1 Screening of Philippine Actinomycetes for

2 Biosurfactant Production

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# Abstract

1. Biosurfactant are non-toxic and biodegradable surface active agents that can replace
2. petroleum-based surfactants used as emulsifier, dispersants and foaming agents. In this
3. study, twenty-eight (28) actinomycetes isolated from volcanic soil samples and distillery
4. effluent, sludge, mud press and coco dust were screened for the production of extracellular
5. biosurfactant. Preliminary screening was done by lipase assay and drop collapse test and
6. showed that 21 isolates are potential biosurfactant producers. Emulsification activity (E24) of
7. the isolates positive for the preliminary tests were determined wherein several isolates
8. exhibited high emulsification activities ranging from 43.58% -- 47.14%. This study implies
9. that actinomycetes have a potential in biosurfactant production and can be further studied
10. for optimization and industrial scale production.

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# Introduction

1. Surfactants are amphiphilic compounds that have the ability to lower the surface tensions
2. between two immiscible compounds. These have hydrophobic and hydrophilic moieties,
3. being used as emulsifiers and foaming agents as well as dispersants and wetting agents[1].
4. Surfactants have important pharmaceutical applications such as medium for the preparation
5. of water and oil soluble drugs, active ingredient in soaps[2] and in the food industry primarily
6. as foam or anti-foam agent[1]. Surfactants having these vast applications in various
7. multimillion industries, however, are mainly derived from petroleum and are mostly toxic and
8. non-degradable.

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1. Biosurfactants from bacteria and yeast are good alternatives to address the deleterious
2. effects to the environment of petroleum/chemical-based surfactants. In microorganisms,
3. these compounds are produced in order to utilize poorly accessible substrates specifically
4. water insoluble carbon sources such as hydrocarbons[3]. Like petroleum-based surfactants,
5. biosurfactants are capable of reducing interfacial tensions and producing foams and
6. emulsions. However, unlike the chemically synthesized surfactants, biosurfactants have
7. high biodegradability, low toxicity, multi-functionality, and environmental capability making
8. these compound a promising substitute for industrial and domestic applications[4]. Moreover,
9. biosurfactants are known to have high activities at extreme temperatures, pH, and salinity;
10. and can be produced at a cheaper cost using renewable substrates[5]. Thus, the production
11. of biosurfactant having its potential multimillion dollar applications and environment
12. compatibility, is now an emerging interest in the field of research.

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1. In this study, biosurfactant from local actinomycetes isolates isolated from volcanic soil
2. samples and distillery effluent, sludge, mud press and coco dust were screened for the
3. presence of biosurfactant via lipase plate assay and drop collapse test. The emulsification
4. index (E24) of the isolates were measured in order to determine promising biosurfactant-
5. producing actinomycetes with potential for industrial scale production.

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60 **Methodology**

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1. **Sampling and Isolation of Actinomycetes**
2. Sludge, mud press, coco dust, and effluent samples from an alcohol distillery plant in the
3. province of Tarlac and volcanic soil samples from different barangays in Albay were
4. collected. Isolation and enumeration of actinomycetes were done by serial dilution and
5. spread plating on starch casein agar (SCA) (soluble starch 1%, casein 0.03%, KNO3 0.2%,
6. MgSO4·7H2O 0.005%, NaCl 0.2%, CaCO3 0.002%, agar 1.8%). The plates were incubated
7. at 30 °C for 7 to 10 days. The cultures were observed and isolated colonies that have the
8. characteristics of actinomycetes being rough and elevated or embedded were picked and
9. streaked to new SCA plates. The isolated actinomycetes were also gram stained and
10. observed under the compound light microscope (Olympus Biological microscope CX-40,
11. Olympus Corporation, Tokyo, Japan) (10X magnification) to check for mycelia formation
12. and purity of the isolates.

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1. **Lipase Plate Assay**. Isolates were streaked on Luria Bertani agar (LBA) plates
2. supplemented with 1% Tween 80 and 0.01% CaCl, The plates were incubated at 37 °C for
3. 5 days and the presence halo of Ca-free fatty acid precipitates around the colonies were
4. observed[6].

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1. **Production of Biosurfactant.** Isolates were inoculated in glucose yeast extract (GYE)
2. (peptone 1%, NaCl 0.5%, glucose 1%, yeast extract 0.3%) broth with 1 mL of spore
3. suspension (0.25 OD) of the actinomycetes isolates, and incubated at ambient temperature
4. (30 °C) at 150 rpm shaking for 7 days. The broth cultures were centrifuged at 4,000 rpm
5. for 10 min to remove the cells and the resulting supernatant were used for the screening of
6. biosurfactant production.

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1. **Drop Collapse Test.** A micro titer plate with 96 wells were coated with 5 uL Pennzoil motor
2. oil (USA). A droplet of supernatant (5 uL) was then placed to each of the wells and were
3. observed after 1 minute[7]. Droplets that collapsed, which got bigger or flat, were positive for
4. biosurfactant production.

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1. **Emulsification Activity.** Equal volume of kerosene and supernatant (3mL) were added
2. in a dram vial and mixed using a high speed vortex mixer for 2 minutes[8]. The vials were
3. allowed to rest for 24 hours and the resulting emulsion layers were measured using a digital
4. caliper. Emulsification activity of the biosurfactant was expressed as emulsification
5. index (E24) computed as percentage of height of emulsified layer (mm) divided by total
6. height of the liquid column (mm).

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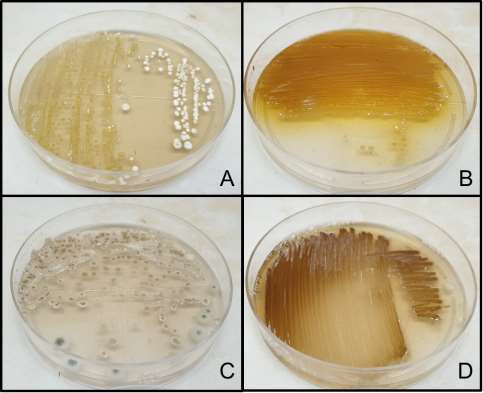
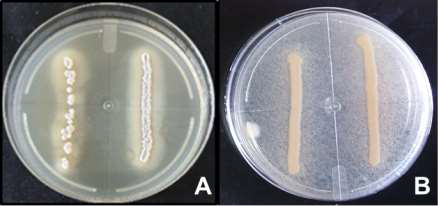
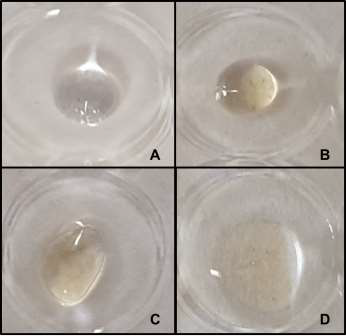
99 **Results**

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1. The isolated actinomycetes were screened for biosurfactant production by lipase plate
2. assay, drop collapse test, and emulsification index (E24) (Table 1).
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4. **Table 1.** Screening for biosurfactant production of actinomycetes isolates using three tests: lipase plate
5. assay, drop collapse test, and emulsification index of isolated actinomycetes.

|  |  |  |  |
| --- | --- | --- | --- |
| **Actinomycetes Isolate** | **Lipase Plate**  **Assay** | **Drop Collapse**  **Test** | **Emulsification Activity** |
| CADT 1 | + | + | + |
| CADT 2 | + | + | + |
| CADT 3 | + | - | - |
| CADT 4 | + | + | + |
| CADT 5 | + | + | + |
| CADT 6 | + | + | + |
| CADT 7 | + | + | + |
| CADT 8 | + | + | + |

|  |  |  |  |
| --- | --- | --- | --- |
| CADT 9 | - | + | + |
| CADT 10 | + | + | + |
| CADT 11 | + | - | + |
| CADT 12 | - | + | + |
| APJ 1 | - | + | + |
| APJ 2 | + | + | + |
| APJ 3 | - | - | + |
| APJ 4 | + | + | + |
| APJ 5 | - | - | + |
| VAA 1 | - | + | + |
| VAA 2 | + | - | + |
| JSM 8 | - | - | - |
| CGS A9 | + | + | + |
| CGS B11 | + | + | + |
| BDY A4 | + | + | + |
| CGS A10 | + | + | + |
| UNO B2 | + | + | + |
| BDY B13 | + | + | + |
| PURO B2 | + | + | + |
| UNO C14 | + | - | - |
| 106 |  |  |  |

1. The following figures show the actual images of actinomycetes isolates (Figure 1) as well
2. as their ability to form Ca-fatty acid precipitates (Figure 2) and collapsed drops (Figure 3)
3. indicating biosurfactant production as supported by their emulsification activities (Figure 4).
4. 110
5. **Figure 1.** Some of the isolated actinomycetes from soil compost, sludge, mudpress, coco dust, slops, waste
6. water and effluent samples. A) CGS A10, B) CADT 1, C) VAA 1, D) CADT 11.
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8. 114
9. **Figure 2.** Lipase plate assay. A) CGS B11, B) Negative control. Opaque halo are precipitates from free fatty
10. acid complexed with calcium indicating the presence of biosurfactant.
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12. **Figure 3.** Drop collapse test. (A) Water, (B) Broth medium, (C) Biosurfactant, (D) Tween 80 (chemical
13. surfactant)..
14. 
15. **Figure 4.** Biosurfactant activity forming emulsion layer. A) Negative, B) Positive, stable emulsion.
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# Discussion

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2. A total of 28 actinomycetes were isolated and purified from soil and distillery samples.
3. Colonies grown on starch casein agar (SCA) were characterized as rough in texture with
4. varying colors and were flat or embedded having white to black aerial spores. Most of the
5. isolates had extensive formation of mycelia as observed under the compound light
6. microscope (10X magnification) and were gram positive. The isolates were observed to
7. have an earthly smell due to production of geosmin[9], which is a known characteristic of
8. actinomycetes.
9. 132
10. Luria Bertani (LB), which is a carbon limited medium[10] was used for lipase plate assay. The
11. addition of 1% polyethylene sorbitan monooleate (Tween 80) allows lipase producing
12. isolates to utilize this anionic surfactant as a major carbon source. Consequently, the free
13. fatty acids from the hydrolysis of Tween 80 can form insoluble complex with the calcium
14. ions present in the medium as shown in Figure 2. This complex can be observed as opaque
15. halo regions surrounding the colonies. Previous study emphasized the significance of lipase
16. and biosurfactant producing bacteria with useful applications in the cosmetics,
17. pharmaceutical, agriculture and food industries[11]. As indicated in Table 1, a total of 21
18. isolates had positive result for lipase plate assay.
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20. Biosurfactants are low molecular weight glycolypids or lipopeptides, which are amphiphatic
21. in nature and capable of dissolution in polar and non-polar compounds[12]. These
22. amphiphatic compounds are known to reduce surface and interfactial tensions between
23. non-identical phases. In microbiological systems, these compounds solubilize hydrophobic
24. molecules such as in oil contaminated soil and water and also increase cell surface
25. hydophobicity for easy uptake of hydrophobic substrates[13]. In the drop test, the isolated
26. actinomycetes were tested qualitatively for its ability to reduce or lower surface tension. In
27. this test, a droplet of cell free supernatant was added in each of the wells of an oil-coated
28. microtiter plate. Collapsed droplets signified lowered surface tension and positive for
29. biosurfactant production while intact droplets (drop beaded up) implied the absence of
30. biosurfactant. Biosurfactant-producting isolates had droplets size that were bigger
31. compared to water (negative control) and comparable with Tween 80 (positive control) as
32. illustrated in Figure 3. Addition of the biosurfactant and Tween 80 caused the drops to
33. spread due to the lowering of surface and interfacial tension between the oily surface and
34. the water. On the other hand, the drop lacking surfactant remains beaded because the oily
35. surface is hydrophobic and therefore the force causes aggregation. A total of 21 isolates
36. showed positive results for drop collapse test as indicated in Table 1.
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38. Biosurfactants being amphiphatic are also known for their ability to form stable emulsions
39. of immiscible liquids through the formation of micelles. These compounds are capable of
40. emulsifying even at very low concentrations having high kinetic stability[13]. In
41. bioremediation, biosurfactant play a major role in extracting oil contaminants by tightly
42. binding to the hydrocarbons for easy removal[14]. In the determination of emulsification
43. activity, emulsions were primarily formed by the binding of kerosene to the hydrophobic
44. moiety of the amphiphilic biosurfactant molecules that in turn form micelles. Emulsification
45. activity varied among the actinomycetes isolates used in this study (data not shown). The
46. emulsification indices were used as basis to choose the best isolate for the optimization of
47. biosurfactant production study . However, it is to note that the consistency of the emulsions
48. were also considered as to being loose, compact or intense. Isolates that had dense
49. emulsions (intense) were considered as better compared to those emulsions containing
50. few (compact) to many (loose) bubbles.
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52. Isolate CGS B11 was chosen to be the best biosurfactant producing actinomycetes, having
53. an emulsification index of 43.58% with intensely dense emulsion consistency. Moreover,
54. CGS B11 had comparatively the fastest observable growth among the isolates that gave
55. intense emulsions being able to grow in GYE standard medium at about 48 hours. Although
56. some isolates such as CGS A10 (E24=46.43%) and BDY B13 (E24=45.71%) had higher
57. intense emulsions, CGS B11 was still chosen because of its faster growth and ability to
58. form finer cells and inoculum size can be easily standardized. With these considerations,
59. CGS B11 is the best isolate for biosurfactant production study to done in the future. Despite
60. the differences in the E24, all the emulsions were stable at room temperature wherein there
61. were no changes in E24 after several hours. Emulsions of this type are applicable in the food,
62. pharmaceutical, agrochemical, personal care and cosmetics industries. There is a rising
63. application of emulsion in food processing industries where emulsions are used to
64. encapsulate, deliver, and protect food components such as oil-soluble flavors, vitamins,
65. colorants, preservatives, and other bioactive ingredients[15].
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# Conclusion

1. Based from the results of the biosurfactant screening experiments, twenty-one (21)
2. actinomycetes isolates were determined to be biosurfactant producers. The isolated
3. actinomycetes had varying emulsification activities wherein several isolates showed
4. emulsification indices of 47.14% - 43.58%. These isolates are promising isolates for
5. optimization studies that can be applied in large industrial scale production of biosurfactant.

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