wastewater could also mean the presence of high organic carbon used for nutrients, and ability of the isolates to form biofilms. According to Jamee and Siddique (2019), some bacteria colonize textile wastewater and utilize dyes as a source of carbon or nitrogen or both. In the process, these bacteria degrade dyes and other pollutants in textile wastewater (Prabba *et al*., 2016; Jamee and Siddique, 2019).

The results of the current study indicate that if the wastewater leaches into surface or groundwater or the food chain, it may cause microbial infections. Though many microorganisms are harmless, some of them act as opportunistic pathogens, taking advantage of susceptible individuals, such as those with a compromised immune system (Jeffrey and Putten, 2011). Some others may act as obligate pathogens.

The isolated fungi in the study were similar to those isolated by Ben *et al.,* (2020) in textile effluents collected from India, as well as to those of Lira *et al,*. (2022) who worked with textile effluents from Brazil. *Penicillium* spare reported to show high tolerance to pollutants such as heavy metals and hydrocarbons in effluents contaminated soils. Prabba *et al*., (2016) reported the isolation of *Aspergillus fumigates*. The detection of the identified microbes in this study shows that the wastewater contained certain compounds that attract microorganisms.

CONCLUSION

The diversity of bacteria and fungi isolated from the textile wastewater and the surroundings of the tie-and-dye industries are among the genera reported in literatures with ability to transform the wastewater to less toxic forms which are safe to release into the environment. The deployment of different indigenous microbial isolates and their consortium can be effective in bioremediation formulation for environmental restoration and sustainability.

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