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Antiseptic Efficacy of A Soap Made from Biosurfactants Isolated from *Bacillus* and *Lactobacillus* against Pathogenic Bacteria

Frédéric Yannick Okouakoua[1,2], Christian Aimé Kayath[1,2*], Nicaise Saturnin Mokémiabeka[1,2], Varelle Bervanie Ngala Elenga[1,2], Digne Nedjea N'goma-Mona[1,2], Ndelani Nkalla Lambi[1,2], Sandra Paola Elenga Wilson[1,2], Christ Dieuveil Bayakissa Malanda[1,2], Rodinet Tsana[1,2], Junior Patrick Sergy Bissoko[1,2], Moïse Doria Kaya-Ongoto[1,2], Duchel Jeanedvi Kinouani Kinavouidi[1,2], Etienne Nguimbi[1,2]

[1]Laboratoire de Biologie Cellulaire et Moléculaire (BCM), Faculté des Sciences et Techniques, Université Marien N'gouabi, Brazzaville, Congo
[2]Institut National de Recherche en Sciences Exactes et Naturelles (IRSEN), Avenue de l'Auberge Gascogne, Brazzaville, Congo
Email: *chriskayath@yahoo.fr

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Abstract

The aim of our study was to use a biosurfactant produced by *Bacillus* and *Lactobacillus* isolates as an antiseptic in the formulation of local soap. A total of 60 isolates were characterized by microbiological techniques (30 *Bacillus* and 30 *Lactobacillus*) and the ability to produce biosurfactants was demonstrated by a hydrocarbon emulsification index (E24). The emulsification indexes (E24) varied from 9% to 100% for *Bacillus* and from 33% to 100% for *Lactobacillus* as well. The antagonistic assay showed that biosurfactants were able to inhibit the formation of biofilms and growth of pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhirium*, *Shigella boydii* and *Proteus mirabilis*. The biosurfactant consortium (BioC) from *Bacillus* consortium and from *Lactobacillus* was able to inhibit biofilm formation and the pathogens growth. The BioC was stable to alkaline pH and the temperatures stability of Biosurfactant was ranging from 50˚C to 90˚C. The soap was made by the cold saponification process using one biosurfactant consortium formulated. This soap has a pH of 10 and showed good cleaning power and good foam stability. Similarly, the soap showed good antiseptic power and disinfection power against all pathogens tested. Handwashing is critical to preventing disease transmission. The persistence of pathogens in waste water was evaluated. The BioS produced showed good disinfection power against all pathogens tested. The valor of reduction on the hands and in the waste water was significantly more than compared to the control soaps used. This soap could be used in the prevention, fighting, and treatment of bacterial and viral infections.

Keywords

Antiseptic, Soap, Biosurfactants, *Bacillus*, *Lactobacillus*, Disinfection, *Pathogens bacteria*

1. Introduction

Biological surfactants are part of key biomolecules that mark the 21st century, with many scientific investigations as to their interest in the industrial world [1]. Amphiphilic microbial molecules with hydrophilic and hydrophobic moieties that partition at liquid/liquid, liquid/gas, or liquid/solid interfaces are known as biosurfactants [2]. Biosurfactants are produced by extensive variations of diverse microorganisms and possess structures with different chemical and surface properties [3]. Such characteristics allow these biomolecules to play a key role in emulsification, foam formation, detergency, and dispersal, which are desirable qualities in different industries [2] [4]. Biosurfactant production is considered one of the key technologies for development in the 21st century [5]. Biodegradability and low toxicity have led to the intensification of scientific studies on a wide range of industrial applications for biosurfactants in the field of bioremediation as well as the petroleum, food processing, health, chemical, agricultural, and cosmetic industries [1] [2].

In addition to their wetting, emulsification, surface tension reduction, and detergency functions [5], biosurfactants have several potential advantages over their synthetic counterparts. These potential advantages include lower toxicity, biodegradability, compatibility with the human skin, stability at extreme conditions (pH, temperature, salinity), and production from cheaper and renewable resources. For these reasons, biosurfactants have received considerable attention in recent decades in the food, environmental protection, textile, oil, agriculture, cosmetic, medical, and pharmaceutical industries [3] [6]. In the pharmaceutical and biomedical industries, thanks to their antibacterial, antifungal and antiviral properties [7], some biosurfactants are used as potential agents in the treatment and fight against many diseases [1] [8] [9]. Likewise, due to multidrug resistance linked to antibiotics and the toxicity of chemical agents, certain biosurfactants have proven to be an essential alternative to synthetic drugs and antimicrobial agents [1]. This is the case of lipopeptides such as surfactin, iturin, and lichenisin produced by *B. subtilis* and *B. licheniformis*, which act as antiviral, antibacterial, and antitumor agents [2] [10]. Surlactin produced by the genus *Lactobacillus*, is known to be used as antimicrobials and antiadhesives [9].

Many reviews have focused on biosurfactant production, their characterization and application in the fields of environmental protection, oil refinery, food and agriculture [1] [2]. However, to our knowledge, a few studies have been assessed the potential application of microbial biosurfactants in the pharmaceutical, cosmetic, and personal care industries [1]. In cosmetics, some

biosurfactants are already used due to their low toxicity and biodegradability in the formulation of some lotions and beauty products [3]. Although biosurfactants are antimicrobial molecules and stable under extreme conditions [8], few studies highlight the use of biosurfactants as antiseptics in the formulation of medicinal soaps.

2. Materiel and Methods

2.1. Foods Sampling

The representative foods and beverages used in this study are: Nsamba (palm wine), Ntoba mbodi (fermented cassava leaves), mbamvou (banana wine), poto-poto (fermented maize), bikedi (fermented roots cassava), mokiki (fermented roots cassava tamised), pili-pili (fermented caspis fructens fruits) and Wilde honey. Samples were collected in five markets in different districts (Bacongo, Talangai, NKombo, Makélekéle, and Moungali).

2.2. Isolation

10 g of each collected sample from fermented foods and beverages was aseptically sampled into a sterile falcon tube. Using sterile physiologic water (0, 85%), the sample was homogenized and distributed into ten flacon sterile tubes. Dilutions were done and the bacterial suspension was streaked on Mossel agar medium supplemented with 4.2 mL of polymyxin B for the growth of *Bacillus* species and on MRS (De Man Rogosa Sharpe) for isolement of Lactic acid bacteria. Enumeration of colonies was done in triplicate on each medium. The plates were incubated at 37˚C for 24 h to 48˚C in aerobic and anaerobic conditions in the incubator.

2.3. Characterization of Strains

In order to characterize the isolates, most cultural and biochemical tests were performed by using microbiological and biochemical standard methods. Each colony from Mossel and MRS, of different appearances, was separately isolated. Purification of the isolates was rigorously done by successive and alternating subcultures. The shape, size, and color of bacterial colonies were studied. The morphological characterization has been done using a light microscope (OPTIKA, Italie). The Gram status of the bacterial isolates has been determined using 3% potassium hydroxide (KOH). A sporulation test was conducted to determine the ability of isolates to form endospores. Oxidase and catalase tests were also conducted for all bacterial strains. Further, all these purified isolated cultures were stored at - 20˚C in Luria Broth and MRS broth containing 20% (v/v) glycerol.

3. DNA Technology Identification

3.1. Genomic DNA Extraction

The extraction and purification of isolate genomic DNA were performed according to the NucleoSpin Microbial DNA (Macherey-NAGEL) kit. Isolates

were grown in 5 mL of LB broth for 24 h at 37˚C with stirring. The DNA purity was assessed by electrophoresis on an agarose gel and by the ratio of optical densities of 260/280nm. 5 µL of each amplification product was mixed with 2 µL of loading buffer (BIOKE). Mixtures were subjected to electrophoresis on 1% agarose gel (w/v). The 10 kb 2-Log (BIOKE) was used as a molecular weight marker.

3.2. *Bacillus* spp. Molecular Identification

The identification of *Bacillus* species was done by the method developed by Kaya Ongoto *et al*. 2019. *B. amyloliquefaciens*, *B. subtilis*, *B. pumilus*, *B. licheniformis*, *B. altitudinis*, *B. mojavensis*, *B. safensis*, and *B. atrophaeus* have all been identified through amplification of the *fibE* gene, which encodes the fibrinolytic enzyme.

3.3. *Lactobacillus* spp. Molecular Identification

The method developed by Song *et al*. (2006) was used in this study with a few modifications. For example, 50 µL of a reaction solution for PCR amplification was composed of 1 U Taq DNA polymerase (Promega Corporation, Madison, WI, USA), 25 µL of Master Mix (dNTPs, MgCl2, enzyme buffer), 10 pmol of primer (**Table 1**) mix comprising one portion of each primer, and 2 µL of template DNA. PCR was carried out for 35 cycles. Each cycle consisted of 95˚C for 20 s for denaturation; annealing and extension were performed for 2 min at 55˚C for multiplex PCR-G, 68˚C for multiplex PCR II-1, 65˚C for multiplex PCR II2, 62˚C for multiplex PCR III, and 60˚C for multiplex PCR IV. A cycle of 74˚C for 5 min was added to the eternal extension. Amplicons were analyzed by electrophoresis on a 2% agarose gel followed by ethidium bromide staining.

3.4. Biosurfactant Production Assay

The emulsifying activity of a biosurfactant is its capability of retaining the emulsion of hydrocarbons or oils in water. 5 mL of a washing cell and 5 mL of acellular supernatant of each isolate were poured into a test tube containing 5 mL (v/v) of gasoline or fuel. The mixture was vigorously shaken for 3 minutes using a vortex mixer (VELP Scientifica, Italy). The tubes were then incubated at room temperature for 24 hours. The height of the emulsion layer and the total height of the mixture were then measured. All the experiments were performed in triplicates and the emulsification index (E24%) was calculated using the standard formula E24% (He/Ht) 100, with He being the emulsion height, Ht the total height of the mixture, and E24% the emulsification percentage after 24 h.

3.5. Biosurfactant Extraction Using Chloroform

The extracellular biosurfactant was extracted by the method developed by Kinouani Kinavouidi *et al*. 2020 has been performed in this study [11] [12].

Briefly one volume of supernatant was added with an equal volume of chloroform (v/v). The mixture is strongly agitated by a vortex. After centrifugation at

Table 1. Primers used in this study in terms of identification of *Lactobacillus* strains.

Oligos names	Sequences 5'…3'	size pb	Groupes of species
Ldel-7 (F)	ACAGATGGATGGAGAGCAGA	450 pb	Groupe II-1
Lac-2 (R)	CCTCTTGCTCGCCGCTACT		
LU-5 (F)	CTAGCGGGTGCGACTTTGTT	400 pb	Groupe II-2
Lac-2 (R)	CCTCTTGCTCGCCGCTACT		
LU-3P (F)	AAACCGAGAACACCGCGTT	350 pb	Groupe III
Lac-2 (R)	CCTCTTGCTCGCCGCTACT		
LU-1P (F)	ATTGTAGAGCGACCGAGAAG	300 pb	Groupe IV
Lac-2 (R)	CCTCTTGCTCGCCGCTACT		
Oligos names	Sequences	species	Target species
LU-5 (F)	CTAGCGGGTGCGACTTTGTT	113 pb	*L. rhamnosus*
RhaII (R)	GCGATGCGAATTTCTATTATT		
Laci-1 (F)	TGCAAGTGGTAGCGTAAGC	210 pb	*L. acidophilus*
23-10C (R)	CCTTTCCCTCACGGTACTG		
Ljen-3 (F)	AAGAAGGCACTGAGTACGGA	700 pb	*L. jensenii*
23-10C (R)	CCTTTCCCTCACGGTACTG		
Lcri-3 (F)	AGGATATGGAGAGCAGGAAT	522 pb	*L. crispatus*
Lcri-2 (R)	CAACTATCTCTTACACTGCC		
Lgas-3 (F)	AGCGACCGAGAAGAGAGGAGA	360 pb	*L. gasseri*
Lgas-2 (R)	TGCTATCGCTTCAAGTGCTT		
Lfer-3 (F)	ACTAACTTGACTGATCTACGA	192 pb	*L. fermentum*
Lfer-4 (R)	TTCACTGCTCAAGTAATCATC		
Lpla-3 (F)	ATTCATAGTCTAGTTGGAGGT	350 pb	*L. plantarum*
Lpla-2 (R)	CCTGAACTGAGAGAATTTGA		
Lreu-1 (F)	CAGACAATCTTTGATTGTTTAG	303 pb	*L. reuteri*
Lreu-4 (R)	GCTTGTTGGTTTGGGCTCTTC		
Lsal-1 (F)	AATCGCTAAACTCATAACCT	411 pb	*L. salivarius*
Lsal-2 (R)	CACTCTCTTTGGCTAATCTT		
LU-5 (F)	CTAGCGGGTGCGACTTTGTT	411 pb	*L. paracasei*
Lpar-4 (R)	GGCCAGCTATGTATTCACTGA		

6000 rpm for 10 min, the non-aqueous phase is recovered. The solvent was allowed to evaporate completely only without heating above 40˚C. The residue is dissolved in a PBS buffer. In terms of ammonium sulfate an overnight culture has been fuged at 13,000 rpm for 15 minutes to separate supernatant and pellet.

Then 15 mL of supernatant were mixed with ammonium sulfate (80%) for 15 minutes. And finally this has been incubated in overnight. Mix has been fuged at 6000 rpm for 30 minutes. Pelet has been hommogenized by using PBS buffer. For both extractions (chloroform and ammonium sulfate) the emulsifying activity is tested in comparison with the supernatant at the start.

3.6. Antibacterial Assay of the Biosurfactants

The antibacterial activity of the produced biosurfactant extract was tested against seven pathogenic strains (*Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella tiphimurium*, *Shigella flexineri* 5a M90T, and *Bacillus cereus*) as recently demonstrated by Bokamba Moukala *et al.* 2020. The antimicrobial activity was evaluated by the agar well diffusion method with some modifications. The antimicrobial activity was done on plate count agar. Wells were aseptically prepared in the Petri dish. The microorganism to be tested was inoculated into the gel. A volume of 75 µl of the biosurfactant extract was deposited onto wells. After an incubation period of 24 hours at 37˚C, the diameter of the inhibition zones was measured. The average of the three measurements was taken to ensure that the results were reproducible. The specific pathogen strain antibiotics were used as positive controls, and the B4 negative biosurfactant producing strain was used as a negative control.

3.7. Antiadhesive Assay of the Biosurfactants

The anti-adhesive activity of the crude biosurfactant extracted from bacilli and lactobacilli isolates against seven microbial strains (*Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella tiphirium*, *Shigella boydii* 5a M90T, and *Bacillus cereus*) was evaluated according to the procedure described by Goma, 2013 with some modifications. Briefly, the wells of a sterile 96-well flat-bottomed polystyrene microtiter plate were filled with 30 µl of the crude biosurfactant. Control wells contained PBS (1X) buffer only. An aliquot of 200 µl of a washed bacterial suspension (108 CFU/mL) was added and incubated in the wells for 24 h at 37˚C. Unattached microorganisms were removed by washing the wells three times with PBS. The adherent microorganisms were fixed with 200 µl of 30% methanol per well, and after 15 min, the plates were emptied and left to dry. Then the plates were stained for 5 min with 200 µl of 2% crystal violet used for Gram staining per well. Excess stains were rinsed out by placing the plate under running tap water. Subsequently, the plates were air dried, and the activity of each biosurfactant was analyzed. The average of the three measurements was taken to ensure that the results were reproducible [13].

3.8. Elaboration of Biosurfactant Soap

- Constitution of consortium.

  During this study, two consortia of biosurfactants was established using 25 mg

of each biosurfactant extract. Con1 was constituted to *Bacillus* strains biosurfactants (FO1, FO2, FO3, FO4, FO5 and FO6) and Con2 contained Lactobacillus strains biosurfactants (FO7, FO8, FO9, FO10, FO11, FO12, FO13, FO14).

The antimicrobial activity, antiadhesive activity and stability of the consortium at pH (10), temperature (50˚C and 90˚C) and during the time were carried out.

- Cold saponification [14]

61.25 g of drinking water was weighed and put in a clean basin, and 23.2 g of sodium hydroxide was subsequently added. The mixture was homogenized using a whisk until completely dissolved. Then this mixture was cooled at room temperature until the temperature was pleasant to the touch. While mixing, 100 g of peanut oil and 75 g of olive oil were successively added. The mixture was subjected to a strong homogenization, always using a whip until the appearance of the trace. At this stage, 10 mL of the consortium of biosurfactants was added. This mixture was molded and dried at room temperature until it solidified, then it was unmolded and stored to carry out the rest of the work. In the study we used four total soaps (made soap, ordinary soap, acid salicylic soap, and one commercialized medicated soap Santex).

Physicochemical Properties of Soap

- **pH**

In essay tubs, 2 g of each soap were measured and transferred separately in 20 mL of distilled water. Melanges were homogenised and allowed to stay for 12 hours. The pH was determined using a pH-mètre. [15].

- **Foaming stability**

1% of each soap solution was prepared and transferred separately in the essay. Each solution was vigorously homogenised for 1 minute and kept for 5 minutes. Finally, foaming height was measured. Foaming stability has been evaluated for 20 min. [15].

- **Cleaning propeties**

One drop of diesel, blood, and bile salt were separately deposed on a filter paper disc. After drying, discs were placed in 1% of each soap solution. The melange was homogenised and incubated at room temperature for 2 minutes. Each disc was retired and rinsed in water, and after drying the disc, the cleaning power of each soap was appreciated [15]. The average of the three measurements was taken to ensure that the results were reproducible.

3.9. Antibacterial Assay of the Soap

The effectiveness of the soap developed has been tested on seven (7) pathogens. To do this, 5 g of each soap was individually transferred into different bottles containing 10 mL of peptone water. The mixtures thus obtained were vigorously homogenized. Then, series of double dilutions were carried out to obtain, respectively, 500 mg, 250 mg, and 125 mg of the concentration for each soap. 75 µL of each concentration was introduced into the wells of an agar previously in-

oculated with the pathogen to be tested. The dishes were left for 1 hour at room temperature to allow diffusion and then incubated at 37˚C. for 24 hours, and the zones of inhibition were measured in mm [16]. A total of four (4) soaps were used (the elaborate soap, the ordinary soap, the salicylic acid soap, and the Santex soap).

4. Hand Decontamination Test

To test the disinfection ability of elaborated soap, hand decontamination tests were conducted on seven reference strains (*P. mirabilis*, *P. aeruginosa*, *S. aureus*, *E. coli*, *S. tiphirium*, *S. boydi* 5a M90T, *and B. cereus*) [17].

- **Prepare Organisms**

Prepare the organism to be used for testing at a concentration greater than 10 × 10^8 CFU/mL for bacteria.

One day before the start of the experiment, pick a single colony from the plate and inoculate 10 mL of LB broth using a sterile loop. Incubate overnight at 37˚C with shaking. In the morning of the experiment, start a fresh culture by adding 1 mL of the overnight culture to 20 mL of fresh LB broth. Incubate for approximately 2.5 h to achieve a cell density greater than 108 CFU/mL.

Use a spectrophotometer to estimate the concentration of the culture.

- **Preparing Volunteers for the Experiment**

Before beginning testing, confirm that the volunteers remain eligible by verbally verifying that they adhered to the seven days, antimicrobial washout period and by visually confirming that they have not developed any breaks or abnormalities on their skin. Using a random number generator, assign each volunteer to use either their right or left hand for sampling on this day of testing. Assign an order in which the handwashing conditions will be performed. Perform a "cleansing wash" once at the start of testing to strip the skin of dirt and oils so that each subsequent test is conducted under equivalent conditions.

To do a cleansing wash, run through each step of the experiment using a blank inoculate (LB broth or PBS only) and take a sample without handwashing.

- **Experimental Procedure**

To test the pH of the skin of each volunteer (to control for variation), place a flat-tipped skin pH probe on the palmar surface of the skin and the web space between the pointer and middle finger. Ensure that the electrode is flat against the skin. Record the pH reading.

- **Spike the hands**.

Have the volunteers cup both hands together. Spike the hands with 1.5 mL of the inoculate by carefully pipetting 750 µL slowly into each palm. Have the volunteers gently rub their hands together until all surfaces of the hands are coated with the inoculate, while subjecting the hands to as little friction as possible.

Have the volunteers hold their hands still and away from their bodies for an additional 30 seconds to allow the inoculate to dry. The inoculate may not dry completely.

- **Wash the hands**

For all the following wash steps, capture the rinse water from the hands in a large sample collection bag.

After inoculation, wash the hands with the next method in the designated order.

For Control A, do not perform a handwashing step and move directly to hand rinse.

For Control B, wash the hands with only sterile water at room temperature through a funnel with a known flow rate. For handwashing with soap, wet the hands with 10 mL of sterile water. Have the volunteers lather their hands with soap and then rub their hands together for an additional 20 seconds. Rinse their hands by pouring 500 mL of sterile water at room temperature.

- **Hand rinse using a modified glove juice procedure**

After handwashing, immediately place each volunteer's hand (*i.e.*, the hand (right or left) selected for testing in into a sample bag containing 75 mL of eluent (PBS) up to the wrist. Hold the top of the bag tightly around the wrist. Have the volunteers gently rub their hands in the solution for 30 seconds, taking care to reach in between the fingers and underneath the fingernails. Massage the hand from outside the bag gently for 30 seconds to ensure that the entire hand is rinsed thoroughly in the eluent, all the way up to the wrist. Seal the bag and process it according to the appropriate assay.

- **Decontamination**.

Before repeating the process with each handwashing method, have the volunteers wash their hands thoroughly in a sink with soap and warm water. Spray the volunteers' hands with 70% ethanol until they are coated on both sides. Allow them to dry. Repeat all steps for each handwashing condition, only using the hand randomly selected.

- **Quantification**

The quantification of each oeganism was done by simple enumeration. After counting the plates, record the estimated CFU/mL for each test for the analyses.

- **Analysis**

The log reduction value of organisms on the hands, for each organism and for each subject and handwashing method, was calculated. For handwashing efficacy, compare the concentration of bacteria in each handwashing sample to control A (no handwashing). For rinse water persistence, compare each rinse water sample to control B (washing with water only). Use the following standard formula:

$$\text{Log Reduction}\left(\text{handwashing}\right)=\log 10\left(\frac{\text{CFU/mL withou thandwashing}}{\text{CFU/mL selected handwashing method}}\right)$$

$$\text{Log Reduction}\left(\text{rinse water}\right)=\log 10\left(\frac{\text{CFU/mL handwashing with wateronly}}{\text{CFU/mL selected handwashing method}}\right)$$

One-way repeated measures analysis of variance (ANOVA) to assess the significant differences in the calculated log reduction values between handwashing

methods and a post-hoc Tukey's HSD test for significant models to pairwise assess significant differences ($p < 0.05$) were used.

5. Results

5.1. Isolation and Characterization of Strains

Isolation on Mossel and MRS media has been used to highlight the presence of bacteria from the genera *Bacillus* and *Lactobacillus*. Thus, 60 isolates were obtained, screened, and purified from raw material: palm wine (5), Ntoba Mbodi (5), Mbamvu(4), honey (9), Bikedi (4), Mokiki (9), Pili pili (9), Ginger wine (5), and Poto poto(10). These isolates have been the subject of various microbiological issues, biochemistry, and molecular biology. The purified isolates were macroscopically and microscopically characterized (data not shown). Sixty (60) isolates were characterized 45 of the 60 isolates are round, and 15 are oval. All isolates retained are gram-positive bacteria. All isolates are sticks. Of the 100% isolates, 50% are mobile and 50% are not. 50% of the isolates are catalase positive and 50% are catalase negative. 20% have a dry texture against 60% creamy and 20% pasty. According to Bergey's manual, the morphological and biochemical characterization of the isolates, 50 were studied and were suspected to be *Bacillus* (30) and *Lactobacillus* (30) species. The sixty isolates were used for molecular identification.

5.2. DNA Technology Identification

The purified isolates were the subject of genomic DNA extraction. Six (6) pairs of primers targeting genes encoding for fibrinolytic enzyme amplified and discriminated against *Bacillus* species, while twenty-two (22) ITS (ARNr 16S-23S) primers amplified and discriminated against *Lactobacillus* species. 870 PCRs were performed with all primer pairs used. As a result, amplifications enable the identification of *Bacillus* isolates FO1 and FO2, which are assigned to *B. safensis*; FO3, FO4, FO5, and FO6 were assigned to *B. subtilis* (**Table 2**).

Using the ITS regions, FO7, FO8, FO9, FO10, FO11, FO12, FO13 and F014 isolates were identified as *L. plantarum*; FO15 and FO16 as *L. paracasei*; FO17 as *L. gasseri*; and FO18 as *L. salivarius* (**Table 2**).

5.3. Biosurfactant Production Assay

To highlight the production of biosurfactant, we performed the emulsification test on strains by using acellular supernatant. This study shows that all 18 strains produced biosurfactants with an emulsification index (EI24) ranging from 68.28% to 94.11% after 24 hours (**Figure 1**).

5.4. Extraction of Biosurfactant Assay

All tested strains produced biosurfactants when they were used for extraction. All strains showed a precipitate at the bottom of the tube (**Figure 2**) and the emulsification index after extraction has been carried on EI24. The extract, after

Table 2. PCR for *Lactobacillus* and *Bacillus* Identification.

Isolates	Origin samples	Strains
FO1	Ntoba Mbodi	*Bacillus. sanfensis*
FO2	Ginger wine	
FO3	Ntoba Mbodi	*Bacillus. subtilis*
FO4	Mokiki	
FO5	honey	
FO6	palm wine	
FO7	Mbamvu	*Lactobacillus plantarum*
FO8	Poto poto	
FO9	Bikedi	
FO10	Mbamvu	
FO11	Mbamvu	
FO12	Pili pili	
FO13	Pili pili	
FO14	Ntoba Mbodi	
FO15	Pili pili	*Lactobacillus. paracasei*
FO16	Poto poto	
FO17	Poto poto	*Lactobacillus gasseri*
FO18	Poto poto	*Lactobacillus salivarius*

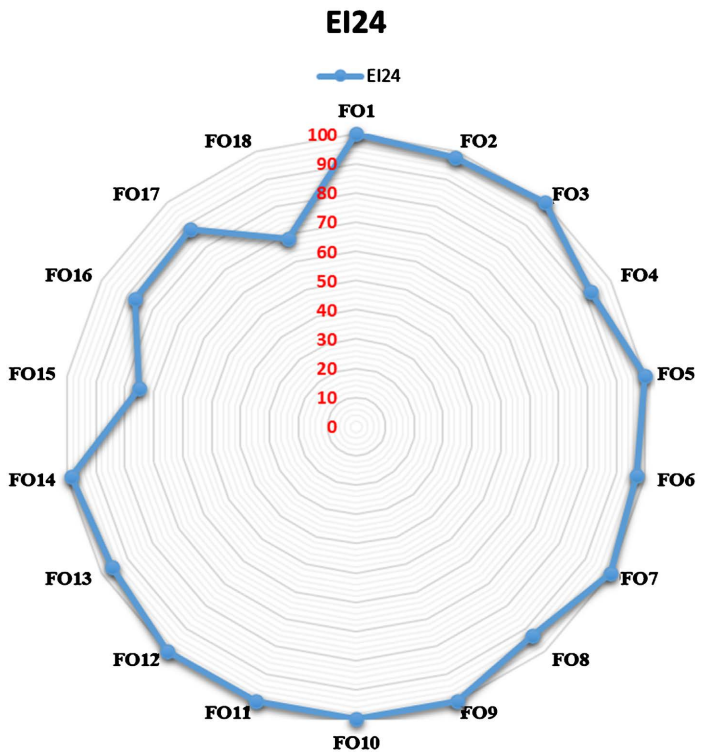


Figure 1. Emulsification index of petroleum hydrocarbon by strains. FO1, FO2, FO3, FO4 FO5 and FO6 (*Bacillus* strains); FO7, FO8, FO9, FO10, FO11, FO12, FO13, FO14, FO15 FO16, FO17 and FO18 (*Lactobacillus* strains).

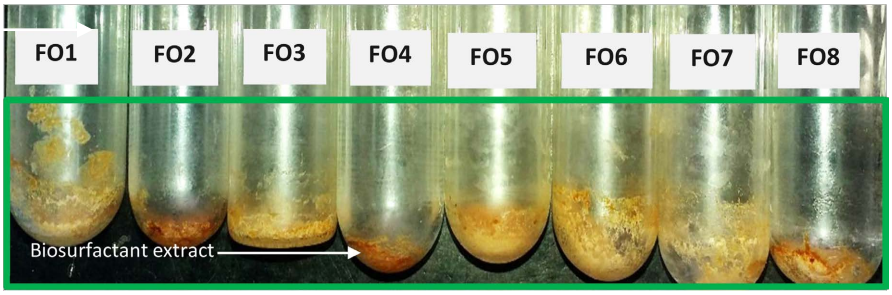


Figure 2. Crude biosurfactant extract after evaporation extracted with chloroform. FO1, FO2, FO3, FO4, FO5, FO6, FO7, and FO8 represented bacterial strains used.

precipitation, suspended in PBS, was able to emulsify gasoline with 100% of EI24 (**Figure 2**).

5.5. Antibacterial Assay of the Biosurfactants

The biosurfactants extracted from strains allowed the inhibition tests to be performed against seven pathogenic strains, both gram positive strains (*S. aureus* and *B. cereus*) and Gram negative strains (*Proteus mirabilis*, *P. aeruginosa*, *E. coli*, *S. typhimirium*, and *S. flexneri* 5a M90T). In addition, the diameters of inhibition of all isolates have been measured. We discovered that all biosurfactant extracts inhibited the target pathogen strains (**Figure 3**). On all selected strains, the inhibition diameter ranged from 3 to 30 mm. The high activity was found with FO1, FO2, FO4, FO6, FO11, FO12, and FO14 against *S. tiphimirium*, *P. mirabilis*, and *B. cereus*. FO3, FO15, FO16, FO7, FO9, FO8 and FO13 showed respectively a good activity against *S. flexneri* 5a M90T, *S. aureus*, *P. aeruginosa*, and *E. coli*. It's important to notice that all biosurfactants obtained were able to inhibit the ability of pathogens strains to adhere on polystyrene plaque using by crystal violet essay.

The diameters of inhibitions obtained made it possible to mount the graph below (**Figure 4**).

The consortiums have been tested. We found that the consortia inhibited the growth of pathogenic bacteria. However, the inhibition was much better when the biosurfactants were combined. (**Figure 5**).

5.6. Anti-Adhesive Assay of the Biosurfactants

In order to test the anti-adhesive activity of the consortium on their ability to form biofilms, the crystal violet assay has been experimented (**Figure 6**). As result biosurfactant consortia were able to inhibit the biofilms formation. This is include consortium 1 (including B. subtilis and B. safensis) (**Figure 6(a)**) and consortium 2 (*L. plantarum, L. gasseri, L. paracasei,* and *L. salivarius*) (**Figure 6(b)**).

5.7. Elaboration of Biosurfactant Soap

In this study, soap was made using a cold saponification process mixed first with

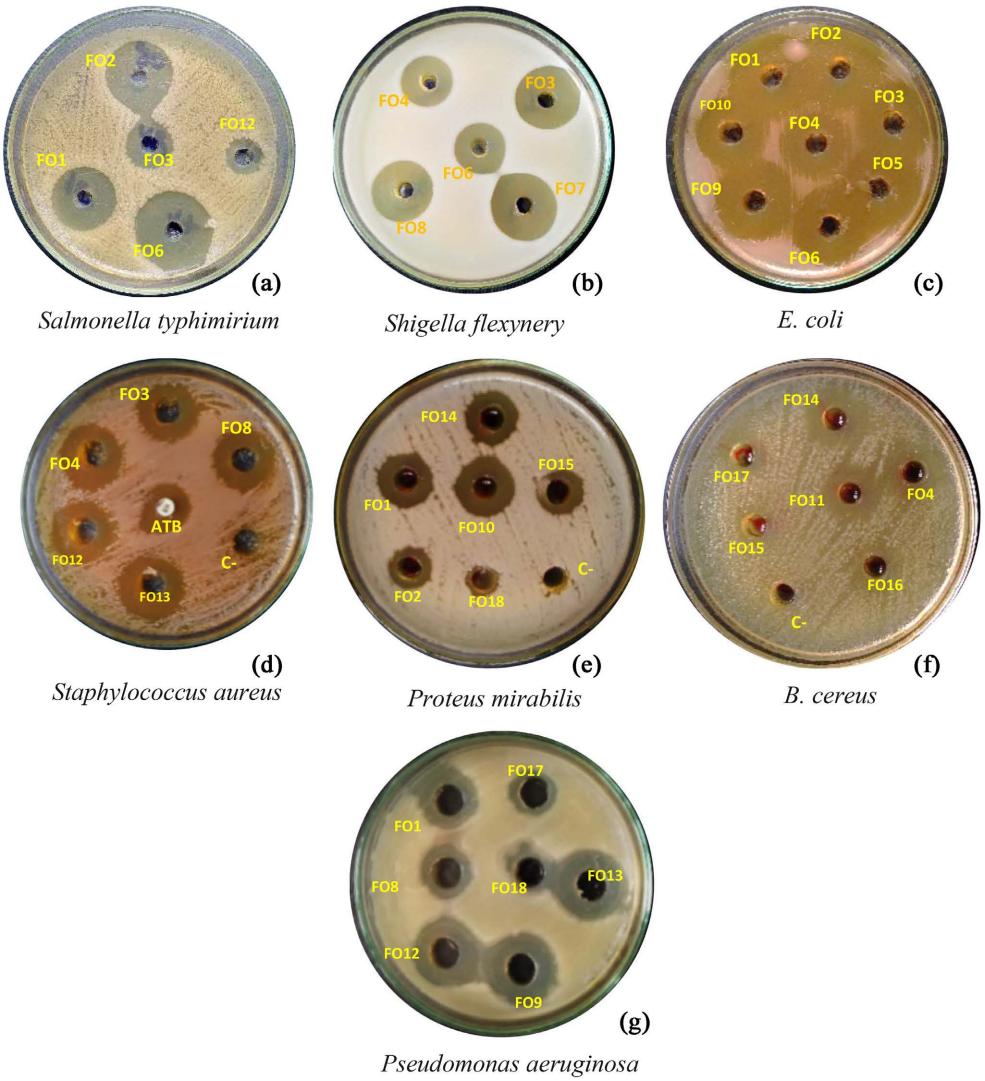


Figure 3. Inhibition profile of biosurfactant (a) *Salmonella typhimirium*, (b) *Shigella flexineri* 5a M90T, (c) *E. coli*, (d) *Staphylococcus aureus*, (e) *Proteus mirabilis*, (f) *B. cereus*, g: *Pseudomonas aeruginosa*. FO1, FO2 (*Bacillus sanfensis* strains) and FO3, FO4, FO5 and FO6 (*Bacillus* subtilis strains), FO7, FO8, FO9, FO10, FO11, FO12, FO13 and FO14 (*Lactobacillus plantarum*), FO15 and FO16 (*Lactobacillus paracasei*), FO17 (*Lactobacillus gasseri*) and FO18 (*Lactobacillus salivarius*).

consortia. One biosurfactant soap has been formulated using a biosurfactant consortium (Cons 1 and Cons 2) (**Figure 7(a)**), Some properties of formulated soaps, like pH, stability of foaming and cleaning ability, were carried. The pH of formulated soaps and soaps controls was 10 (**Figure 7**). Biosurfactants consortium soaps (BCS) has been demonstrated high aptitude to removal salt bile, blood, and diesel oil. In addition, BCS was compared with salicylic soap and ordinary soap, BCS was the most interesting in forming soap suds (Data not shown).

The biosurfactant consortium showed considerable activity against all target strains, and the inhibition halo diameters ranged from 5 to 17 mm. The biosurfactant consortium used showed good stability to pH, temperature and during

the store time ($p > 0.05$). All biosurfactants conserved all biologic activities after all treatment. A biosurfactant consortium demonstrated potent anti-cell adhesion activity against *E. coli*, *B. cereus*, *S. aureus*, *P. mirabilis*, *S. typhimurium*, and *S. flexineri* (*Data no shown*).

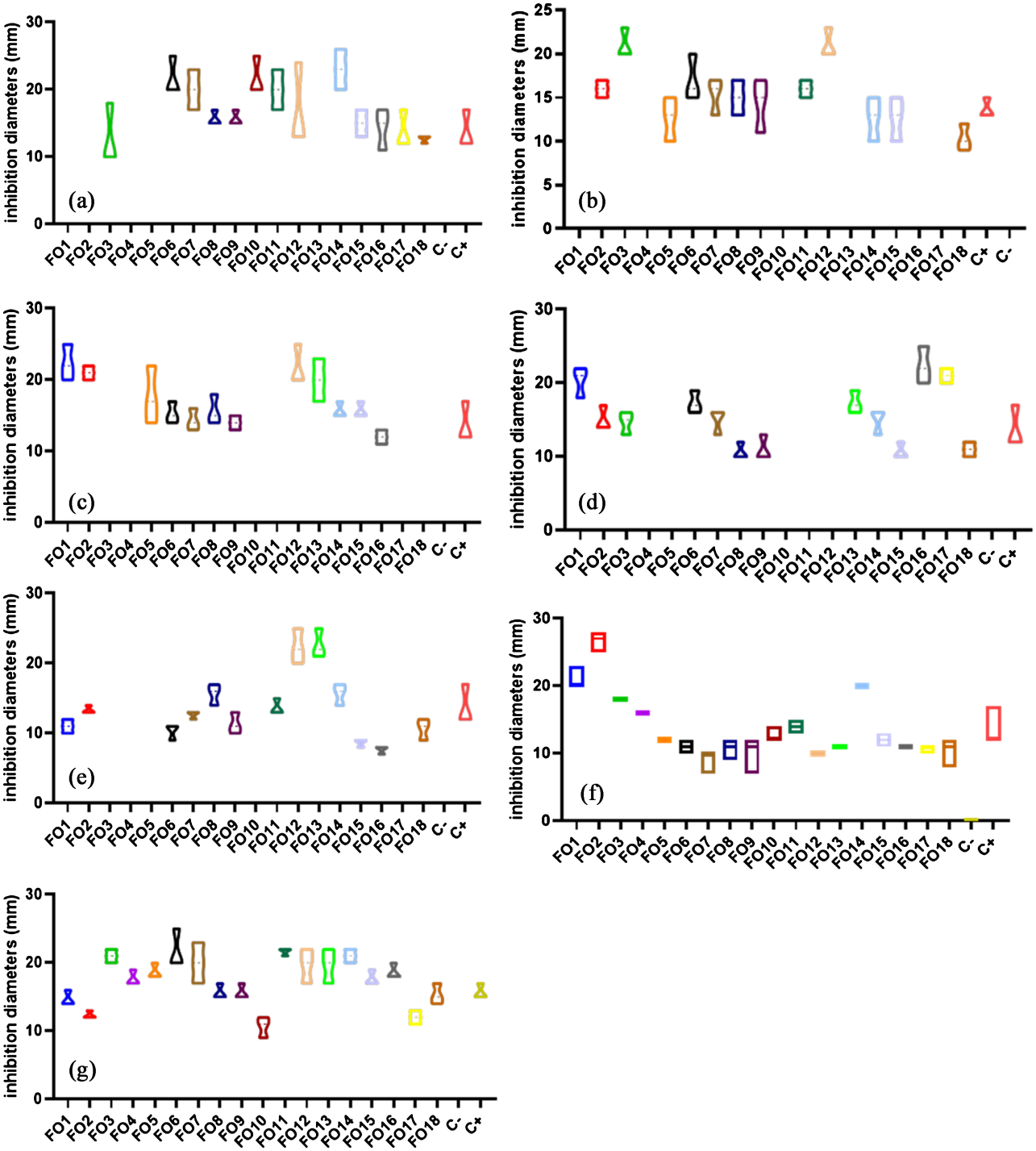


Figure 4. Antagonistic activity of biosurfactants on pathogens growth. (a) *E. coli*, (b) P aeruginosa, (c) *S. flexineri,* (d) *S. typhimirium*, (e) *B. cereus*, (f) *P. mirabilis*, (g) *S. aureus*, FO1, FO2, FO3, FO4 FO5 and FO6 (*Bacillus* strains); FO7, FO8, FO9, FO10, FO11, FO12, FO13, FO14, FO15 FO16, FO17 and FO18 (*Lactobacillus* strains).

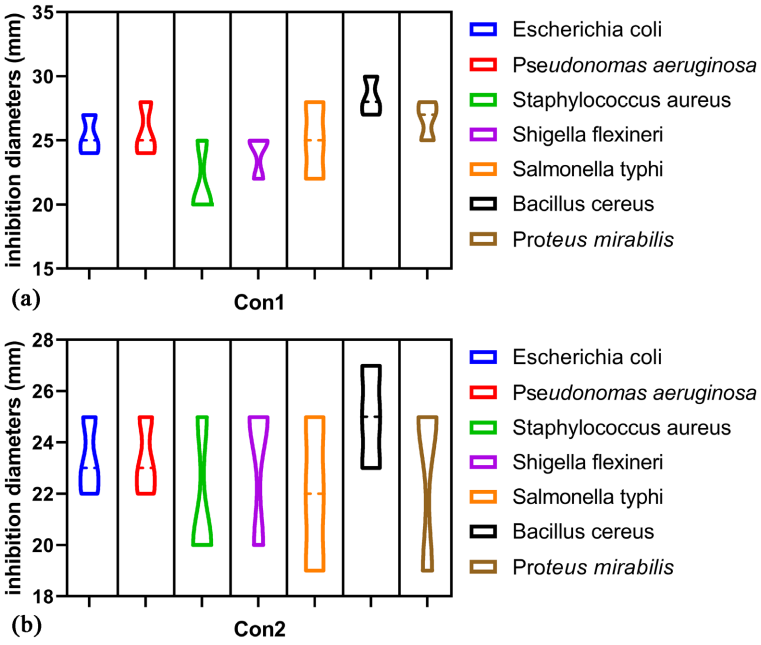


Figure 5. Antagonistic activity of biosurfactants consortia on pathogens growth. (a) Con1: Consortium including B. *subtilis and B. safensis*. (b) Cons2: *L. plantarum, L. gasseri, L. paracasei, and L. salivarius*.

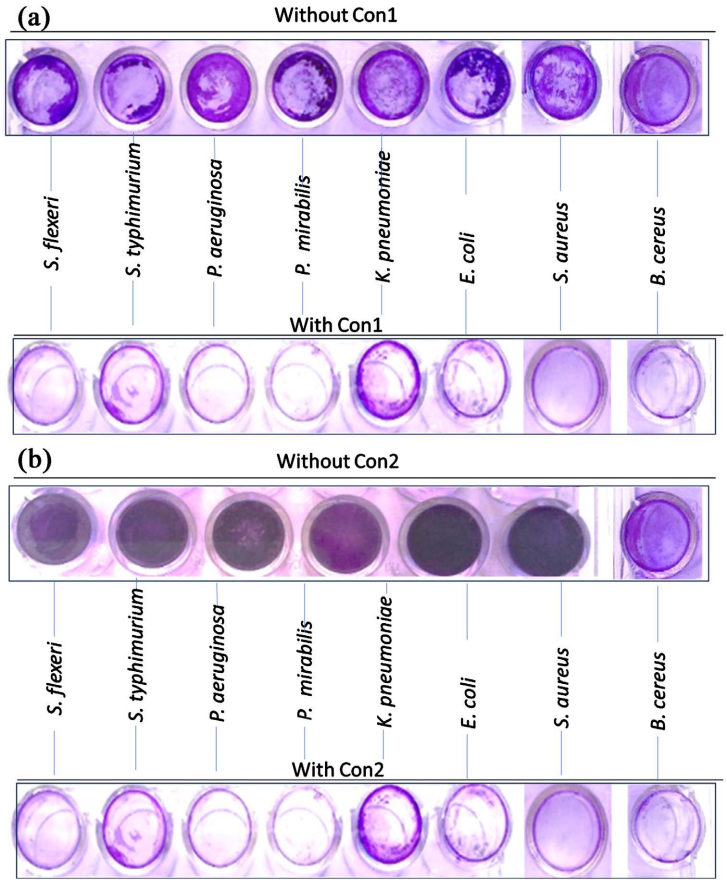


Figure 6. Antiadhesive activity of *Bacillus* and *Lactobacillus* strains in the presence of biosurfactants consortium. Con1: Consortium including B. *subtilis and B. safensis*. Cons2: *L. plantarum, L. gasseri, L. paracasei, and L. salivarius*.

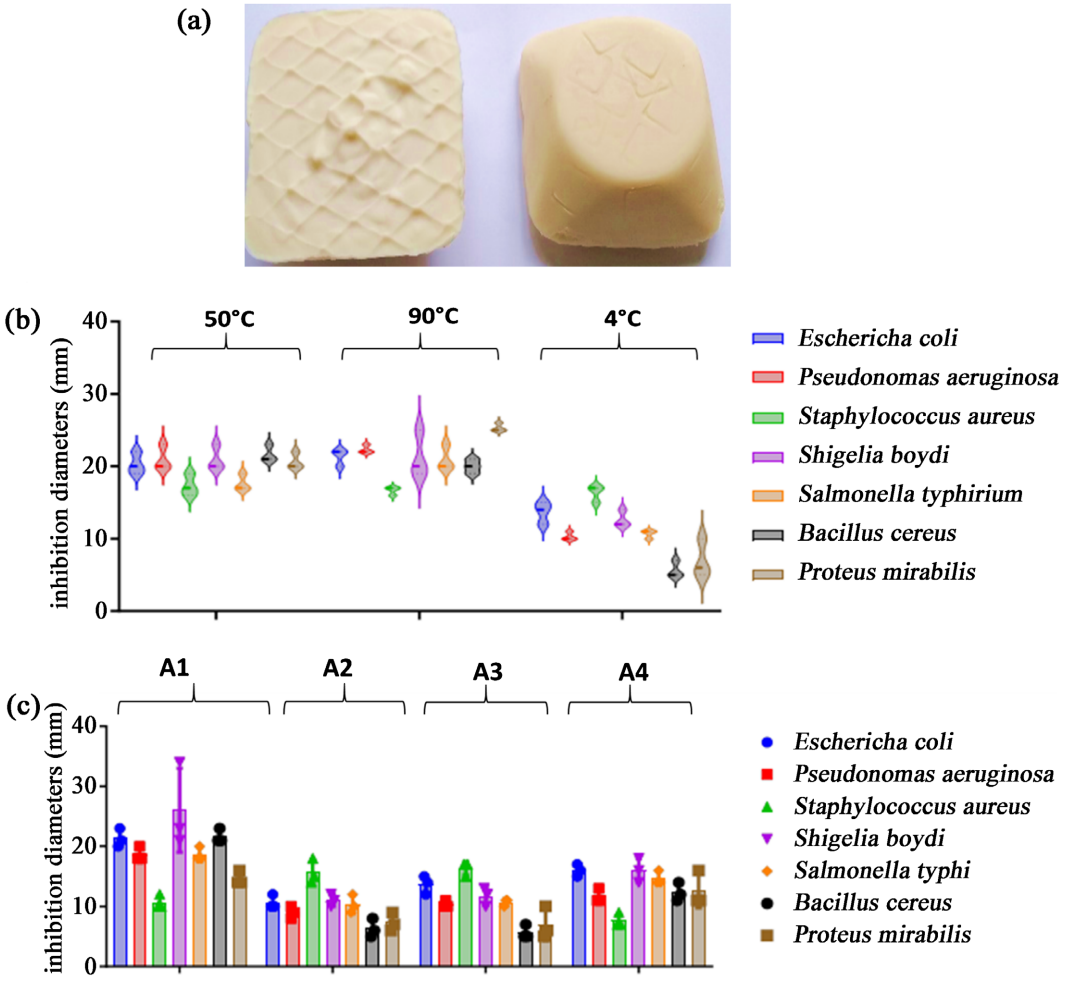


Figure 7. Elaboration of soap with biosurfactant (a) and Stability of biosurfactant consortium with Temperature (b) and pH (c), A1: pur Con1, A2: Cons1 in Soap at pH10, A3: Con1 with soap after one month at pH 10, A4: Con1 with soap after one month at pH 10.

5.7.1. Antibacterial Assay BCS

In order to carry the antibacterial potential of formulated biosurfactant soap, the antibacterial activity was investigated against *P. mirabilis*, *P. aeruginosa, S. aureus*, *Escherichia coli*, *S. tiphirium*, *S. flexineri* 5a M90T, and *Bacillus cereus*. The result shows that BCS was able to inhibit all target strains (**Figure 8**) more than three control soaps (ordinary soap, salicylic acid soap, and medicated santex soap) ($p < 0.05$). The inhibition diameters varied from 10 to 20 mm (**Figure 8**) and this was higher at 250 mg/mL with the same strains (**Figure 8(b)**).

5.7.2. Handwashing Efficiency of Elaborated Soap

The handwashing property of manufactured soap has been investigated.

Here, the protocol was completed with volunteers, who were each tested using both the 7 reference strains. Significant differences were found between handwashing results with all strains (**Figure 9**). For *E. coli*, handwashing with biosurfactant soap resulted in significantly greater log reductions than handwashing with the control soaps (ordinary soap and salicylic acid soap) used and with

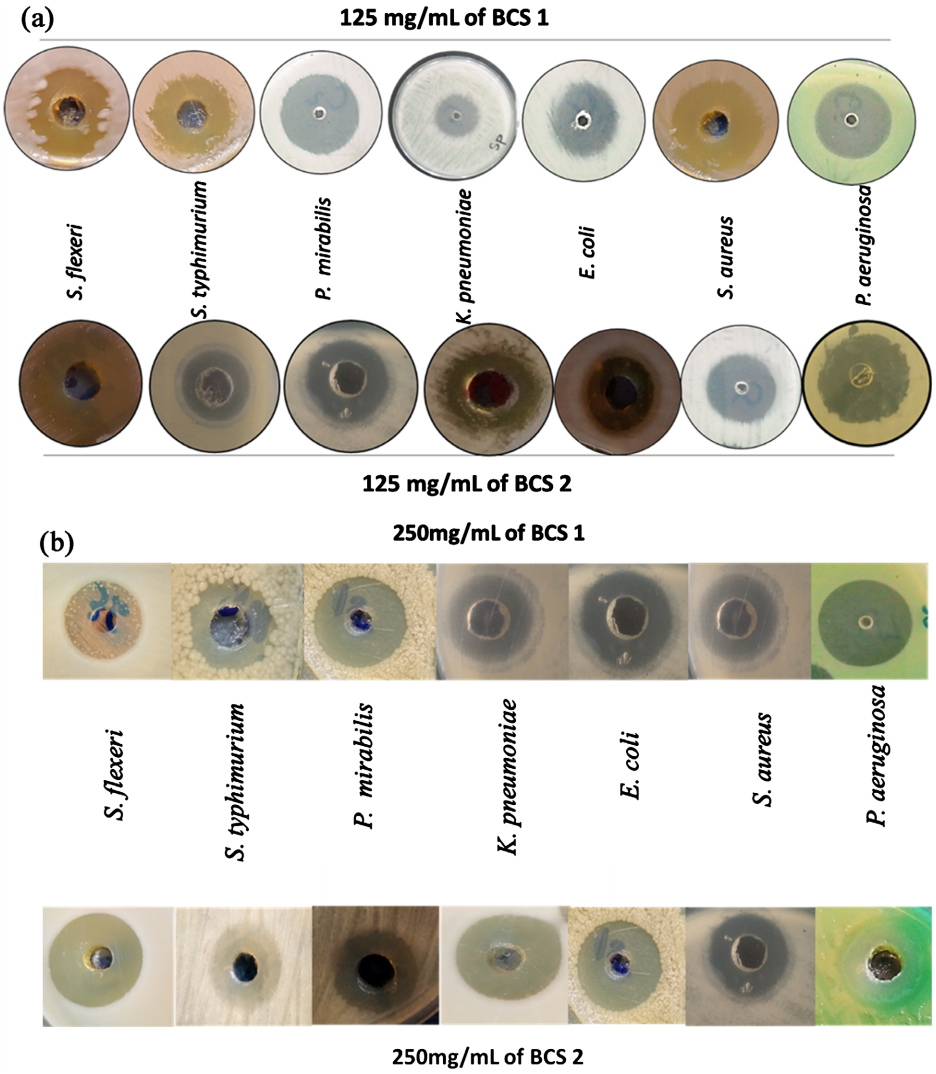
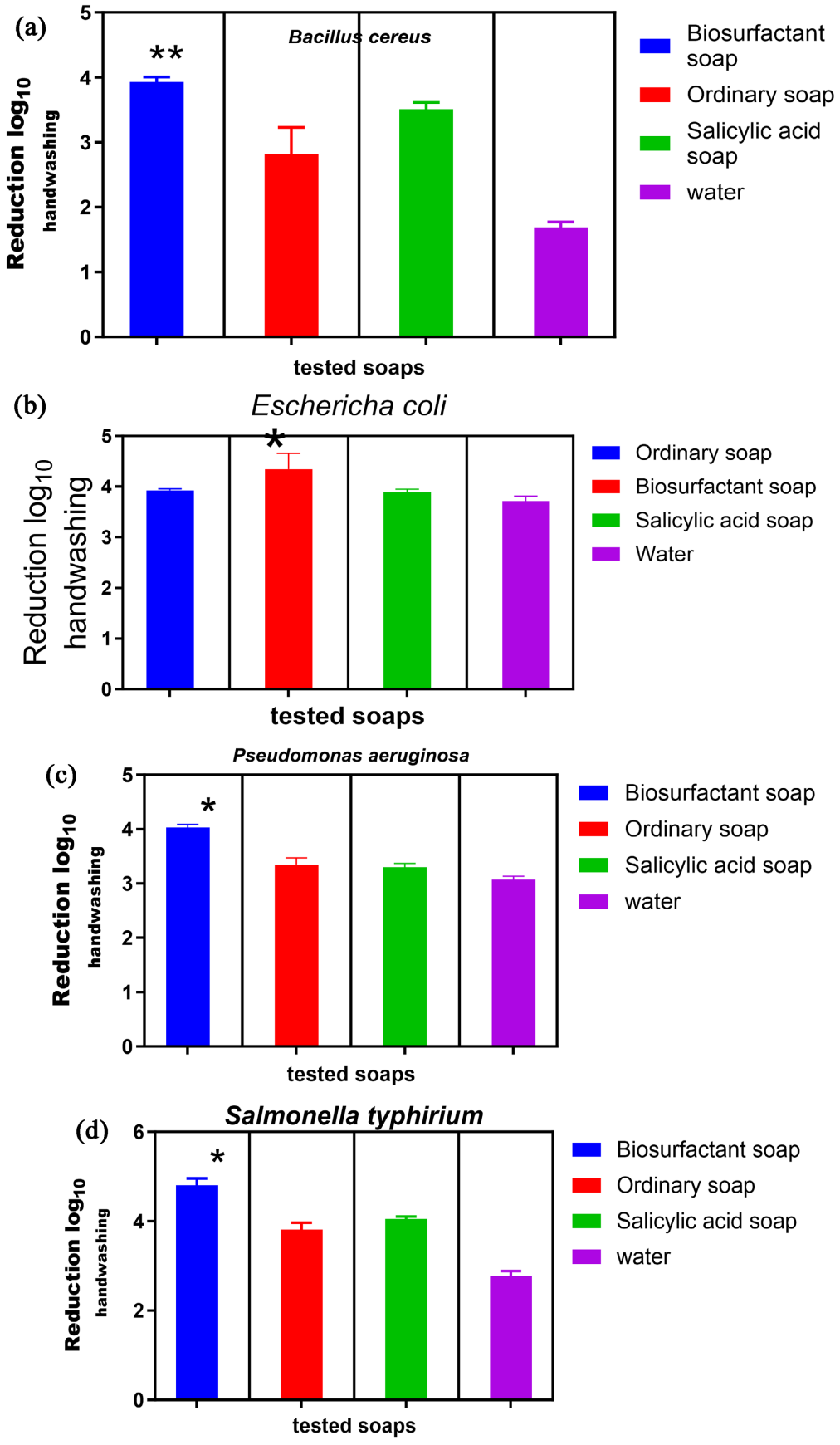


Figure 8. Profile of inhibition activity of BCS a: *Shigella flexineri* 5a M90T; b: *Pseudomonas aeruginosa*, c: *Proteus mirabilis* and d: *Staphylococcus aureus.* Consortium including B*. subtilis and B. safensis*. Cons2: *L. plantarum, L. gasseri, L. paracasei, and L. salivarius.*

water only (red log = 4.342 $p < 0.009$). In the rinse water, the biosurfactant soap resulted in a greater log reduction of *E. coli* persisting in the rinse water than an ordinary soap and salicylic acid soap (red log = 1.257 $p < 0.001$). In the case of *B. cereus* compared to handwashing with only water or with ordinary soap and salicylic acid soap, handwashing with biosurfactant soap demonstrated a high reduction of organisms during handwashing (red log = 3.926 $p < 0.05$) and in rinse water (red log = 1.537 $p < 0.05$). The same pattern was found with *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella tiphimurium*, and *Shigella boydii* 5a M90T. Handwashing using the biosurfactant soap shows a greater significant log reduction of organisms than handwashing using control soaps or with only water. On the hands, the log reduction value of target strains ranged from 4.505 for *Staphylococcus aureus* to 4.807 for *Salmonella typhirium*, 4.788 for *Shigella boydii*, 5.283 for *Proteus mirabilis*, and 4.03 for *Pseudomonas*


(a)
Bacillus cereus
**
Reduction log10 handwashing
tested soaps
Biosurfactant soap
Ordinary soap
Salicylic acid soap
water
(b)
Eschericha coli
*
Reduction log10 handwashing
tested soaps
Ordinary soap
Biosurfactant soap
Salicylic acid soap
Water
(c)
Pseudomonas aeruginosa
*
Reduction log10 handwashing
tested soaps
Biosurfactant soap
Ordinary soap
Salicylic acid soap
water
(d)
Salmonella typhirium
*
Reduction log10 handwashing
tested soaps
Biosurfactant soap
Ordinary soap
Salicylic acid soap
water

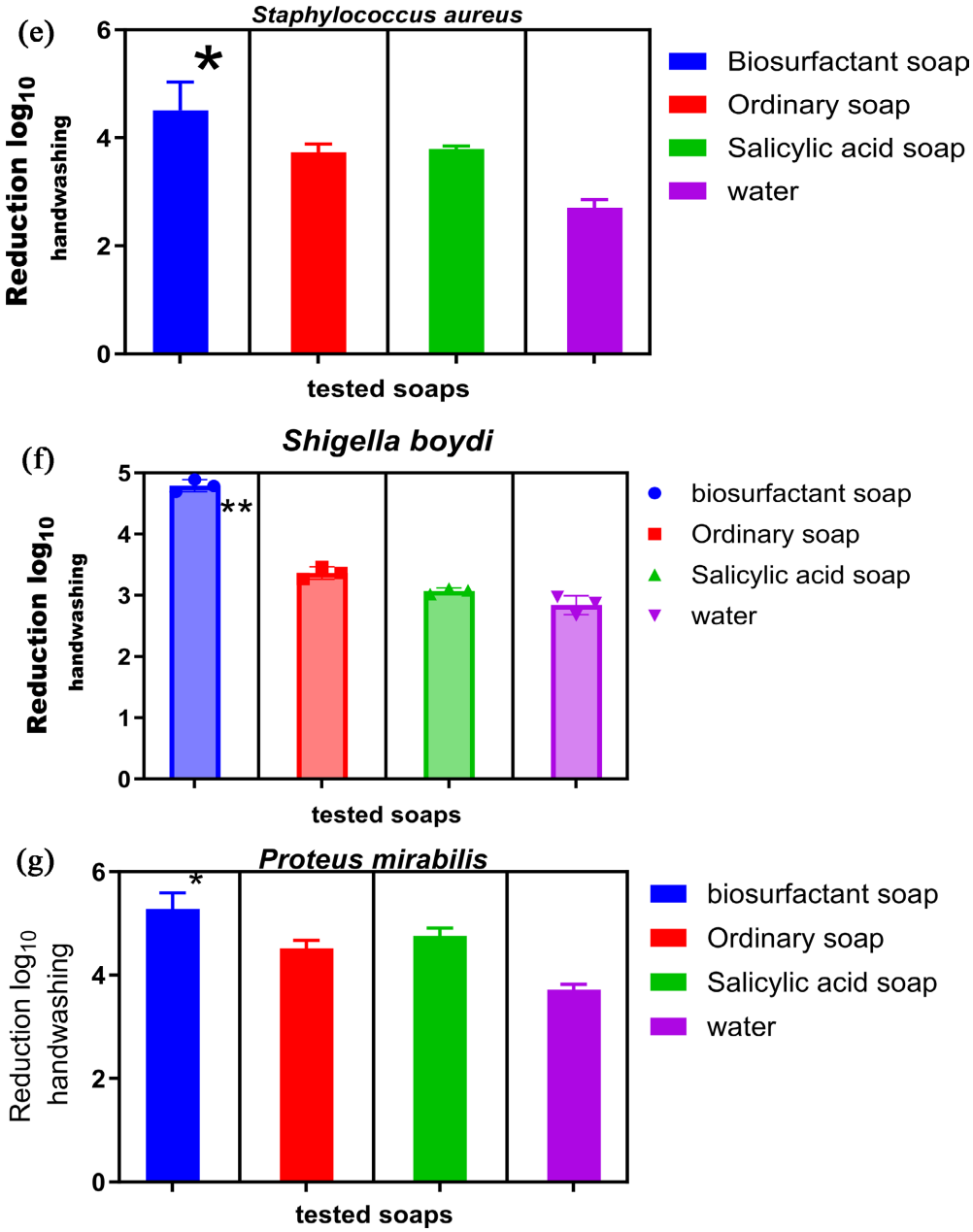


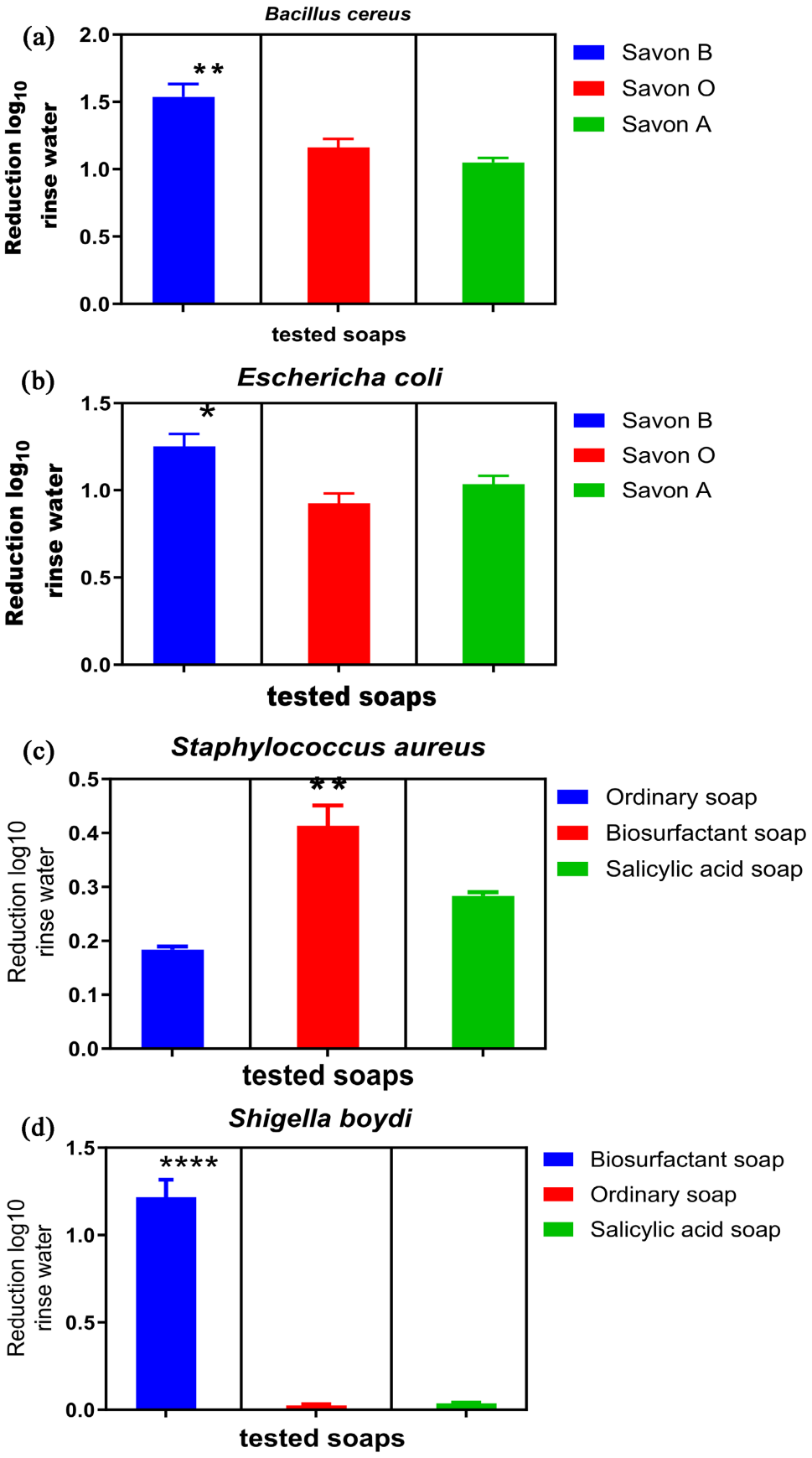
Figure 9. Hand washing results.

aeruginosa ($p < 0.05$). In rinse water, biosurfactant soap resulted in a greater log reduction than all other soaps used ($p < 0.05$). The result shows a considerable diminution of the persistence of all strains in a biosurfactant rinse water (**Figure 10**).

The valor of log reduction of target strains on the hands ranged from 0.413 for Staphylococcus aureus, 1.396 for *Salmonella typhirium*, 1.217 for *Shigella boydii*, 1.113 for *Proteus mirabilis*, and 0.545 for *Pseudomonas aeruginosa*.

6. Discussion

In this work, the main goal was to show more benefits from using biosurfactants. The role of biosurfactants should be considered again. Indeed, in many departments


(a)
Bacillus cereus
Reduction log10 rinse water
2.0
1.5
1.0
0.5
0.0
**
tested soaps
Savon B
Savon O
Savon A
(b)
Eschericha coli
Reduction log10 rinse water
1.5
1.0
0.5
0.0
*
tested soaps
Savon B
Savon O
Savon A
(c)
Staphylococcus aureus
Reduction log10 rinse water
0.5
0.4
0.3
0.2
0.1
0.0
**
tested soaps
Ordinary soap
Biosurfactant soap
Salicylic acid soap
(d)
Shigella boydi
Reduction log10 rinse water
1.5
1.0
0.5
0.0
****
tested soaps
Biosurfactant soap
Ordinary soap
Salicylic acid soap

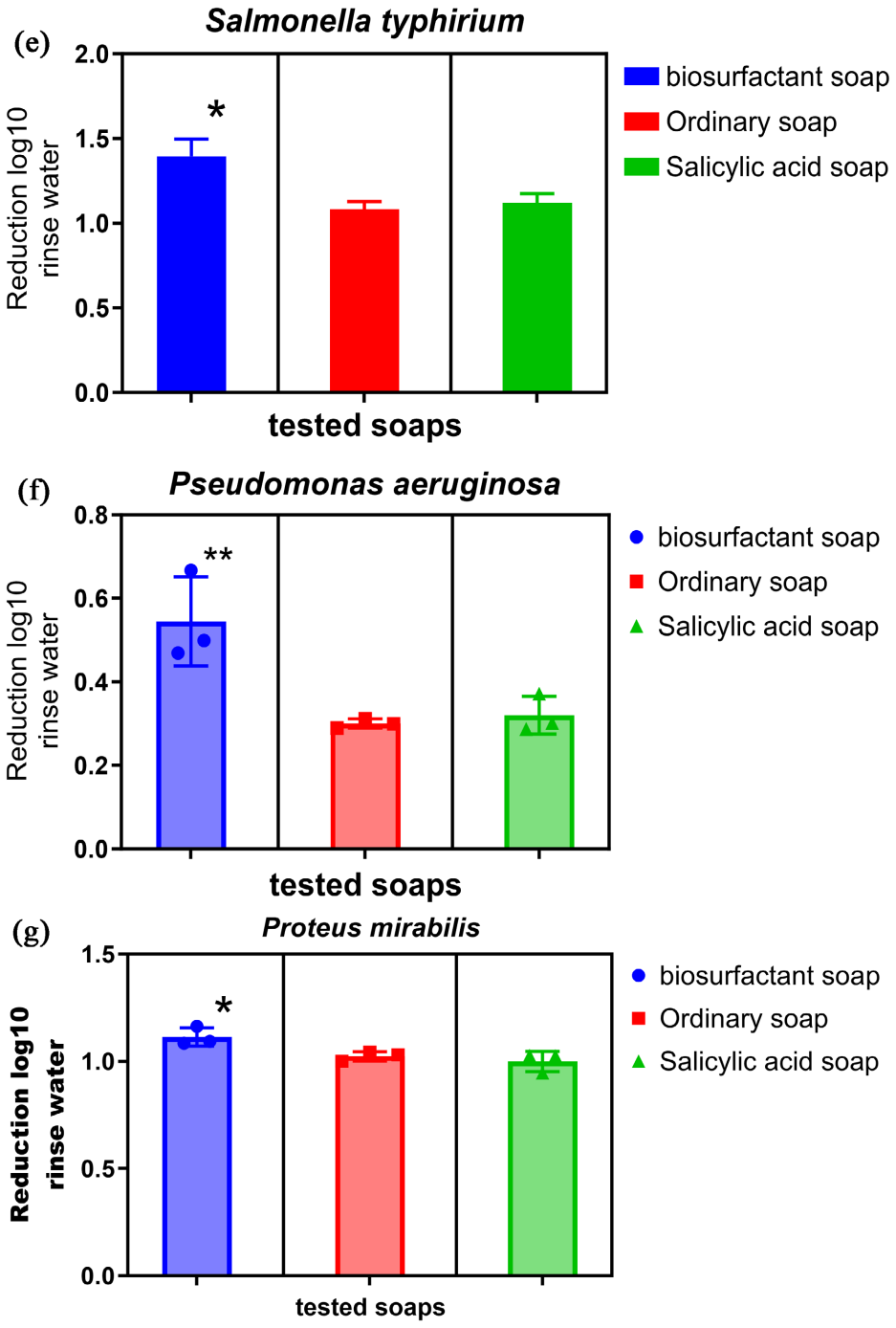


Figure 10. Reduction of persistence of organisms in rinse water.

of the Republic of Congo, Congolese are consumers of fermented beverages and sometimes seek new tastes, novel sensations, and good protective beverages [18].

In this work, 60 isolates (*Lactobacillus* and *Bacillus*) were found using local foods. Previous studies have reported that bacteria of the genera *Lactobacillus* and *Bacillus* are isolated from local and fermented foods [19] [20] [21].

Direct identification by housekeeping genes PCR has been used to identify isolates. L. plantarum (66,66%) was associated with CMBM2, CMP7, VC4,

CMBVOU1, M19, CO2, CMNS4 and TM13 for Lactobacillus isolates, and B. subtilis (50%) was associated with 43, 4, G7, and NM23 for Bacillus isolates. The use of the fibE gene for Bacillus identification [20] and ARNr (16S-23S) ITS for Lactobacillus detection by PCR assays has been documented [22] [23].

In this work, we have clearly shown the ability of *Lactobacillus* and *Bacillus* isolates to produce biosurfactants through an emulsification test with gasoline. Emulsification indexes (E24) ranged from 64.28% to 100% and from 33.33% to 100% were obtained from the cell culture and the Lactobacillus supernatant, respectively. We also showed the ability of Bacillus to produce biosurfactants whose emulsification index (E24) varied from 20% to 94.11% for the culture and from 9.09% to 100% for the supernatant. Previous work shows that bacteria are able to produce two types of biosurfactants; extracellular biosurfactants and cell membrane-bound biosurfactants [9]. The results obtained from this study are similar to those of Moukala *et al.* in 2019, which showed the ability of *Lactobacillus* isolates isolated from fermented foods to produce biosurfactants by the gasoline emulsification test with emulsification indices (E24) varying from 20 to 90% [19]. Similarly, Elenga *et al.* in 2021 showed the ability of Bacillus to produce biosurfactants through an emulsification test with gasoline whose emulsification indexes (E24) varied from 10% to 100% [24]. All isolates produce extractible biosurfactants.

The biosurfactants extracted with HCl are able to inhibit the cell adhesion and the growth of pathogenic bacteria, including *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella tiphirium*, *Shigella boydii* 5a M90T, and *Bacillus cereus*. Moukala and Elenga have respectively shown the potential of *Bacillus* and *Lactobacillus* biosurfactants to inhibit pathogens [19] [24]. The ability of Lactobacillus and Bacillus biosurfactants to inhibit biofilm formation [13] [25] [26]

The ability of microorganisms to secrete and the presence of biosurfactants in the extracellular medium may account for the absence of pathogenic bacteria in the same fermented foods [19] [27]. Lactobacillus spp. and Bacillus spp. are also able to secrete other biomolecules such as bacteriocins, which can inhibit the growth and adhesion of the bacterial pathogens mentioned above [28] [29]. Biosurfactants have already been proposed as a preservative in the food industry [1] [2]. The probiotic effect and ability of Lactobacillus and Bacillus bacteria to inhibit the growth of pathogenic bacteria has previously been demonstrated [12] [22].

The consortium of biosurfactants was established in this study and biological activities were investigated. The results showed that the biosurfactant consortium was easily able to inhibit the growth and biofilm formation of pathogens. Some studies have reported that biosurfactants can be associated with acting in synergy [30]. The stability of biosurfactants consortium to temperature (50 and 90˚C), pH (10) and during the conservation (2 months). The consortium tested has conserved the biological activities after all treatments. Biosurfactants can withstand higher temperatures as well as alkaline pH [31].

Using the cold saponification process, the biosurfactant consortium soap was made. Some physico-chemical parameters of madding soap were investigated, like pH, foaming stability, and cleansing properties. The pH of all soaps in this study was 10, and the biosurfactant soap produced showed a strong, stable foam compared to the control soaps used (ordinary soap and salicylic soap). The product soap possesses good activity to remove blood, bile salt, and diesel dirt. A study reported that the pH of kindly soaps is comprised of a range of 7 to 10 [32]. The high stability of foam and the good aptitude of cleaning could be attributed to biosurfactants present in soap because the biosurfactants are described as foaming and cleansing agents [3] [6].

The antiseptic property of biosurfactant soap was researched against Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, *Salmonella* tiphirium, Shigella boydi 5a M90T, and Bacillus cereus.

The biosurfactant soap inhibited more pathogens than the control soaps (ordinary soap, salicylic acid soap, and medicated santex soap). The minimal inhibitory (MIC) concentration was found to be 125 mg/mL for *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus* and 250 mg/mL for *Salmonella tiphirium*, *Bacillus cereus*, and *Shigella boydii* 5a M90T. It's important to note that ordinary soap and salicylic acid soap showed inhibit activity against some pathogens, but this activity was less than biosurfactant soap. This potential could be assigned to biosurfactants by possessing antimicrobial activity [33].

Biosurfactants can be versatile in their interactions with pathogenic bacteria being variously beneficial, neutral or antagonistic in their effect. This would go a long way in making biosurfactants a commercially successful compound of the current century. In addition, the ability to decontaminate the hands of the soap produced was evaluated on seven (7) pathogens as well as their ability to persist in rinsing water. The results obtained show that the soap with biosurfactant extracts was able to effectively reduce the microorganisms on the hands of the volunteers compared to the control soaps ($p < 0.001$). This observation was the same for the persistence of pathogenic microorganisms in the rinse water for all the microorganisms tested compared to ordinary soap and soap with salicylic acid ($p < 0.001$). Our results are similar to those of Janice *et al.* in 2008, who showed that antiseptic soaps were able to reduce the contamination of pathogens like *Shigella flexneri* and *Escherichia coli* on the hands [34]. Similarly, Solomon *et al.* in 2021 reported that handwashing with soap reduced hand contamination with pathogens leading to diarrheal disease in the Ethiopian community among children 0 - 5 years old [35]. The log reduction values of *Escherichia coli* obtained with the biosurfactant soap were 4.125 on the hands and 1.117 in the rinse water. These results are different from those obtained by Wolfe and Lantagne in 2017, who found the log reduction values of less than 3 on the hands and 0.28 in the water [17] [36]

Handwashing is essential for disease prevention [37]. This protocol can be used to generate evidence about handwashing efficacy and rinse water persis-

tence [23]. This method can be adapted to test a wide range of surrogate organisms and handwashing methods [23].

7. Conclusions

This present work has contributed to the valorization of biosurfactants produced by bacteria of the *Bacillus* and *Lactobacillus* genera isolated from different foods in the Republic of Congo.

This study demonstrated that biosurfactants can be secreted directly into the extracellular medium. *Lactobacillus* and *Bacillus* were isolated from local foods in this study. The biosurfactant extracts with HCl are able to inhibit biofilm formation and the growth of pathogenic bacteria such as Proteus mirabilis, Pseudomonas aeruginosa, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella tiphirium*, *Shigella boydii* 5a M90T, and *Bacillus cereus*.

The consortium of biosurfactants was established in this study and biological activities were investigated. The results showed that the biosurfactant consortium was easily able to inhibit the growth and biofilm formation of pathogens. Some studies have reported that biosurfactants can be associated with acting in synergy. The stability of biosurfactants consortium to temperature (50˚C and 90˚C), pH (10) and during the conservation (2 months). The consortium tested has conserved the biological activities after all treatments. Biosurfactants can resist higher temperatures and alkaline pH. Biosurfactants are produced under various growth and environmental conditions and are reported to be mainly involved in increasing the solubility and availability of various water-immiscible substrates.

The soap produced showed an antiseptic power directed against all the pathogens tested. Similarly, the results of the hand decontamination test for this soap are very encouraging. In addition, the soap is able to significantly reduce the persistence of microorganisms in the rinse water. This promising soap could be used in households, in the prevention of diarrheal diseases related to dirty hands in children from 0 to 5 years old, and in the cleaning of hands and equipment in clinical and hospital settings. In addition, the biosurfactants produced could be applied as biopreservatives, biocontrol agents, and why not in the prevention of transmission of covid-19. Finally, eight strains of *Bacillus* were identified based on DNA technology targeting genes encoding fibrinolytic enzymes like *B. safensis*, *B. subtilis*, and *B. pumilus*. On the other hand, 10 *Lactobacillus* strains were identified using PCR of the ITS regions of 16S-23S rDNA such as *L. plantarum* and *L.* paracasei.

This work also constitutes a scientific support, a reference tool and a source of information that may be of interest to future scientists and researchers who would like to deepen the analysis.

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Through the project INT0098, the IAEA had provided a package that includes detection equipment, namely real-time RT-PCR and kits, together with reagents and laboratory consumables, as well as biosafety supplies such as personal protection equipment and laboratory cabinets for the safe handling and analysis of samples. This equipment was also essential for detecting, tracking and studying the coronavirus that causes COVID-19 disease.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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