*VNUHCM Journal of Engineering and Technology 2026, 9(3): 3267-3275*

Open Access Full Text Article

*Research article*

# Enhancing the cleaning ability of soapberry through fermentation with biosurfactant-producing bacteria isolated from Garbage Enzyme

**Duy K. TRAN[1,2], Hien T.T. NGUYEN[1,2], Gia H. BAO[1,2], Oanh N. HUYNH[1,2], Dung M. HOANG[1,2,*]**



**ABSTRACT**

The development of natural-based products is a contemporary trend, with biological cleaning agents playing a pivotal role in this movement. Increasing environmental concerns and the demand for safer, biodegradable alternatives to synthetic chemicals have further accelerated interest in such eco-friendly solutions. Garbage enzymes, which are biologically derived through the fermentation of organic waste, have emerged as promising candidates due to their sustainability and multifunctional applications, particularly in cleaning and household sanitation. In addition to its ability to degrade organic residues, garbage enzyme are also highly likely to harbor Bacillus species, which are well known for their ability to produce biosurfactants with strong surface-active properties. This study aims to isolate and screen Bacillus strains with biosurfactant-producing capabilities. A total of 20 strains were isolated from garbage enzyme. Among these, three strains exhibiting the highest biosurfactant production were selected based on cleaning capacity with oil spreading assay, a rapid and reliable method for assessing surface activity. 16S rRNA sequencing identified these three strains (B5, B6, and B8) as belonging to the genus Bacillus. Notably, after three days of cultivation, strains B5 and B6 expressed the gene encoding fengycin (lipopeptide biosurfactant with strong surface activity), while strain B8 exhibited both fengycin and iturin production. Fermentation assays utilizing soapberry with the three isolated strains demonstrated enhanced oil spreading and improved EC24 emulsification compared to fermentation with each individual strain and the soapberry extract without any added strains. However, the use of multiple strains in co-fermentation did not demonstrate higher biosurfactant production or EC24 values compared to single-strain fermentation. These findings underscore the potential of the three isolated Bacillus strains for the development of efficient, sustainable and biologically based cleaning products.

**Key words:** Bacillus, biosurfactant, fermentation, soapberry

[1]*Faculty of Chemical Engineering, Ho Chi Minh University of Technology (HCMUT), 268 Ly Thuong Kiet Street, Dien Hong Ward, Ho Chi Minh City, Vietnam*

[2]*Vietnam National University Ho Chi Minh City, Linh Xuan Ward, Ho Chi Minh City, Vietnam*

**Correspondence**

**Dung M. HOANG**, Faculty of Chemical Engineering, Ho Chi Minh University of Technology (HCMUT), 268 Ly Thuong Kiet Street, Dien Hong Ward, Ho Chi Minh City, Vietnam

Vietnam National University Ho Chi Minh City, Linh Xuan Ward, Ho Chi Minh City, Vietnam

Email: hoangmydung@hcmut.edu.vn

**History**

- Received: 24-04-2025
- Revised: 11-08-2025
- Accepted: 03-07-2026
- Published Online: 04-09-2026

**DOI : https://doi.org/10.32508/vnuhcmjet.v9i3.1502**

Check for updates



## INTRODUCTION

Bacterial biosurfactants are amphipathic molecules containing both hydrophilic and hydrophobic parts, synthesized by a variety of microorganisms, including water (both marine and freshwater) and soil, and they can thrive across a wide range of temperatures, pH levels, and salinities [1], particularly species of Bacillus and Pseudomonas [2,3]. Surfactants can be categorized based on different factors, such as their origin (chemical or natural), the charge on their headgroup (anionic, cationic, non-ionic, and zwitterionic), and their solubility (water or lipid solubility) [4,5]. Compared to chemical surfactants, biosurfactants offer benefits such as reduced toxicity, enhanced biodegradability, and greater stability under harsh conditions [6]. These compounds are widely used in industries like enhanced oil recovery, bioremediation, food processing, and pharmaceuticals. Furthermore, they can be produced from renewable resources, supporting sustainability and circular bioeconomy [2].

Biosurfactants play a pivotal role in the survival and growth of the microorganisms that produce them within microbial communities in their respective environments. These roles include exhibiting antibacterial, antifungal, antiviral activities, antioxidant properties, inhibiting biofilm formation. The antibacterial effects of biosurfactants are primarily mediated through mechanisms that alter cell membrane permeability, disrupt cellular membranes, and inhibit bacterial ATP synthesis. Gram-positive bacteria are generally more susceptible to biosurfactants compared to Gram-negative bacteria due to the more permeable structure of their cell membranes. Lipopeptides, such as fengycin and iturin, demonstrate antifungal activity at low concentrations by inducing programmed cell death, while at higher concentrations, they cause membrane disruption in fungal cells. Rhamnolipid and surfactin have been shown to impede the adhe-

**Cite this article :** TRAN D K, NGUYEN H T, BAO G H, HUYNH O N, HOANG D M. **Enhancing the cleaning ability of soapberry through fermentation with biosurfactant-producing bacteria isolated from Garbage Enzyme**. *VNUHCM J. Eng. Technol. 2026; 9(3): 3267-3275.*

sion and biofilm formation of target bacteria on surfaces like polystyrene and stainless steel. Furthermore, surfactin and fengycin exhibit antiviral activity, including against SARS-CoV-2, by interacting with the lipid components of the viral envelope, thereby disrupting the membrane and inactivating the virus. Lipopeptides produced by Bacillus, such as surfactin, display significant antioxidant activity, attributable to the presence of hydroxyl groups, which support the survival of these bacterial strains under extreme conditions[3,7]. The lipopeptide are synthesized during the early stages of spore formation. Additionally, under unfavorable conditions, bacteria have the ability to form biofilms. Rhamnolipids play a crucial role in the preservation of biofilm architecture by modulating cell-cell interactions and the attachment of bacterial cells. This contributes to the stabilization of the biofilm structure, thereby ensuring the persistence and survival of the bacteria within the biofilm matrix[8]. Biosurfactants contribute significantly to maintaining the growth rate of microorganisms in oil-rich nutrient environments. In such systems, microbial proliferation is typically restricted to the interfacial region between the aqueous and oil phases. However, in the case of biosurfactant-producing bacteria, the generation of emulsions—mediated by the biosurfactants—enhances the interfacial area, thereby promoting more extensive colonization and metabolic activity within the oil phase[9]. Nowadays, natural products are highly valued. Garbage Enzyme (GE) is a fermented product made from kitchen waste, with high applicability, and is gradually being used widely in household activities due to its economic benefits and convenience. GE is produced from the fermentation process of kitchen waste such as vegetables and fruits combined with water and sugar in a ratio of 1:3:10 for at least 3 months. GE is widely used in household cleaning, wastewater treatment, and agriculture. Bacillus has been found to be an important microorganism, accounting for a high proportion in GE, with a function that supports compost processing[10]. For the reasons mentioned above, GE is a high-probability source for isolating Bacillus strains with the ability to produce biosurfactants. Soapberry is commonly used as a natural cleaning agent due to its saponin content, which has cleansing, foaming, and emulsifying properties. The fermentation process of soapberry by Saccharomyces cerevisiae (ADY) and Levilactobacillus brevis enhances its foaming ability and improves the stability of the extract[11].

This study focused on isolation, screening, and identification of spore-forming bacterial strains capable of producing biosurfactants. These strains were then used in the fermentation of soapberry to enhance the cleaning ability of the soapberry extract.

## MATERIALS AND METHODS

**Isolation of spore-forming bacteria from GE**: 1 mL of the GE prepared from orange peel, pomelo peel, lemon peel, lemongrass, soapberry, and Indian borage was cultured in 24 mL of Luria Broth (LB) medium (Himedia, India) and incubated at 37°C for 36 hours. The culture is then heat-treated at 70°C for 1 hour. 100 $\mu$L of the heat-treated culture was 10-fold diluted and spread on LB agar plates to isolate spore-forming bacterial strains. After 24 hours, colonies were picked and sub-cultured several times[12].

**Investigation of Biosurfactant Production by Isolated Strains:** The LB medium was supplemented with 4% molasses (v/v) and 2% olive oil (v/v) to create favorable environmental conditions for screening strains capable of producing biosurfactants, as well as to monitor the timing of biosurfactant production. Inoculation was performed at a rate of 10% (v/v), and cultures were incubated at room temperature. The culture broth was collected to evaluate biosurfactant production.

**Oil Spreading Assay method**: 500 $\mu$L of used motor oil, collected from the Laboratory of Process & Equipment at Ho Chi Minh City University of Technology (HCMUT) and stained with Sudan III, was added to 30 mL of distilled water in a Petri dish to form a thin oil layer. Then, 50 $\mu$L of the culture or culture supernatant was gently dropped into the center of the oil layer. If biosurfactants were present in the culture, they would displace the oil, forming a clear zone. The diameter of the cleaning zone on the oil surface correlates with biosurfactant activity and is referred to as oil displacement activity.

**Emulsification Capacity Assay**: Emulsification index (E24) of the fermentation broth was determined by thoroughly mixing 2 mL of the broth with 2 mL of n-hexane in a test tube. The mixture was vortexed vigorously for 2 minutes and then left undisturbed. After 24 hours, the height of the stable emulsion layer was measured. The E24 was calculated as the percentage ratio between the height of the emulsion layer and the total height of the mixture column. These test results provide insight into the cleaning potential and surface activity of the fermentation broth[13].

**Bacterial identification:** After isolation, screening, the selected strains were activated in liquid LB medium. After 3 days of incubation, the bacterial biomass was harvested. The biomass was then subjected to DNA extraction using the Tri-RNA Reagent

(Favogen, Taiwan). Briefly, the biomass was treated with 1 mL of Tri-Agent and 0.2 mL of chloroform. DNA was extracted from the organic phase, and 0.3 mL of absolute ethanol was added. The mixture was shaken and incubated for 3 minutes, followed by centrifugation at 2000 × g for 5 minutes at 4°C. The DNA was washed with 1 mL of a mixture containing 0.1 M sodium citrate in 10% ethanol, shaken for 30 minutes at room temperature, and then cold centrifuged at 2000 × g for 5 minutes. The DNA was resuspended in 30 $\mu$L of Tris-EDTA buffer (TE). The extracted DNA was used for amplification of the 16S rRNA by using universal primers: F- AGAGTTTGATCCTGGCTCAG, R- GGTTACCTTGTTACGACTT. The PCR product was then sent to Nam Anh Company for DNA sequencing. The raw sequencing data were analyzed by MEGA X software to identify the bacterial species.

**Biosurfactant-encoding gene expression**: After 3 days of culture, the bacterial biomass was harvested. The biomass was then subjected to RNA extraction using the Tri-RNA Reagent (Favogen, Taiwan). Briefly, the biomass was treated with 1 mL of Tri-Agent and 0.2 mL of chloroform. RNA was collected in aqueous phase. The aqueous phase is mixed with cold isopropyl alcohol, using 0.5 mL of isopropyl alcohol. The mixture is incubated at room temperature for 10 minutes, followed by centrifugation at 2600 × g for 30 minutes at 4°C. After precipitation, the supernatant is discarded, and the RNA pellet is washed with 75% ethanol. The sample is then vortexed and subjected to centrifugation at 7500 × g for 5 minutes at 4°C. The RNA pellet is subsequently air-dried and dissolved in TE. The extracted RNA was then reversed-transcribed into cDNA using a reverse transcription kit (Nanohelix, Korea). The resulting cDNA was used as a template to investigate the expression of the iturin fengycin and surfactin gene (Table 1) via PCR using the Ready-2x-Go [Tag] kit (NanoHelix, Korea). The presence of the surveyed gene expression is determined by the presence of the corresponding band on the 1% agarose gel (Biobasic, Canada) electrophoresis.

**Soapberry fermentation**: 5 g of dried soapberries (Dak-Lak, Vietnam) were finely ground and dissolved in 250 mL of distilled water, with the addition of 4% molasses (Tin cay, Vietnam). The mixture is then sterilized by autoclaving and inoculated with 10% culture at a concentration of $10^8$ CFU/mL of B5, B6, B8, or B5+B6+B8. The fermentation process is conducted over a period of 7 days using a static fermentation method.

**Statistic:** The data were analyzed using One-way ANOVA test to compare the differences between groups in the experiment, with statistical significance assessed through Tukey's post-hoc analysis.

## RESULTS

**Isolation and selection of spore-forming bacteria with the ability to produce biosurfactant:** Before proceeding with the isolation process, GE was cultured in liquid LB medium for 48 hours at 37°C to enhance bacterial growth. Subsequently, the culture was heat-treated at 70°C to screen for spore-forming strains. The isolation results yielded 20 strains, consisting of white, opaque, and smooth-surfaced colonies. The isolated strains were cultured in LB medium supplemented with molasses and olive oil for 7 days to provide a favorable environment to produce biosurfactant. The oil spreading assay method was used to screen and test the ability to clean oil surfaces. After 7 days, 5 strains exhibiting oil-cleaning activity were selected. 3 strains with high biosurfactant production were selected and labeled as B5, B6, and B8 (Fig.1). The DNA of all three strains B5, B6, and B8 was extracted and purified, then subjected to a PCR reaction for amplification with the universal 16S rRNA primers. The samples were sequenced, and the gene sequences were analyzed using MEGA X version 11.0.13. The sequences were compared with the NCBI database. The identification results for strain B5 showed a high similarity with the Bacillus velezensis strain with a similarity level of 99.5%. This species has great potential in biosurfactant production and the ability to inhibit plant pathogenic microorganisms. B. velezensis is an important member of Rhizobacteria, which are plant growth-promoting bacteria (PGPR) that can enhance plant growth and control soilborne diseases [17]. It is also known for its ability to survive in harsh environments and is often used in agricultural and environmental applications as a biological control agent. Most B. velezensis strains can produce secondary metabolites with antimicrobial activity, such as surfactin, fengycin, bacillomycin D, macrolactin, bacillaene, difficidin, oxydifficidin, plantazolicin, amylocyclin, and bacilysin. Strain B6 showed a high similarity in the 16S rRNA gene sequence compared to Bacillus velezensis, Bacillus subtilis, and Bacillus amyloliquefaciens strains published on GenBank, with a similarity level of 99.43%. They are known for their ability to produce biosurfactants and powerful enzymes. These Bacillus strains produce a wide range of lipopeptide-type biosurfactants, among which surfactin, iturin, and fengycin

**Table 1: Primer list**

| Gene | Name of primer | Sequence | Size | |
|---|---|---|---|---|
| Iturin D | Itu-F | ATGAACAATCTTGCCTTTTTA | 1203bp | [14] |
| | Itu-R | TTATTTTAAAATCCGCAATT | | |
| Fengycin | FenA-F | CCATCGGCACTTATTACGGAAAC | 126bp | [15] |
| | FenA-R | TGCGGACAAAGGACAGGAACT | | |
| Surfactin | Sfp-F | ATGAAGATTTACGGAATTTA | 675bp | [16] |
| | Sfp-R | TTATAAAAGCTCTTCGTACG | | |

are widely studied[18]. Strain B8 showed a high similarity with two Bacillus sp. strains and B. siamensis strains published on GenBank, with a similarity level of 100%. B. siamensis was recently discovered and shares characteristics with other species in the Bacillus group, including the ability to produce enzymes and biological compounds with potential in plant protection. B. siamensis produces surfactin, a common type of lipopeptide biosurfactant. B. siamensis HoB1 is a promising candidate for enhancing heavy oil recovery through biosurfactant production and biodegradation of oil. The biosurfactant produced by this strain demonstrates high stability, even under harsh temperature conditions[19].

**Kinetic Dynamics of Biosurfactant Synthesis in Three Isolated Strains**: All three strains (B5, B6, and B8) were cultured in LB medium (10% v/v) supplemented with molasses and olive oil to investigate the production kinetics of biosurfactants. The culture was incubated at room temperature and agitation at 200 rpm. The bacterial supernatant was collected every 24 hours for a total of 7 days, and its oil-cleaning capacity was assessed using the Oil Spreading Assay method. The diameter of the oil-cleaning zone was measured using a digital caliper. The results revealed a temporal variation in the diameter of the oil-cleaning zone for all three strains (B5, B6, and B8) over the 7-day period (Figure 2).

For strain B5, the data indicated a significant increase in the diameter of the oil-cleaning zone from day 2 to day 6, peaking on day 6 ($62.17 \pm 1.63$ mm), which was statistically significant ($p < 0.05$) compared to earlier days. However, a marked decline in the cleaning capacity was observed on day 7 ($42.13 \pm 3.27$ mm), representing a reduction of approximately 32% compared to day 6. Additionally, fluctuations in the diameter of the oil-cleaning zone were observed across the study period, with an increase of approximately 20 units from day 2 to day 3, a nearly 9-unit increase from day 4 to day 5, and a sharp decline from day 6 to day 7. This decline may be attributed to the depletion of nutrients in the culture medium after 6 days, leading to a reduction in biosurfactant synthesis. Alternatively, the accumulation of metabolic by-products and biological inhibitors could have adversely impacted the development and oil-cleaning activity of strain B5 in the later stages of the experiment.

The results indicated that strain B5 exhibited superior oil-cleaning capacity compared to strains B6 and B8 on day 6 ($62.17 \pm 1.63$ mm). However, strains B6 and B8 demonstrated greater stability in their oil-cleaning abilities up to day 7, with a gradual increase in the diameter of the oil-cleaning zone and no significant fluctuations in the measurements, unlike strain B5, which experienced a sudden drop on day 7. Consequently, strain B5 may be selected for optimal cleaning efficiency on day 6, while strains B6 and B8 would be preferable for stable biosurfactant production over extended periods. Gusmao et al. showed that when Candida glabrata UCP1002 was cultivated in a 100 mL aqueous medium with 5% plant fat waste, the biosurfactant concentration reached 7 g/L after 144 hours of incubation. This concentration was twice that achieved using waste coconut oil. Consequently, optimizing nutrient sources for these strains is crucial to improving biosurfactant production efficiency[20].

**Surfactin, iturin and fengycin gene expression in isolated strains**: Surfactin, a cyclic lipopeptide, exhibits exceptional surface-active properties, significantly reducing surface tension in water even at low concentrations[21]. This compound demonstrates a wide range of biological activities, including antibacterial, antiviral, and antitumor effects[22]. Its potential applications extend across various sectors, including environmental, food, and biomedical industries[23]. Despite these advantages over synthetic alternatives, the widespread production and use of surfactin face challenges due to high production costs

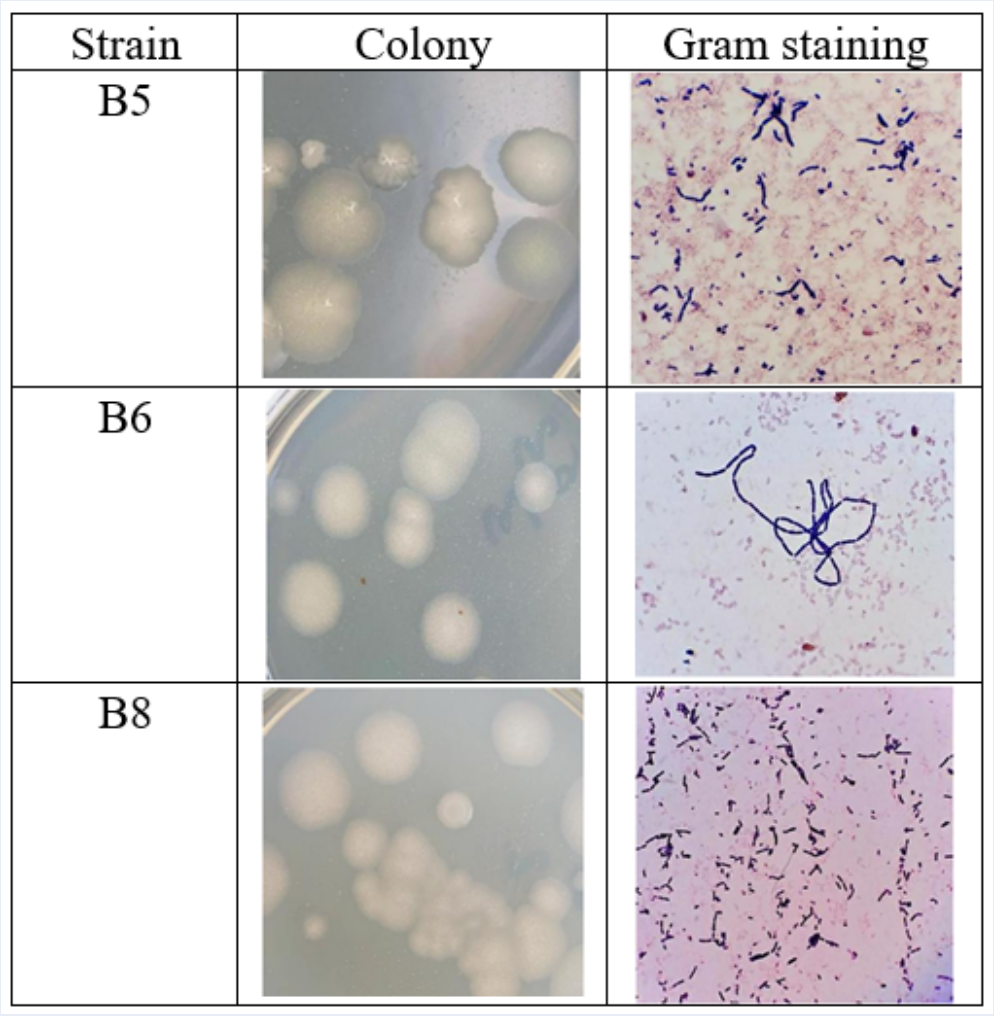


**Figure 1**: Macroscopic and microscopic details of selected biosurfactant producing strains [Source: authors' group]

and low yields[21]. Current research efforts are focused on optimizing fermentation processes and exploring biotechnological approaches to enhance surfactin production[23]. Iturin, a cyclic lipopeptide with strong biosurfactant activity, foaming ability, and the ability to reduce surface tension in solutions, as well as antimicrobial activity. These compounds are commonly used in industrial and environmental applications due to their unique and beneficial properties, such as oil pollution treatment, cleaning processes, and food preservation. These compounds have demonstrated the ability to enhance oil recovery from oil wells and facilitate oil transportation through pipelines[24,25]. Additionally, the diverse range of applications of iturin in the cosmetics industry, such as using it as an emulsifier, anti-wrinkle agent, and antimicrobial, highlights its adaptability[26]. Fengycin is a surfactant peptide with strong antibacterial properties and foaming ability, and it also has applications in pollution treatment and food preservation. Furthermore, fengycin shows more pronounced antagonism against filamentous fungi compared to other lipopeptides, making it useful for treating various plant diseases without the side effects of chemical pesticides, and it also has potential applications in the food and cosmetic industries[27].

B5, B6 and B8 were grown in liquid LB medium. After 3 days, the biomass of the strains was collected. The biomass was then subjected to extraction to obtain mRNA. The obtained cDNA was used to amplify the Fen-A, Sfp, and Itu-A using 35 cycles with the Ready-2x-Go [Tag] kit (NanoHelix) and corresponding primers for Fen-A, Sfp, and Itu. The re-

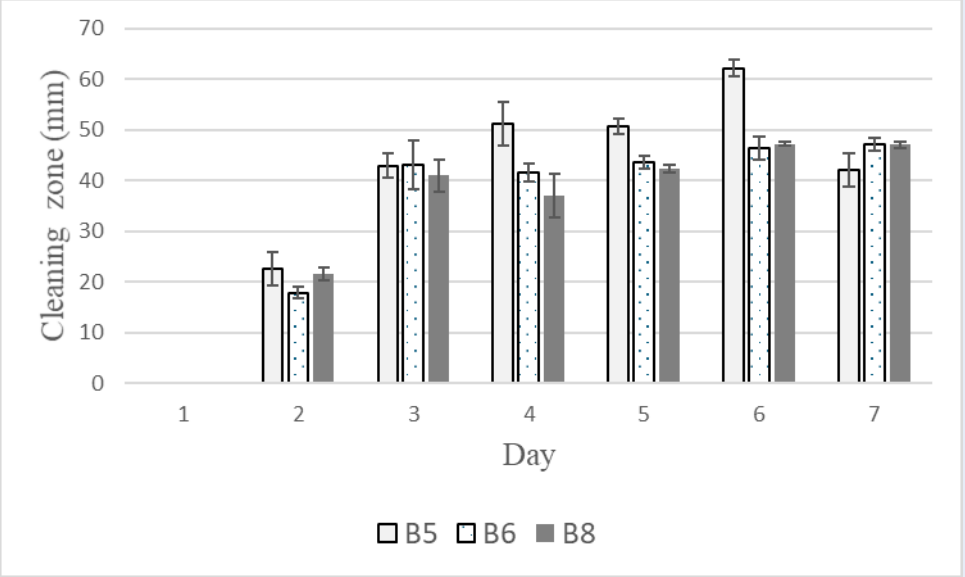


**Figure 2**: Kinetic dynamics of biosurfactant synthesis in three isolated strains [Source: authors'group]

sults showed that all three strains, B5, B6, and B8, express the Fen-A gene. For strain B8, iturin was expressed (Figure 3). This indicates that strain B8 has the potential to produce multiple types of biosurfactants. Therefore, combining all three strains in the production process can diversify the types of biosurfactants, leading to better and more effective cleaning capabilities compared to single biosurfactant types.

**Soapberry fermentation**:

**Table 2: 2. Oil surface cleaning zone of soapberry samples after 6 days of fermentation with different strains. Different letters in the same column indicate statistically significant differences ($p < 0.05$, Tukey's test).**

| Inoculation | Cleaning zone (mm) |
|---|---|
| | Soapberry |
| B5 | $61.80 \pm 1.47^{a}$ |
| B6 | $57.60 \pm 1.01^{b}$ |
| B8 | $52.30 \pm 0.66^{c}$ |
| B5 + B6 + B8 | $57.23 \pm 0.32^{b}$ |
| Control (without bacteria) | $51.10 \pm 0.80^{c}$ |

In the soapberry fermentation process over a 6-day period, the results indicated that all samples exhibited the ability to clean the oil surface, with the B5 strain showing the highest cleaning diameter ($61.80 \pm 1.47$ mm). Soapberries contain a substantial amount of saponins, which are compounds known for their surface-active properties. The molecular structure of saponins consists of both hydrophilic and lipophilic components, enabling them to disrupt the bonds between oil and water molecules. In addition to the cleaning efficiency of the biosurfactants produced by the strains, the saponins in the soapberry can generate foam when dissolved in water, which could potentially enhance the cleaning effect. Moreover, the foam reduces the surface tension of water, thereby increasing its ability to interact with and clean oil layers more effectively (Table 2). Statistical analysis revealed that the cleaning diameter of the soapberry fermentation liquid for the B5 ($61.80 \pm 1.47$ mm), B6 ($57.60 \pm 1.01$ mm), and B5+B6+B8 ($57.23 \pm 0.32$ mm) samples were significantly higher compared to the control ($51.10 \pm 0.80$ mm). This difference can be attributed to the biosurfactants produced by the added strains during fermentation. However, the B8 sample did not exhibit a significant difference from the control. The increased cleaning diameter of the soapberry samples supports the hypothesis that, in addition to the biosurfactants produced by the strains, a considerable amount of saponin in the soapberry fruit peel contributes to enhanced cleaning and foaming ability during fermentation.

The emulsification capacity (EC24) in soapberry fermented with three strains, B5, B6, and B8, achieved the highest value. This result is statistically significant when compared to soapberry fermented with the single B5 strain and is higher than that of soapberry

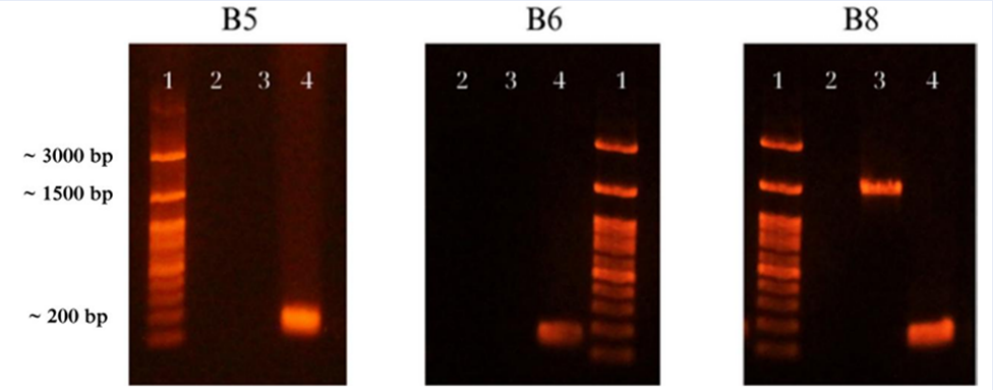


**Figure 3**: Expressionof biosurfactant genes in B5, B6 and B8. 1- Ladder, 2- Surfactin, 3-Iturin, 4-Fengycin [Source: authors' group]

fermented with the individual B6 and B8 strains, although this difference lacks statistical significance (Table 3). In the oil surface cleaning assay, soapberries fermented with B5 exhibited superior cleaning performance compared to soapberries fermented with the three strains concurrently. Notably, the Oil Spreading Assay method is particularly sensitive to substances that reduce surface tension, which aids in assessing the oil spreading capacity, the disruption of the oil film on water surfaces, and the ability to reduce surface tension. However, efficient surface tension reduction does not necessarily correlate with good emulsification capacity. Conversely, the Emulsification Capacity Assay (E24) method evaluates the ability to sustain a mixture of two immiscible phases (e.g., oil and water) by forming stable, small emulsion particles (micelles). Emulsifiers not only reduce the interfacial tension between the phases but must also maintain the emulsion's stability over time. Therefore, a substance with good surface tension reduction capability does not automatically indicate good emulsification performance, and similarly, effective emulsification does not inherently imply efficient cleaning ability[28]. Thus, the results from the two experiments suggest that strain B5 produces biosurfactants with excellent oil surface cleaning properties, although the emulsification capacity of these biosurfactants is lower than that observed when fermenting soapberry with all three strains simultaneously.

**Table 3: EC24 of soapberry samples after 6 days of fermentation with different strains. Different letters in the same column indicate statistically significant differences ($p < 0.05$, Tukey's test).**

| Sample | EC24 (%) |
|---|---|
| Soapberry B5 | $54.9 \pm 0.5^{a}$ |
| Soapberry B6 | $55.8 \pm 0.9^{ab}$ |
| Soapberry B8 | $55.5 \pm 1.4^{ab}$ |
| Soapberry B5 + B6 + B8 | $57.3 \pm 0.5^{b}$ |
| Control-Soapberry Control-SDS (1%) | $53.2 \pm 0.90^{ac}$ $53.8^{a}$ |

## CONCLUSION

The results from the soapberry fermentation product indicated that three Bacillus sp. strains (B5, B6, B8), selected from an initial screening of 20 isolates from GE, exhibit strong biosurfactant-producing capabilities. All three strains are spore-forming and demonstrate promising outcomes in cleaning assays. Strain B5 yielded the highest performance with the largest cleaning zone diameter on day 6. Genetic analysis revealed that both B5 and B6 strains expressed the fengycin gene, while strain B8 expressed both the fengycin and iturin genes, which are responsible for the production of distinct biosurfactants. The combination of all three strains was significantly larger than that of the control sample. Additionally, the E24 emulsification index of the fermented soapberry product was highest when all three strains were used, surpassing the results of other samples. These findings underscore the potential of fermented soapberry as a natural biosurfactant source in the development of cleaning products.

## LIST OF ABBREVIATIONS

GE: Garbage Enzyme
LB: Luria Broth
TE: Tris-EDTA
PCR: Polymerase Chain Reaction

DNA: Deoxyribonucleic Acid
RNA: Ribonucleic Acid

## CONFLICTS

The authors affirm that there are no conflicts of interest associated with this paper.

## AUTHOR CONTRIBUTIONS

Duy K. TRAN and Hien T.T. NGUYEN carried out the experiments and wrote the manuscript. Gia H. BAO and Oanh N. HUYNH provided comments on the manuscript. Dung M. HOANG conceived the idea, designed the experiments, and revised the final manuscript.

## ACKNOWLEDGEMENTS

We acknowledge Ho Chi Minh City University of Technology (HCMUT), VNU-HCM for supporting this study with grant number SVOISP-2024-KTHH – 77.

## REFERENCES

1. Dias M, Nitschke M. Bacterial-derived surfactants: an update on general aspects and forthcoming applications. Brazilian Journal of Microbiology. 2023;54(1):103–126.
2. Dini S, Bekhit AD, Roohinejad S, Vale JM, Agyei D. The physicochemical and functional properties of biosurfactants: a review. Molecules. 2024;29(11):2544–2544.
3. Snj P, Tolker-Nielsen T. Multiple roles of biosurfactants in structural biofilm development by Pseudomonas aeruginosa. Journal of Bacteriology. 2007;189(6):2531–2540.
4. Nakama Y. Surfactans. Cosmetic Science Technology. 2017;p. 231–275.
5. Moldes AB, Rodríguez-López L, Rincón-Fontán M, López-Prieto A, Vecino X, Cruz JM. Synthetic and bio-derived surfactants versus microbial biosurfactants in the cosmetic industry: An overview. International Journal of Molecular Sciences. 2021;22(5):2371–2371.
6. Aboelkhair H, Diaz P, Attia A, Engineering. Environmental comparative study of biosurfactants production and optimization using bacterial strains isolated from Egyptian oil fields. Journal of Petroleum Science. 2022;216:110796–110796.
7. Ron EZ, Rosenberg E. Natural roles of biosurfactants: Minireview. Environmental microbiology. 2001;3(4):229–265.
8. Davey ME, Caiazza NC, 'toole O, A G. Rhamnolipid surfactant production affects biofilm architecture in Pseudomonas aeruginosa PAO1. Journal of bacteriology. 2003;185(3):1027–1063.
9. Shreve G, Inguva S, Gunnam S. Rhamnolipid biosurfactant enhancement of hexadecane biodegradation by Pseudomonas aeruginosa. Molecular Marine Biology. 1995;4(4):331–338.
10. Jiang J, Wang Y, Yu D, Yao X, Han J, Cheng R. Garbage enzymes effectively regulated the succession of enzymatic activities and the bacterial community during sewage sludge composting. Bioresource Technology. 2021;327:124792–124792.
11. Nguyen QD, Nguyen LQD, Nguyen NN, T-N-L. Green removal of unpleasant volatiles from soapberry (Sapindus mukorossi) extracts by two-phase microbial fermentation fortified with pomelo peel waste. SC Advances. 2019;13:13282–91.
12. Hoang DM, Tran TT, Bui PH, Thanh N. Potential Concrete Healing Activity of Bacillus marisflavi Isolated in Vietnam. J Chemical Engineering Transactions. 2023;106:643–651.
13. Cooper DG, Goldenberg BG. Surface-active agents from two Bacillus species. Applied Environmental Microbiology. 1987;53(2):224–233.
14. Grover M, Nain L, Singh SB, Saxena AK. Molecular and biochemical approaches for characterization of antifungal trait of a potent biocontrol agent Bacillus subtilis RP24. Current Microbiology. 2010;60:99–106.
15. Zhao J, Zhang C, Lu J, Lu Z. Enhancement of fengycin production in Bacillus amyloliquefaciens by genome shuffling and relative gene expression analysis using RT-PCR. Canadian Journal of Microbiology. 2016;62(5):431–437.
16. Perumal V, Yao Z, Kim JA, Kim HJ, Kim JH. Purification and characterization of a bacteriocin, BacBS2, produced by Bacillus velezensis BS2 isolated from Meongge Jeotgal. J Microbiology Biotechnology. 2019;.
17. Ruiz-Garcia C, Bejar V, Martinez-Checa F, Llamas I, Quesada E. Bacillus velezensis sp. nov., a surfactant-producing bacterium isolated from the river Vélez in Málaga, southern Spain. International Journal of Systematic Evolutionary Microbiology;.
18. Wang J, Xing J, Lu J, Sun Y, Zhao J, Miao S. Complete genome sequencing of Bacillus velezensis WRN014, and comparison with genome sequences of other Bacillus velezensis strains. Journal of Microbiology Biotechnology. 2019;.
19. Zhang J, Feng W, Xue Q. Biosurfactant production and oil degradation by Bacillus siamensis and its potential applications in enhanced heavy oil recovery. J International Biodeterioration Biodegradation. 2022;169:105388–105388.
20. Gusmao CAD, Rufino RD, Sarubbo LA. Laboratory production and characterization of a new biosurfactant from Candida glabrata UCP1002 cultivated in vegetable fat waste applied to the removal of hydrophobic contaminant. World Journal of Microbiology Biotechnology;.
21. Shaligram NS, Singhal RS. Surfactin-a review on biosynthesis, fermentation, purification and applications. Food Technology Biotechnology;.
22. Seydlová G, Svobodová J. Review of surfactin chemical properties and the potential biomedical applications. Central European Journal of Medicine. 2008;3:123–156.
23. Banat IM, Franzetti A, Gandolfi I, Bestetti G, Martinotti MG, Fracchia L. Microbial biosurfactants production, applications and future potential. Applied Microbiology Biotechnology;.
24. Geetha SJ, Banat IM, Joshi SJ. Biosurfactants: Production and potential applications in microbial enhanced oil recovery (MEOR). Biocatalysis Agricultural Biotechnology;.
25. Varjani S, Pandey A, Upasani VN. Petroleum sludge polluted soil remediation: Integrated approach involving novel bacterial consortium and nutrient application. Science of The Total Environment. 2021;763:142934–142934.
26. Deleu M, Razafindralambo H, Popineau Y, Jacques P, Thonart P, Paquot M. Interfacial and emulsifying properties of lipopeptides from Bacillus subtilis. Colloids Surfaces A: Physicochemical Engineering Aspects;.
27. Morikawa M, Hirata Y, Imanaka T. A study on the structure-function relationship of lipopeptide biosurfactants. Biochimica et Biophysica Acta -Molecular Cell Biology of Lipids. 2000;1488(3):211–219.
28. Yin Y, Wang P, Wang X, Wen J. Construction of Bacillus subtilis for efficient production of fengycin from xylose through CRISPR-Cas9. Frontiers in Microbiology. 2024;14:1342199–1342199.

*VNUHCM Journal of Engineering and Technology 2026, 9(3): 3267-3275*

Open Access Full Text Article

*Bài báo nghiên cứu*

# Enhancing the cleaning ability of soapberry through fermentation with biosurfactant-producing bacteria isolated from Garbage Enzyme

**Duy K. TRAN[1,2], Hien T. T. NGUYEN[1,2], Gia H. BAO[1,2], Oanh N. HUYNH[1,2], Dung M. HOANG[1,2,*]**

Use your smartphone to scan this QR code and download this article

**TÓM TẮT**

Sự phát triển của các sản phẩm có nguồn gốc tự nhiên là một xu hướng hiện nay, trong đó các tác nhân tẩy rửa sinh học đóng vai trò then chốt. Những lo ngại ngày càng tăng về môi trường cùng với nhu cầu tìm kiếm các lựa chọn an toàn hơn, có khả năng phân hủy sinh học để thay thế hóa chất tổng hợp đã thúc đẩy mạnh mẽ sự quan tâm đối với các giải pháp thân thiện với môi trường. Garbage enzyme, được tạo ra theo con đường sinh học thông qua quá trình lên men chất thải hữu cơ, đã nổi lên như những ứng viên đầy tiềm năng nhờ tính bền vững và khả năng ứng dụng đa chức năng, đặc biệt trong lĩnh vực làm sạch và vệ sinh hộ gia đình. Bên cạnh khả năng phân hủy các chất hữu cơ, garbage enzyme cũng rất có khả năng chứa các loài *Bacillus*, vốn được biết đến rộng rãi với khả năng sản xuất các chất hoạt động bề mặt sinh học.

Nghiên cứu này nhằm phân lập và sàng lọc các dòng *Bacillus* có khả năng sinh chất hạot động bề mặt. Tổng cộng 20 dòng đã được phân lập từ garbage enzyme. Trong số đó, ba dòng có mức sản xuất chất hoạt động bề mặt cao nhất được lựa chọn dựa trên khả năng tẩy rửa đánh giá bằng thử nghiệm lan dầu, một phương pháp nhanh và đáng tin cậy để đánh giá hoạt động bề mặt. Giải trình tự gen 16S rRNA xác định ba dòng này (B5, B6 và B8) đều thuộc chi *Bacillus*. Đáng chú ý, sau ba ngày nuôi cấy, các dòng B5 và B6 biểu hiện gen mã hóa fengycin (một biosurfactant dạng lipopeptide có hoạt tính bề mặt mạnh), trong khi dòng B8 biểu hiện đồng thời fengycin và iturin. Các thí nghiệm lên men sử dụng bồ hòn cùng với ba dòng phân lập cho thấy khả năng lan dầu được tăng cường và chỉ số nhũ hóa EC24 được cải thiện so với lên men từng dòng riêng lẻ và so với bồ hòn không bổ sung vi khuẩn. Tuy nhiên, việc sử dụng nhiều dòng trong đồng lên men không cho thấy mức sản xuất chất hoạt động bề mặt hoặc giá trị EC24 cao hơn so với lên men đơn dòng. Những kết quả này nhấn mạnh tiềm năng của ba dòng *Bacillus* đã phân lập trong việc phát triển các sản phẩm tẩy rửa có nguồn gốc sinh học hiệu quả, bền vững.

**Từ khoá:** Bacillus, bồ hòn, chất hoạt động bề mặt, lên men

[1]*Khoa Kỹ thuật Hóa học, Đại học Bách khoa Thành phố Hồ Chí Minh (HCMUT), 268 Lý Thường Kiệt, Phường Diên Hồng, Thành phố Hồ Chí Minh, Việt Nam*

[2]*Đại học Quốc gia Thành phố Hồ Chí Minh, Phường Linh Xuân, Thành phố Hồ Chí Minh, Việt Nam*

**Liên hệ**

**Dung M. HOANG**, Khoa Kỹ thuật Hóa học, Đại học Bách khoa Thành phố Hồ Chí Minh (HCMUT), 268 Lý Thường Kiệt, Phường Diên Hồng, Thành phố Hồ Chí Minh, Việt Nam

Đại học Quốc gia Thành phố Hồ Chí Minh, Phường Linh Xuân, Thành phố Hồ Chí Minh, Việt Nam

Email: hoangmydung@hcmut.edu.vn

**Lịch sử**

- Ngày nhận: 24-04-2025
- Ngày sửa đổi: 11-08-2025
- Ngày chấp nhận: 03-07-2026
- Ngày đăng: 04-09-2026

**DOI : https://doi.org/10.32508/vnuhcmj-et.v9i3.1502**

Check for updates



**Trích dẫn bài báo này:** TRAN D K, NGUYEN H T T, BAO G H, HUYNH O N, HOANG D M. **Enhancing the cleaning ability of soapberry through fermentation with biosurfactant-producing bacteria isolated from Garbage Enzyme**. *VNUHCM J. Eng. Technol. 2026; 9(3):3267-3275.*