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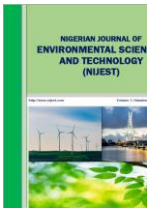

Biosurfactant Producing Bacteria from Effluent of Selected Textile Industries

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ABSTRACT

The aim of this study is to isolate, screen and molecular characterize biosurfactant producing bacteria from effluent of selected textile industries. Effluent samples were collected from ABY textile industrial company, Agege Motor Road, Mushin Lagos state and from Sunflag Nigeria Limited, Surulere Lagos state. Bacteria isolation from the effluents was done by serial dilution and spread plate method on Nutrient agar medium then passes through incubation process for 24 hours for colonies to be present. Screening method used are hemolytic, drop collapse and oilspread techniques to check for biosurfactant present in the effluent samples, which results as positive, negative, gamma, alpha and beta. Gram staining was done to the isolates and viewed under microscopic view showing mostly of Gram-positive bacillus and also Gram negative bacillus. Biochemical test was done by checking the biochemical characteristics such as citrate, glucose, lactose, H2S, gas, motility, indole, urease, oxidase, catalase, mannitol, spore former, pigmentation, vokes and prokeur. Molecular Characterization was done showing, Bacillus tropicus, Lysinibacillus fluoroglycofenilyticus, Paenibacillus sonchi, and Bacillus tianshenii. The PCR analysis of amplified, full length bacterial 16SrRNA fragments from the isolates obtained. The suspected organisms isolated from the samples includes; Bacillus thuringesis, Bacillus subtilis, with different Bacillus spp. The result of the gram staining done from the isolate collected from the textile industries is mostly Gram-Positive Bacilli showing the predominant Bacilli present in the effluents from the industries.

Keywords: Effluent, Screening, Biosurfactant, Isolate, Textile

1.0. Introduction

Biosurfactants are the amphiphilic compounds with the ability to accumulate between fluid phases and are produced on microbial cell surfaces or can be secreted extracellularly **(**Karanth *et al*., 1999; Anuraj *et al*., 2018**).** The hydrophilic moiety of the biosurfactants can be a carbohydrate, an amino acid, a phosphate group, or alike compounds whereas the hydrophobic moiety is mostly the fatty acid carbon chain.

This property helps reducing the interfacial and surface tensions, making them potential candidates for enhancing oil recovery **(**Sarkar *et al*., 1989; Anuraj *et al*., 2018**).** A number of microorganisms have been stated to produce a number of classes of biosurfactants such as glycolipids, lipopeptides, phospholipids, neutral lipids or fatty acids and polymeric biosurfactants **(**Makkar, and Rockne, 2003; Yin *et al*., 2009; Anuraj *et al*., 2018**).**

Biosurfactants are mainly classified according to their chemical structure and their microbial origin. The main classes of biosurfactants are glycolipids, phospholipids, polymeric biosurfactants and lipopeptides (surfactin). The best-known glycolipids are rhamnolipids, sophorolipids and trehalolipids (Makkar, and Rockne, 2003; Anuraj *et al*., 2018)

Many microorganisms produce biosurfactants that includes *Candida antartica*, *Acinetobacter species*, *Pseudomonas eruginosa*, and *Bacillus* species. Biosurfactants production is influenced by many factors like temperature, pH, aeration, nature of nitrogen and carbon source and C: N Ratio (Fakruddin, 2012).

Biosurfactants are delivered by various microorganisms, for example, microscopic organisms, growths and yeast. Biosurfactants pick up consideration as hydrocarbon disintegration operators in the 1960s, and their

applications have been enormously reached out in the previous five decades as an enhanced other option to compound surfactants particularly in sustenance, pharmaceutical and oil industry. It was evaluated that more than 10 million tons of compound surfactants and microbial biosurfactants were delivered each year. Despite the fact that the sort and amount of the microbial surfactants created depends chiefly on the maker living being, factors like nitrogen and carbon, temperature, air circulation and follow components likewise influence their production by the life form (Banat *et al*., 2010).

Biosurfactants are amphiphilic compounds produced in living surfaces, mostly on microbial cell surfaces or excreted extracellular hydrophobic and hydrophilic moieties that confer the ability to accumulate between fluid phases, thus reducing surface and interfacial tension at the surface and interface respectively (Cunha *et al*., 2004; Fakruddin, 2012). They possess the characteristic property of reducing the surface and interfacial tension using the same mechanisms as chemicals surfactants (Singh *et al*., 2007; Fakruddin, 2012).Surfactants are the active ingredients found in soaps and detergents with the ability to concentrate at the air- water interface and are commonly used to separate oily materials from a particular media due to the fact that they are able to increase aqueous solubility of Non-Aqueous Phase Liquids (NAPLS) by reducing their surface/ interfacial tension at air–water and water–oil interfaces(Yin *et al*., 2009; Fakruddin, 2012).

Biosurfactants are mainly classified according to their chemical structure and their microbial origin. The main classes of biosurfactants are glycolipids, phospholipids, polymeric biosurfactants and lipopeptides (surfactin). The best-known glycolipids are rhamnolipids, sophorolipids and trehalolipids (Yin *et al*., 2009 and Makkar *et al*., 2003; Fakruddin, 2012).

Biosurfactants present many advantages when compared to their chemical counterparts, since they have higher biodegradability and lower toxicity (Fakruddi, 2012). These biomolecules can also be produced under milder conditions of temperature and pressure and present increased effectiveness and resistance to variation in environmental conditions (Cameotra, 2002 and Souza *et al*., 2004).

Biosurfactants are good emulsifying agents and can be produced from renewable raw materials (Fakruddi, 2012, Mulligan, 2009). The properties of biosurfactants when compared to their chemically synthesized counterparts and broad substrate availability made them suitable for commercial applications. Microbial surfactants are identified with their surface movement, resilience to pH, temperature and ionic quality, biodegradability, low poisonous quality, emulsifying and demulsifying capacity and antimicrobial action (Chandran *et al*., 2010).

2.0 Methodology

2.1. *Materials and Reagents*

Sterile containers, Petri dishes, Paper tape, Cotton swab, Inoculating loop, Spatula, Ethanol, Syringe, Microscope glass slide, Nutrient Agar, McConkey, conical flask, sterile distilled water, cell suspension, shaker incubator, micropipettes, microtitre, nutrient agar slant, Whatman's filter paper, distilled water, aluminum foil.

2.2. *Selection of Sampling Sites for the Isolation of Bacteria*

Effluent samples were collected in a screwed capped sterilized bottle from two selected textile industries.
Sample A - ABY Textile Industrial Company Limited, 359-360, Agege Motor Road, Mushin Lagos.
Sample B - Sunflag Nigeria Limited, Surulere, Lagos state, Nigeria.

2.3. *Sterilization of Glassware*

All glassware was sterilized with autoclave at 121^{o}C for 15 minutes to disinfect all forms of contaminations.

2.4. *Media Preparation*

The Nutrient agar was prepared according to the method of (Terrones-Fernandez *et al.*, 2023).

2.5. *Preparation for Serial Dilution*

90 to 100ml of distilled water was measured into conical flask which served as diluents for each sample and 9ml of these diluents was measured into 7 test tubes, the diluents was autoclaved at 121^{o}C for 15mins together with the media for 15minutes.

2.6. *Examination of the Samples for Bacteria*

It was examined according to the method of (Chaprao *et al*. 2018).

2.7. *Screening Method for Identifying Bacteria That Have Biosurfactant Properties*

2.7.1. *Total Heterophobic Bacteria Count (THBC)*
The total heterotrophic bacterial count (THBC) was determined using the method of (Coax *et al.*, 2024)

2.7.2. *Surface Active Bacteria Count (SABC) or Hemolytic Analysis*
Screening for surface-active bacteria was conducted according to (Tabatabaee *et al*., 2005)

2.8. *Identification of Bacteria from Textile Effluents*

2.8.1. *Gram Staining Technique*
It was carried out according to the method of Fawole and (Oso, 2004).

2.8.2. *Bacteria Characterization/Identification of the Isolates*
This was carried out using standard microbiological method of Joshi and (Deshpande, 2010).

2.9. *Biochemical Test For Identification of Bacteria From Textile Effluents*

2.9.1. *Motility Indole Urease (MIU) agar:* It was prepared and carried out according to the method of (Kumala, 2006)

2.9.2. *Klinger Iron Agar (KIA):* It was prepared and carried out according to the method of (Tibebu *et al.*, 2020)

2.10. *Molecular characterization*

The following tests were carried out for the molecular characterization of the isolates;

DNA Extraction
Polymerase Chain Reaction (PCR)
Sequencing

2.10.1. *DNA Extraction Preparation From Bacteria Effluent*
DNA was extracted and prepared according to the method of (Gaaib *et al*., 2011)

2.10.2. *PCR Amplification of the 16SrRNA gene (27F and 1492R)*
Polymerase chain reaction was carried out according to the method of (Hanshew *et al*., 2013)

3.0 Result and Discussion

3.1. *Morphological Characteristics of Bacteria Isolates from Textile Effluents*
Isolates were obtained from ABY textile industrial company, Agege Motor Road, Mushin Lagos- state and from Sunflag Nigeria Limited, Surulere Lagos- state. Table 1 shows the numbers of colonies isolated from the effluent samples. Most of the isolates are round with few filamentous in shape. The isolates are dominantly smooth in surface appearance while just five are dull in appearance as shown in Table 1.
In this research work, biosurfactant producing bacteria were isolated, screened and characterized from textile industry effluent. The morphological characteristics of the isolates revealed a dry, shiny and moist texture. The shape of the isolates was round, oval, filamentous and rhizoid. Some of them were transparent while others appeared opaque. The sizes ranges from small to moderate and large with curled, entire, smooth, lobate, hairy, rough edges. The assays used in the screening of the isolates showed positivity to the ability of the isolates to produce surface active compounds (biosurfactant) initially determined by drop collapse test which confirms that the biosurfactant is not cell-bound since the drop collapsed, in contrast, the drop lacking biosurfactant remains beaded due to the hydrophobicity of the oil surface that caused aggregation of droplets (Mohammed, 2015).

The randomly selected waste water samples were taken from textile industrial areas for isolation, screening and molecular characterize biosurfactant producing bacteria which may be present in effluent samples. The effluent samples were isolated and inoculated on nutrient agar and incubated, screening was carried on the isolates after incubation to check for the biosurfactant producing bacteria present, morphological and

biochemical characterization of the bacteria isolates obtained from different textile industrial effluents revealed the following genera; *Bacillus thuringesis*, *Bacillus subtilis* and various types of *Bacillus spp.*

There was 96 morophologically distinct bacterial isolates isolated from 12 different sludge, textile effluent and dye contaminated soil samples. Generic composition of the 96 isolates comprised of *Bacillus* spp, *Enterobacteriaceae*, *Pseudomonas* spp, *Micrococcus* spp, *Alcaligenes* spp, *Aeromonas* spp, *Staphylococcus* spp and *Lactobacilus* spp (Sahasrabhude et al., 2014). Not does textile effluents and soil contaminated with dye have been the source of *Bacillus* spp, its ubiquitous nature is well established from a report which is suggestive of its source being the waste water generated from carpet industry in Khairabad, Uttar Pradesh, India. (Olukanmi et al., 2016) investigated the potential of effluent adapted and non-adapted bacterial isolated from textile industrial wastewater and outlet in Nigeria, Africa. Likewise, a similar study conducted by (Nig) Ltd, Odogunyan Industrial Estate Ikorodu, Lagos State, Nigeria to explore dye decolourising potential of adapted strains under immobilized condition on agar-agar in a bioreactor. The strains were characterized as Bacillus subtilis. (Mahmood et al., 2012) Screened textile effluents, sludgeand affected soil samples from Hudiara drain near Nishat Mills Limited from Ferozepur Road Lahore, Pakistan to isolate and identify potential dye degrading bacterial strains. The isolates were identified as *Bacillus subtilis*, *Bacillus cereus*, *Bacillus mycoides*, *Bacillus* sp, *Pseudomonas* sp, and *Micrococcus* sp by morphological and biochemical tests. Effluents from textile industries contain different types of dyes, which because of high molecular weight and complex chemical structures, show low level of biodegradability and Metals enter rivers via a variety of sources, such as natural chemical weathering of rocks, atmospheric deposition, agricultural activities, mining and improper disposal of untreated waste (Olayinka and Alo, 2004; Asare et al., 2019). Hence, direct deposition of these effluents into sewage networks, produce disturbances in biological treatment processes (Babu et al., 2000; Mohammad, 2015).

Table 1: Morphological characteristics of selected bacteria isolates

S/N	Textile name	Shape	Elevation	Edge	Surface Appearance	Pigment color	Optical Characteristics	Texture
1	ABY	Filamentous	Umbonate	Lobate	Smooth	White	Opaque	Dry
2	ABY	Round	Flat	Entire	Dull	Yellow	Opaque	Moist
3	ABY	Irregular	Flat	Entire	Smooth	Milky	Transparent	Dry
4	ABY	Round	Flat	Undulate	Smooth	Milky	Transparent	Dry
5	ABY	Round	Raised	Smooth	Smooth	Milky	Opaque	Moist
6	ABY	Round	Flat	Curled	Smooth	Milky	Transparent	Shiny
7	Sunflag	Filamentous	Convex	Filamentous	Smooth	White	Opaque	Shiny
8	Sunflag	Round	Flat	Entire	Dull	Milky	Transparent	Dry
9	Sunflag	Round	Raised	Entire	Smooth	Milky	Opaque	Shiny
10	Sunflag	Round	Raised	Entire	Smooth	Milky	Opaque	Shiny
11	Sunflag	Filamentous	Convex	Lobate	Smooth	Milky	Opaque	Dry
12	Sunflag	Round	Flat	Entire	Smooth	Milky	Transparent	Shiny
13	ABY	Round	Flat	Entire	Smooth	Cream	Opaque	Dry
14	ABY	Filamentous	Convex	Lobate	Smooth	Milky	Opaque	Dry
15	ABY	Filamentous	Umbonate	Lobate	Smooth	White	Opaque	Dry
16	ABY	Round	Flat	Undulate	Smooth	Milky	Transparent	Dry
17	ABY	Round	Flat	Entire	Dull	Yellow	Opaque	Moist
18	ABY	Round	Flat	Entire	Dull	Milky	transparent	Dry
19	ABY	Round	Flat	Curled	Smooth	Milky	Transparent	Shiny
20	ABY	Round	Raised	Entire	Smooth	Milky	Opaque	Shiny
21	Sunflag	Round	Flat	Entire	Smooth	Cream	Opaque	Dry
22	Sunflag	Round	Flat	Curled	Smooth	Milky	Transparent	Shiny
23	Sunflag	Round	Raised	Entire	Smooth	Milky	Opaque	Shiny
24	Sunflag	Round	Flat	Entire	Dull	Yellow	Opaque	Moist
25	Sunflag	Round	Flat	Curled	Smooth	Milky	Transparent	Shiny
26	Sunflag	Filamentous	Umbonate	Lobate	Smooth	White	Opaque	Dry

Table 2 shows the Gram reaction and cell morphology, providing insights into the cell shape and arrangement of the isolates. This is crucial for identifying bacteria types and understanding their roles in effluent treatment processes. Thirteen Gram positive and twelve (12) bacterial isolates were obtained with nineteen (19) rod, four (4) cocci and two coccobacillus were observed.

Table 2: Gram Reaction, and Cell Morphology (Microscopic Characteristics)

S/N	Textile name	Gram reaction	Cell shape	Cell arrangement
1	ABY	_	Rod	Double
2	ABY	_	Rod	Single
3	Sunflag	+	Rod	Double
4	Sunflag	_	Cocci	Cluster
5	Sunflag	_	Rod	Double
6	ABY	+	Rod	Chain
7	ABY	+	Rod	Single
8	Sunflag	_	Rod	Double
9	Sunflag	_	Rod	Chain
10	ABY	+	Coccibacillus	Single
11	ABY	+	Coccibacillus	Single
12	ABY	+	Rod	Tetrad
13	ABY	+	Rod	Cluster
14	ABY	_	Cocci	Cluster
15	ABY	_	Rod	Chain
16	ABY	+	Rod	Chain
17	ABY	+	Cocci	Double
18	ABY	+	Rod	Chain
19	ABY	_	Rod	Double
20	ABY	+	Rod	Cluster
21	ABY	_	Rod	Double
22	Sunflag	+	Rod	Double
23	Sunflag	_	Cocci	Cluster
24	ABY	_	Rod	Chain
25	Sunflag	+	Rod	Chain

N.B: + Positive, - Negative

3.3. *Hemolytic and Drop Collapse Tests*

The screening for biosurfactant using haemolytic test confirmed eighteen isolates with Gamma, five (5) alpha and two Beta while drop collapse method confirmed biosurfactant in eight (8) isolates and negative in seveneteen (17) isolates as shown in Table 3.

A similar study was carried out to explore potential dye decolourizing bacterial strains from the textile industry waste located in Erode and Tripur districts, Tamil Nadu, India.

Blood agar lysis as a primary method to screen biosurfactant producing organism cannot be reliable because the positivity of the haemolysis is not specific, this is because other lytic enzyme could lead to zone clearance and also the diffusion restriction in surfactant can inhibit the formation of a zone of clearance as reported by (Youseff et al., 2015). The effluent samples collected for this project work shows positive hemolysis (beta and alpha) but for other screening tests carried out shows negative and small size of clear zone when measured. The effluent samples collected shows positive hemolysis having negative and no clear zone when other tests (drop collapsed assay and oil spread assay) were conducted.

For the oil drop assay result were in corroboration with the drop collapsed assay results. The strains found positive in the oil drop assay result as shown were also positive for drop collapsed assay. These results confirmed the presence (for strains with positive results) and absence (for strains with negative results) of surface active compound (biosurfactant) in the cell free culture broth.

A similar study was carried out by Morikawa et al. (2000) on the area of oil displacement in oil spreading assay and is directly proportional to the concentration of the biosurfactant in the solution. However in this study there was no quantitative study conducted on biosurfactant concentration versus oil spreading activity, therefore the presence of good producer of biosurfactants bacteria were detected when positive in the three screening tests conducted.

Table 3: Hemolytic and Drop collapse result

S/N	ISOLATE CODE/ TEXTILE NAME	HEMOLYTIC	DROP COLLAPSE
1	ABY	Gamma	_
2	ABY	Gamma	+
3	Sunflag	Beta	+
4	Sunflag	Gamma	_
5	Sunflag	Alpha	+
6	ABY	Gamma	_
7	ABY	Alpha	+
8	Sunflag	Gamma	_
9	Sunflag	Alpha	+
10	ABY	Gamma	_
11	ABY	Gamma	_
12	ABY	Gamma	_
13	ABY	Gamma	_
14	ABY	Alpha	+
15	ABY	Gamma	_
16	ABY	Gamma	_
17	ABY	Beta	+
18	ABY	Gamma	_
19	ABY	Gamma	_
20	ABY	Gamma	_
21	ABY	Alpha	+
22	Sunflag	Gamma	_
23	Sunflag	Gamma	_
24	ABY	Gamma	_
25	Sunflag	Gamma	_

Keywords: *+ Positive, - Negative*

3.4. *Biosurfactant Production Assessment*

Four bacterial isolates with prominent biosurfactant were identified using 16SrRNA primer for sequencing of extracted DNA and PCR of the isolates as shown in Table 4.

PCR is a technique that is used to amplify trace amounts of DNA (and in some instances, RNA) located in or on almost any liquid or surface where DNA strands may be deposited. Universal PCR primers selected annealed the 16S rRNA sequences. The 16SrRNA gene is widely used for microbial identification and phylogeny construction. After digestion with HAE III restriction enzyme, the sizes of the terminal fragments amplified with the PCR primer. The combination of PCR primer and enzyme gave the largest number of fragments with unique sizes. This analysis indicated that the universal primer pair could theoretically anneal to the largest fraction of the 16SrRNA sequences of *Bacillus* spp. However, the resulting PCR product was relatively short, and there were too few unique fragments produced upon digestion of the product with the enzymes. PCR products generated the expected fragments.

This present work shows that gram positive *bacillus* constituted the majority of species in the textile industrial effluent samples.

Table 4: 16SrRNA identification of Biosurfactant producing bacteria isolates

S/N	Sample code	Identified organism	% identity	Sequence ID
1	ABY textile	*Bacillus tropicus*	96	NR- 157736
2	Sunflag textile	*Lysinibacillus fluoroglycofenilyticus*	100	NR- 148289
3	Sunflag textile	*Paenibacillus sonchi*	100	NR- 115751
4	ABY textile	*Bacillus tianshenii*	96	NR- 133704

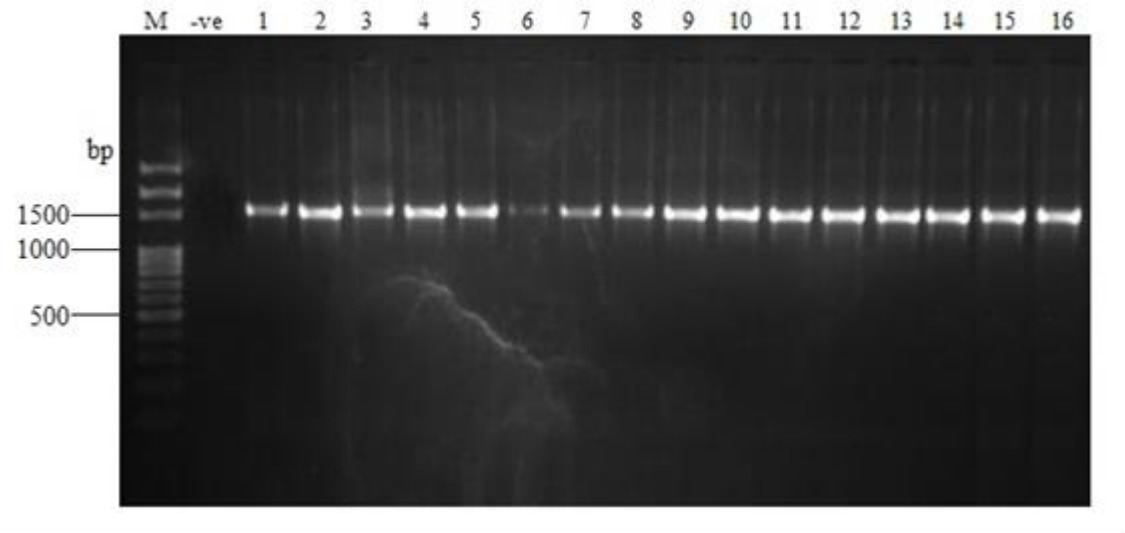

Figure 1: Agarose Gel Electrophoresis of Bacterial Isolates

4.0. Conclusions

Textile industry effluent samples were screened by haemolytic assay, oil spread and drop collapse assay to determine their biosurfactant producing ability. This process was followed by the Polymerase Chain Reaction in order to amplify the16SrRNA gene of the bacteria and lastly the determination of the molecular characterization of these bacteria is mostly Gram positive and Gram negative bacteria. Bacilli is predominant in the effluent samples collected for the project work, which are most of *Bacillus spp, Bacillus thuringesis*, and *Bacillus subtilis*.
Techniques provided for the identification, screening, and characterization of beneficial microorganism possesses a high capability to produce high amount of biosurfactant.

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