# Journal of Applied Sciences

ISSN 1812-5654

science alert

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OPEN ACCESS Journal of Applied Sciences

ISSN 1812-5654
DOI: 10.3923/jas.2019.776.781

# Research Article
# Screening Selected Detergents for Use as Positive Control in Assessing for Biosurfactant Production

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## Abstract

**Background and Objectives:** Chemically synthesized surfactants used as positive controls in assessing for biosurfactant production are expensive and not readily available in microbiology laboratories in Nigeria. The objectives of this study included comparing the response values of selected detergents to screening methods used in assessing for biosurfactant production with those of standard surfactants. **Materials and Methods:** Glycerol-mineral salts medium was used for culturing *Pseudomonas fluorescens* for biosurfactant production. At the end of the production period, the medium and 1% of selected detergents (Klin, Ariel and Cussons Morning fresh) were screened for surfactant activity using surface tension, foaming capacity, oil spread diameter and drop collapse activity. **Results:** The surface tension of the medium used for culturing *P. fluorescens* for biosurfactant production was reduced from 55.21-34.63 mN $m^{-1}$, the foaming capacity was 58.83% and the oil spread diameter was about 15 mm. The detergent solution having the least surface tension and the largest oil spread diameter was cussons morning fresh (17.84 mN $m^{-1}$ and 70 mm, respectively), while the detergent having the highest foaming capacity was Ariel (82.6%). The drop collapse activity of the medium and the detergent solutions were all positive. **Conclusion:** The results obtained for the detergents were comparable with those of some chemically synthesized surfactants. This indicates that readily available and inexpensive detergents can be used in place of chemically synthesized surfactants as positive controls in assessing for biosurfactant production.

Key words: Detergents, biosurfactant production, positive controls, chemically synthesized surfactants, screening for surfactant activity

Citation: Lekiah Pedro Peekate and Happy Boreh, 2019. Screening selected detergents for use as positive control in assessing for biosurfactant production. J. Applied Sci., 19: 776-781.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Biosurfactants are surfactant (surface-active agents) produced by living things, especially bacteria and yeast[1-3]. Surfactants produced by micro-organisms are capable of lowering the surface tension of the culture broth in which the organisms are growing[1,4]. Lowering of surface tension along with other activities such as emulsification of hydrophobic compounds and foaming is used in screening micro-organisms for biosurfactant production[4,5]. Lowering of surface tension (or interfacial tension) between two liquids or between a liquid and a solid, emulsification of hydrophobic compounds and foaming are inherent properties of chemically synthesized surfactants[6,7]. Surfactants can thus be used as positive controls in assessing biosurfactant production by an investigated micro-organism.

Chemically synthesized surfactants that have been used in assessing for biosurfactant production include Sodium dodecyl sulphate (SDS) and Triton® X-100 [8-10]. Surfactants such as SDS and Cetyltrimethylammonium bromide[11] are not readily available in many microbiology laboratories in Nigeria. However, surfactants are components of some detergents that are commercially produced in Nigeria. Commercially produced detergents are readily available for purchase from the local markets. Locally produced detergents even act as surfactants, since they produce foaming and can thus reduce the surface tension of water.

The price of chemically synthesized surfactants such as Triton® X-100, Tween®-20 and Sodium dodecyl sulfate range from $ 59-62 per 10 g as of November[12], 2018. As of 11th November, 2018, the exchange rate of the Naira (₦; the Nigerian currency) to the Dollar[13] is ₦ 306.15 per $ 1. Thus the price of the surfactants in Naira, minus importation cost will range from ₦ 18,062.85-₦ 18981.30 per 10 g. This amount is the take home salary per month of many civil workers in Nigeria. For scientists working in Nigerian laboratories, chemically synthesized surfactants are thus expensive for them. Also, chemically synthesized surfactants are only readily available to Nigerian industries making use of them by way of importation. Locally and commercially produced detergents may thus be used as positive controls by scientists in assessing biosurfactant production by investigated micro-organisms growing in broth culture.

Biosurfactant production by an investigated micro-organism growing in a broth medium is usually assessed using assays and methods such as oil spreading assay, drop collapse assay, reduction in surface tension, emulsification capacity and foaming capacity[14-16]. The extent to which selected detergents react to these assays and methods can be used as a guide in assessing if an investigated micro-organism growing in a broth culture has produce biosurfactant and in assessing the strength of biosurfactant produce. The aim of this study was thus to investigate the use of readily available detergents as positive controls in assessing for biosurfactant production by microorganisms.

## MATERIALS AND METHODS

**Biosurfactant production using a *Pseudomonas* species:** Yellowish green pigment producing *Pseudomonas fluorescens* isolated by Peekate and Abu[17] was obtained for this study. The identity of the isolate was confirmed through Gram-staining and microscopic examination and the following physicochemical/biochemical tests: Catalase, oxidase, motility, citrate utilization, indole production, MRVP (Methyl Red-Vogues Proskauer), blood haemolysis, casein hydrolysis, lecithinase production and fermentation tests using glucose, lactose, maltose, xylose and glycerol.

The isolate was cultured for biosurfactant production using the glycerol-mineral salts medium used in Peekate *et al.*[18] (Table 1, 2). The pH of the medium was adjusted to 5.5 as specified. A broth culture of the isolate was first prepared by transferring colonial growth obtained from its stock culture into 200 mL sterile nutrient broth (Titan Biotech Ltd., Rajasthan, India), which was then incubated at ambient temperature (29-31°C) for 48 h. After incubation, 10 mL of the broth culture was inoculated into 100 mL of the sterile glycerol-mineral salt medium in a 250 mL capacity conical flask. Air was bubbled through the content

Table 1: Composition of the glycerol-mineral salt medium

| Composition | Concentration |
|---|---|
| Glycerol (% v/v) | 3.00 |
| $KH_2PO_4$ (g $L^{-1}$) | 4.03 |
| $MgSO_4.7H_2O$ (g $L^{-1}$) | 0.40 |
| NaCl (g $L^{-1}$) | 1.00 |
| $CaCl_2.2H_2O$ (g $L^{-1}$) | 0.10 |
| $NaNO_3$ (g $L^{-1}$) | 4.46 |
| TES (% v/v) | 0.10 |
| pH adjusted to | 5.5 |

TES: Trace elements solution

Table 2: Composition of the TES

| Trace element salts | g $L^{-1}$ |
|---|---|
| $MnSO_4.H_2O$ | 1.5 |
| $FeSO_4.7H_2O$ | 0.5 |
| $CuSO_4.5H_2O$ | 0.2 |
| $Na_2MoO_4.2H_2O$ | 0.1 |
| $ZnSO_4.7H_2O$ | 1.5 |
| $H_3BO_3$ | 0.3 |

Table 3: Composition of the detergents used

| Detergents | Ingredients | Manufacturer |
|---|---|---|
| Ariel | Surfactants, builders, oxygen based bleaching agents, polycarboxylates, enzymes, optical brighteners and perfumes | Procter and Gamble, Nigeria Limited, Ibadan, Oyo state, Nigeria |
| Klin | Linear alkylbenzene sulfonate*, sodium tripolyphosphate, sodium carbonate and sodium sulphate | Natural Prime Resources Nigeria Ltd., Agbara, Ogun state, Nigeria |
| Cussons morning fresh | Anionic surfactants, hydrotropes, salts, perfume, preservatives and colours | PZ Cussons Nigeria, Plc., Ilupeju, Lagos, Nigeria |

*Alkylbenzene sulfonates are anionic surfactants. Source: Hibbs[19]

of the flask with the aid of an aquarium pump (Sea Star, HX-106A) connected to drip set tubes and sterile syringe. The setup was operated for 7 days, after which the content of the flask was screened for biosurfactant activity.

**Screening for surfactant/biosurfactant activity:** At the end of the biosurfactant production period, the broth culture medium was screened for surfactant activity using (1) Surface tension determination, (2) Foaming capacity, (3) Oil spread diameter and (4) Drop collapse activity. These were also carried out for the un-inoculated culture medium, distilled water and selected detergents produced in Nigeria. The detergents used include Klin, Ariel and Cussons morning fresh. The composition of the detergents is presented in Table 3. A 1% (w/v or v/v) solution of each detergent was prepared using distilled water and subjected to the screening.

**Determination of surface tension:** The capillary rise method was used in determining the surface tensions of the test solutions (broth culture medium, un-inoculated culture medium, distilled water and detergent solutions). A sterile capillary tube of about 0.2 cm in diameter was used to measure the rise in height of the test solutions. The rise in height was then used to calculate the surface tension with the aid[20] of Eq. 1:

$$\gamma = \frac{rhdg}{2} (mN\ m^{-1}) \quad (1)$$

where, 'r' is the radius of the capillary tube in cm, 'h' is the rise in height in cm of the liquid, 'd' is the broth density in g $mL^{-1}$ and 'g' is the acceleration due to gravity in cm $sec^{-2}$, i.e., 980 cm $sec^{-2}$.

**Foaming capacity:** The foaming capacity of a test solution was determined by transferring 10 mL of the solution into three graduated measuring cylinders, which were then vortex rigorously for 1 min. The foaming height and the total height were measured and used to calculate the foaming capacity (FMC). The calculation was done[16] using Eq. 2:

$$FMC = \frac{\text{Foaming height}}{\text{Total height}} \times 100\ (\%) \quad (2)$$

**Oil spread diameter:** About 40 mL of water were poured into Petri dishes and oil films generated on the surface of the water by applying 10 drops of diesel oil. A drop of the test solution was placed in the centre of the oil films and the diameter of the ensuing zone of clearance was measured.

**Drop collapse activity:** Each well in a ceramic well plate was coated with a drop of used-engine oil. The well plate was then incubated at 35°C for about 1 h. After incubation, two drops of the test solutions were transferred into the different oil-coated wells. The shapes of the drops were observed after about 1 min, with the aid of a magnifying lens. Test solutions whose drops collapsed (or became flat) were recorded as positive for inherent presence of surfactant, while those whose drops remained intact (rounded) were recorded as negative for presence of surfactant.

## RESULTS

**Confirmation of the identity of the *Pseudomonas* isolate:** The yellowish green *Pseudomonas* isolate appeared as Gram-negative rods under the microscope was motile and reacted positive to catalase, oxidase, citrate utilization, casein hydrolysis and lecithinase production tests. The bacterium produced beta-haemolysis on blood agar and was negative for indole, methyl red and Vogues-Proskauer tests. The isolate did not ferment lactose and maltose. However, it produced only acid from fermentation of glucose, xylose and glycerol.

**Surface tension of test solutions:** The surface tension of the test solutions (the detergents, broth culture, un-inoculated culture medium and distilled water) is presented in Table 4. From the Table 4, it can be seen that the mean surface tension of the medium used for culturing *P. fluorescens* for biosurfactant production was reduced from 55.21-34.63 mN $m^{-1}$ and the detergent solution having the least surface tension (17.84 mN $m^{-1}$) was cussons morning fresh.

Table 4: Surface tension of the test solutions determined through the capillary rise method

| | ST (mN $m^{-1}$) (Replicates) | | | |
|---|---|---|---|---|
| Test solutions | 1 | 2 | 3 | ST (Mean (mN $m^{-1}$)) |
| Ariel (1% w/v) | 22.05 | 23.28 | 23.77 | 23.03 |
| Klin (1% w/v) | 19.60 | 22.05 | 22.79 | 21.48 |
| CMF (1% v/v) | 17.64 | 17.64 | 18.23 | 17.84 |
| Broth culture | 37.73 | 30.87 | 35.28 | 34.63 |
| UIB | 53.90 | 58.80 | 52.92 | 55.21 |
| Distilled water | 61.74 | 63.70 | 68.60 | 64.68 |

CMF: Cussons Morning fresh, UIB: Un-inoculated broth, ST: Surface tension

Table 5: Foaming Capacity (FC) of the test solutions

| | FC (%) (Replicates) | | | |
|---|---|---|---|---|
| Test solutions | 1 | 2 | 3 | FC (Mean (%)) |
| Ariel (1% w/v) | 84.54 | 84.69 | 78.57 | 82.60 |
| Klin (1% w/v) | 83.15 | 72.22 | 78.08 | 77.82 |
| CMF (1% v/v) | 72.94 | 78.95 | 80.00 | 77.30 |
| Broth culture | 57.14 | 55.26 | 64.10 | 58.83 |
| UIB | 0 | 0 | 0 | 0 |

CMF: Cussons morning fresh, UIB: Un-inoculated broth

Table 6: Oil Spread (OS) activity of the test solutions

| | OS (mm) (Replicates) | | | |
|---|---|---|---|---|
| Test solutions | 1 | 2 | 3 | OS (Mean (mm)) |
| Ariel (1% w/v) | 50 | 60 | 65 | 58.3 |
| Klin (1% w/v) | 50 | 50 | 60 | 53.3 |
| CMF (1% v/v) | 70 | 70 | 70 | 70.0 |
| Broth culture | 11 | 15 | 20 | 15.3 |
| UIB | 0 | 0 | 0 | 0.0 |

CMF: Cussons morning fresh, UIB: Un-inoculated broth

**Foaming capacity of test solutions:** The foaming capacity of the test solutions is presented in Table 5. From the Table 5, it can be seen that the mean foaming capacity of the broth culture was 58.83% and the detergent solution having the highest foaming capacity (82.6%) was Ariel.

**Oil spread diameter of test solutions:** The oil spread diameter of the test solutions is presented in Table 6. From the Table 6, it can be seen that the oil spread diameter of the broth culture was about 15 mm and the detergent solution having the largest oil spread diameter (70 mm) was cussons morning fresh. On determination of oil spread diameter, when a drop of the broth culture was placed on the oil films, a clearing of about 5 mm in diameter was noticed. After about 40 sec, a spontaneous increase in the size of the clearing occurred-like a bursting phenomenon. In the case of the detergent solutions, a large clearing occurred immediately the drops were applied. In both cases, the enlarged clearing reduced in size gradually till it disappeared. As a result of this, the measured oil spread diameter was a quick estimated measurement taken.

**Drop collapse activity of test solutions:** The drop collapse activity of the broth culture and detergent solutions was positive, while that of the un-inoculated medium and distilled water was negative.

## DISCUSSION

Chemically synthesized surfactants are usually used as positive controls during assessing for biosurfactant production and for comparison purposes when assessing for the activity of biosurfactant been produced[8-10]. Chemically synthesized surfactants are however expensive and not readily available in laboratories. The use of readily available detergents containing surfactants was thus investigated for use as positive controls in the assessment of biosurfactant production. Three detergents containing surfactants were used for this study; Ariel, Cussons morning fresh and Klin. Solutions (1% w/v or 1% v/v) of the detergents were subjected to the various screening methods used in the assessment of biosurfactant production. This was done so as to determine if the detergent solutions could be used as positive controls during the assessment of biosurfactant production and also to determine which of them would be more appropriate for use as a positive control.

In the assessment of surfactant activity of the detergent solutions, all the detergent solutions were positive for drop collapse activity and they all had low surface tension. Cussons Morning Fresh (CMF) solution had the lowest surface tension (17.84±0.34 mN $m^{-1}$) and largest oil spread diameter (70 mm), while Ariel (ARL) had the highest foaming capacity (82.60±3.49%). The extent of reduction of surface tension is one of the most common measured properties of surfactant solutions[21]. The surface tensions of solutions of some chemically synthesized surfactant (2% conc.) are as follows: Sodium dodecyl sulphate (SDS) -28.76 mN $m^{-1}$, Triton® X-100 (TX)-29.95 mN $m^{-1}$, Cocamidopropyl betaine (CB)- 29.93 mN $m^{-1}$ [22]. On comparing the extent of reduction of surface tension of the solutions of the detergents used in this study with that of SDS, TX and CB, it was seen that the solutions of the detergents had lower surface tension values. The solutions of the detergents can thus be used in place of chemically synthesized surfactants as positive controls in the assessment for the presence of biosurfactant in a broth culture.

The ARL had the highest foaming capacity; however there was not much difference between its foaming capacity and that of CMF. On the other hand, though the extent of reduction of surface tension obtained with CMF was more pronounced than that obtained using ARL, ARL solution still

had a value lower than the chemically synthesized surfactants mentioned above. Klin solution had the lowest oil spread activity and though its surface tension was lower than that of ARL, the difference was minimal. Thus solutions of ARL and CMF are preferable than solution of Klin for use as positive controls in assessing biosurfactant production by an investigated microorganism.

The reaction pattern of the *Pseudomonas fluorescens* isolate obtained for this study was confirmed to be similar to the reaction pattern usually exhibited by *Pseudomonas* species to the physicochemical/biochemical tests used. The confirmation was arrived at by comparing the results with information in selected literature and texts[23-26]. *Pseudomonas fluorescens* is a known biosurfactant producer[27-29]. The broth culture of the bacterium in this study at the end of the operation period for biosurfactant production was positive for drop collapse activity, with surface tension of 34.63±3.48 mN $m^{-1}$, foaming capacity of 58.83±4.66% and oil spread diameter of 15±5 mm. In a previous study carried out by Peekate and Abu[30], the surface tension of a broth culture of *P. fluorescens* in which biosurfactant was produced was reduced to 30.64 mN $m^{-1}$ and the oil spread diameter ranged from 30-40 mm. A solution of the positive control (Tipol®) used in that study had a surface tension of 29.55 mN $m^{-1}$ and an oil spread diameter of 58 mm. In another study on biosurfactant production by *P. fluorescens*, surface tension values of 30 and 35 dyne $cm^{-1}$ were obtained for cell free broth culture and immobilized cell culture, respectively[27]. Note: dyne $cm^{-1}$ is equivalent to mN $m^{-1}$. A surface tension of 29.0 mN $m^{-1}$ has been obtained for cell free culture containing biosurfactant produced by *P. fluorescens* where glycerol was used as the carbon source[28]. It can be observed that the surface tension obtained in this study for the broth culture of *P. fluorescens* in which biosurfactant was produced is almost similar to those obtained in the other research works. However, the oil spread diameter obtained in this study and in Peekate and Abu[30] were different. This could be attributed to the difference in the quantity of diesel oil used in the oil spread determination test. Also the surface tension of the positive controls used in this study was lower than that of Tipol® used in Peekate and Abu[30] but the oil spread diameter of one of the positive control (Ariel) tallied with that of the Tipol®.

## CONCLUSION

Readily available and relatively inexpensive surfactant containing detergents produced industrially in Nigeria including cussons morning fresh, Ariel and Klin were investigated in this study for use as positive control in assessing biosurfactant production by micro-organisms. The results obtained from this research shows that the detergents can be used in place of chemically synthesized surfactants as positive controls during assessment for biosurfactant production by an investigated micro-organism.

## SIGNIFICANCE STATEMENT

Chemically synthesized surfactants used as positive controls in assessing biosurfactant production by micro-organisms are relatively expensive and not readily available in Microbiology laboratories. Readily available inexpensive detergents containing surfactants have been shown in this research work to serve as substitutes for chemically synthesized surfactants for use as positive controls during the assessment of biosurfactant production by micro-organisms.

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