Asian Journal of Green Chemistry 10 (2026) 464-482

Asian Journal of Green Chemistry

Original Research Article View Article Online | View Journal

Biosurfactant Production Using Coconut Meal by Indigenous *Bacillus velezensis* ES7.3: Genetic Characterization and Antifungal Activity against *Fusarium oxysporum*

Farah Aisyah Nafidiastri[1,2], Salamun[3,4,5,6,*], Ninik Fadhillah[7], Ni'matuzahroh [3,4,5], Fatimah[3,4,5], Almando Geraldi[3,4,5], Amalia Rizky Febriyanti[3]

[1]*Doctoral Program of Mathematics and Natural Sciences, Faculty of Science and Technology, Universitas Airlangga, Surabaya, 60115, Indonesia*
[2]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Surabaya, Ketintang Surabaya, 60231, Indonesia*
[3]*Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, 60115, Indonesia*
[4]*Research Group of Applied Microbiology and Bioresource Technology, Universitas Airlangga, Surabaya, 60115, Indonesia*
[5]*University of Co-E-Research Center for Bio-Molecule Engineering, Universitas Airlangga, Surabaya, 60115, Indonesia*
[6]*Institute of Tropical Diseases, Universitas Airlangga, Surabaya, 60115, Indonesia*
[7]*Faculty of Science and Technology, Sunan Ampel Islamic University, Surabaya, Indonesia*

ARTICLE INFORMATION

Submitted: 2025-10-11
Revised: 2025-11-20
Accepted: 2025-12-13
Published: 2025-12-21
Manuscript ID: AJGC-2511-1859
DOI: 10.48309/AJGC.2026.556599.1859

KEYWORDS

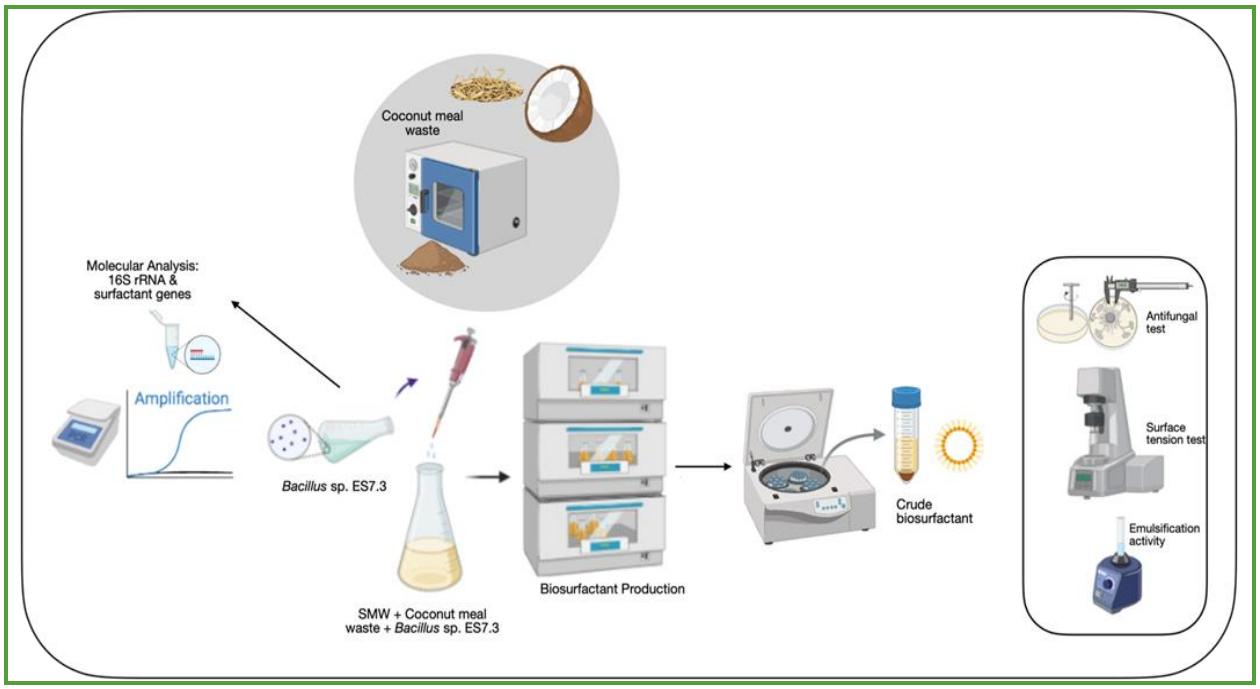
Bacillus velezensis
Biosurfactant
Lipopeptide
Coconut meal
Antifungal activity
Fusarium oxysporum

ABSTRACT

Bacillus sp. are a group of bacteria that produce biosurfactants on agroindustrial substrates. This study aimed to determine the species name of *Bacillus* sp. ES7.3 and biosurfactant-related genes. Additionally, the growth response of *Bacillus* sp. ES7.3 on coconut meal media, the activity of the crude biosurfactant extract, and the effectiveness of crude biosurfactant extract production on coconut meal and glucose were compared. The antifungal activity of *Bacillus* sp. ES7.3 was tested. The research included 16S rRNA gene detection, biosurfactant activity screening, biosurfactant biosynthesis gene detection, crude biosurfactant extract production from coconut meal, synthetic mineral water + 2% glucose media for comparison, and antifungal activity testing. The results of species identification showed that *Bacillus* sp. ES7.3 isolate was 99.31% homologous with *Bacillus velezensis* strain CBMB205. In turn, the biosurfactant activity of *B. velezensis* ES7.3 isolate was characterized by the formation of a clear zone around the colony on blood agar media. Furthermore, surfactin thioesterase biosynthesis genes srfAD and ituD were detected. Meanwhile, *B. velezensis* ES7.3 grown on coconut meal media produced a biomass of 2.9 mg/mL after 48 h of incubation. The production of crude biosurfactant by *B. velezensis* ES7.3 grown on coconut meal media was more effective than that of the 2% glucose Synthetic Mineral Water media substrate, as indicated by a greater percentage of emulsification activity and a lower surface tension on coconut meal media. Regarding the antifungal activity, *B. velezensis* ES7.3 was able to suppress the growth of *Fusarium oxysporum* by more than 50%.

Corresponding author, E-mail: salamun@fst.unair.ac.id (Salamun).
Tel.: +6281332198122.

Graphical Abstract



Introduction

Indonesia is an agricultural country with vast areas of farmland and abundant natural resources. The Indonesian government has developed various programs to improve agricultural production processes. This policy aims to ensure national, sustainable food security to meet the increase in demand due to an ever-increasing population. Therefore, crop yields must be maximized. However, a successful harvest depends on adequate nutrient availability and a supportive environment. The most limiting environmental factors for agriculture in Indonesia include extreme weather conditions, pH, high ambient temperature, and the presence of pathogens that can affect the yield and quality of agricultural products. Plant pathogens that often attack plants include various bacteria and fungi groups, as well as a range of insect pest species, which farmers rely mainly on synthetic fungicides and pesticides to combat. However, using these products poses a severe threat to the surrounding environment because the active ingredients involved are hazardous chemicals, including the compounds used as surfactants to reduce surface tension and increase the spreading and wetting properties of a liquid. Thus, the use of synthetic surfactants has a negative impact because of their high level of toxicity and low biodegradability in the environment [1]. Alternative solutions are urgently required that are environmentally friendly and have superior properties, namely, biosurfactants, to replace conventional chemical surfactants. Biosurfactants are amphiphilic molecules produced by living organisms, including microbial groups such as bacteria, molds, and yeasts. These compounds can be applied in various fields, including agriculture, industry, cosmetics, health, food, and bioremediation in oil-polluted environments [2].

Members within the genus *Bacillus* are bacteria capable of synthesizing cyclic lipopeptide-type biosurfactants through Non-

Ribosomal Peptide Synthesis (NRPs). Cyclic lipopeptides are amphiphilic peptides composed of hydrophilic oligopeptides (with *L*- and *D*-amino acids) linked to hydrophobic fatty acid chains [3]. Notable differences in the resulting cyclic lipopeptides include the type and sequence of amino acids in the peptide, as well as the branching of the fatty acid chain. Cyclic lipopeptides can be classified into three families: (1) iturin, (2) fengycin and plipastatin, and (3) surfactin [4,5]. Several bacterial species in the genus *Bacillus* reportedly produce lipopeptide biosurfactants, including *Bacillus velezensis* ES4.3, *Bacillus mojavensis* EG6.4, and *Bacillus subtilis* BK7.1 [6-9].

Using waste material as a nutrient source in fermentation media for biosurfactant production is an attractive alternative to reduce production costs and sustainably protect the environment. Agroindustrial waste contains high levels of carbohydrates, lipids, and proteins [10]. Furthermore, several studies using agroindustrial wastes as nutrients in fermentation media for biosurfactant production have been reported, including the use of corn cob waste [11], grape shoots [12], sugarcane bagasse [13], aqueous extracts of orange, potato, and banana peels, and banana peel molasses [14], pineapple peel [2], and date molasses [15]. However, research on using coconut meal waste as a substrate for biosurfactant production media by *Bacillus* ES7.3 isolates has never been conducted. Therefore, in this study, *Bacillus* ES7.3 isolates were utilized that use coconut meal media as a source of organic compounds, vitamins, and minerals for growth and biosurfactant synthesis. Biosurfactant production by microorganisms can be demonstrated by screening for biosurfactant activity through hemolytic or emulsification activity, surface tension, critical micelle concentration, drop collapse test, oil spreading [16,17], and detection of biosurfactant biosynthesis-related genes [18]. Moreover, genes involved in the biosynthesis of various lipopeptides from the iturin, fengycin, and surfactin families can be detected using Polymerase Chain Reaction (PCR) with specific primers [4].

Therefore, this study was conducted to identify the bacterial species of *Bacillus* sp. ES7.3, which can be developed as a candidate green biocide agent against the plant pathogenic fungus *F. oxysporum*, the genes involved in the biosynthesis of biosurfactants, and the activity of crude biosurfactant extract on coconut meal medium. Lastly, the effectiveness of crude biosurfactant production by *Bacillus* sp. ES7.3 isolates was compared when grown on coconut meal media against those observed when 2% glucose Synthetic Mineral Water (SMW) media substrate was used.

Experimental

Molecular identification of Bacillus sp. ES7.3 by 16S rRNA gene

The *Bacillus* sp. ES7.3 16S rRNA gene was identified in isolates grown on SMW media and coconut meal and incubated at 37 °C in an incubator at 120 rpm for 24 h. *Bacillus* sp. ES7.3 DNA was isolated using the CTAB method and visualized using electrophoresis [19]. The 16S rRNA gene was amplified using SelectCycler II Gradient Thermal Cycler (Select BioProducts, USA) beginning with the addition of GoTaq Green Master Mix (Promega, Madison, USA) and 16S rRNA primers 27F and 1492R. The PCR cycle was as follows: initial denaturation at 94 °C for 2 min, followed by denaturation at 92 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 1 min, with final elongation for 35 cycles at 72 °C for 5 min. The PCR products were visualized using electrophoresis on a 1% agarose gel stained with ethidium bromide under ultraviolet light and subsequently analyzed by 1st

Base DNA Sequencing Service, Malaysia. Amplicons were then sequenced and analyzed for similarity with GenBank data using National Center for Biotechnology Information (NCBI) BLASTn [20]. The data were analyzed for kinship relationships by constructing a phylogenetic tree using Molecular Evolutionary Genetics Analysis (MEGA) software version 6 [21]. MEGA software was developed for comparative analysis of DNA and protein sequences aimed at inferring molecular evolutionary patterns of genes, genomes and species over time [21,22].

Detection of biosurfactant-encoding genes B. velezensis ES7.3

The same procedure was used for 16S rRNA identification to identify the surfactin gene using srfA-D gene primers designed in-house with the Thermo Fisher Scientific OligoPerfect Primer Designer application for cloning.

Screening of biosurfactant activity by hemolytic test

Screening of biosurfactant activity was performed qualitatively through a hemolytic test on Blood Agar medium to determine the ability of bacteria to produce biosurfactants. *Bacillus* sp. ES7.3 (24 h) was inoculated on Blood Agar using the dot method and incubated for 24 to 72 h. Observations were made every 24 h to record any changes. A positive indicator shows that a clear zone will form around the three isolates, indicating their ability to lyse blood cells.

Production of crude biosurfactant B. velezensis ES7.3

The production of biosurfactants was initiated using SMW by dissolving 3 g $(NH_4)_2SO_4$, 10 g NaCl, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.01 g $CaCl_2$, 0.001 g $MnSO_4 \cdot H_2O$, 0.001 g H_3BO_3, 0.001 g $ZnSO_4 \cdot 7H_2O$, 0.001 g $CuSO_4 \cdot 5H_2O$, 0.005 g $CoCl_2 \cdot 6H_2O$, and 0.001 g $NaMoO_4 \cdot 2H_2O$ into 900 mL of distilled water. Elemental phosphate and iron were prepared by separately dissolving 5 g KH_2PO_4 and 2 g K_2HPO_4, and 0.0006 g $FeSO_4 \cdot 7H_2O$ into 50 mL of distilled water, respectively. Meanwhile, the coconut meal substrate was prepared by weighing 100 g of coconut meal and dissolving it in 1000 mL of distilled water. The isolate culture medium was prepared by mixing 2% coconut meal substrate solution with 86.4 mL of SMW. The control medium was prepared using SMW + 2% glucose and coconut meal substrate. All media were sterilized by autoclaving for 15 min at 121 ℃ and a pressure of 1 atm. Cultures (4%) of *Bacillus* ES7.3 isolate, with an OD value of 0.5, were added to the media. The emulsification activity and surface tension were measured at 24 h intervals until 144 h of incubation.

Antifungal activity of B. velezensis ES7.3 against F. oxysporum

In vitro antifungal activity tests were conducted using the swab method to evaluate the ability of *Bacillus* sp. ES7.3 isolates to inhibit the growth of the pathogenic fungus *F. oxysporum*. For that purpose, *F. oxysporum* isolates were rejuvenated on Potato Dextrose Agar (PDA) medium for 5 days and cut into small blocks with a cutter. *Bacillus* sp. ES7.3 isolates were cultured in Nutrient Broth (NB) medium for 72 h and then centrifuged. The culture forms tested included the supernatant fraction without cells, the sonication pellet fraction, and pure culture. The three culture forms were spread on PDA medium in a Petri dish, and a hole was made in the center of the medium to place an *F. oxysporum* inoculum block. Observations were made daily during the 144 h incubation period, and the growth area of *F. oxysporum* was calculated using a predetermined formula.

Data analysis

Nucleotide sequence data were obtained to identify encoding genes and compared with the GenBank database at NCBI using the Basic Local Alignment Search Tool (BLAST) program. For gene detection, data on protein-coding genes and proteins that make up *Bacillus* sp. ES7.3 isolates were obtained through BLASTx analysis, while those for species exploration based on DNA isolation results were obtained through BLASTn analysis. In addition, data in the form of bands obtained from gene amplification via PCR were analyzed descriptively. Data on biosurfactant production by *Bacillus* sp. ES7.3 isolates, emulsification activity, surface tension in biosurfactant production with coconut meal media, and fungal growth inhibition activity were analyzed statistically and descriptively. Normality (Shapiro–Wilk) and homogeneity (Levene test) tests were used, followed by continuous analysis of variance.

Results and Discussion

Bacillus sp. ES7.3 molecular identification by the 16S rRNA Gene

Bacillus sp. ES7.3 isolate was identified at the molecular level using the 16S rRNA gene to determine the species name of the isolate. DNA isolation results for *Bacillus* sp. ES7.3 are shown in Figure 1. The results showed that *Bacillus* sp. ES7.3 was approximately 1,500 bp in size.

The DNA sequences obtained were analyzed using BioEdit software and BLASTn and aligned with sequences in the NCBI GenBank database. The results of the sequencing analysis are summarized in Table 1.

The results of BLASTn analysis (Table 1) showed that *Bacillus* sp. ES7.3 is similar to *B. velezensis* strain CBMB205 (99.31%) under GenBank accession number NR 116240.1. This homology rate was slightly greater than that between *B. velezensis* strain FZB42 and B. amyloliquefaciens strain NBRC 15535 (99.30 %).

Phylogenetic tree analysis

Phylogenetic tree analysis was conducted to determine the kinship relationship of isolates of *B. velezensis* ES7.3 to other types of *Bacillus* isolates. The results of this analysis are shown in Figure 2.

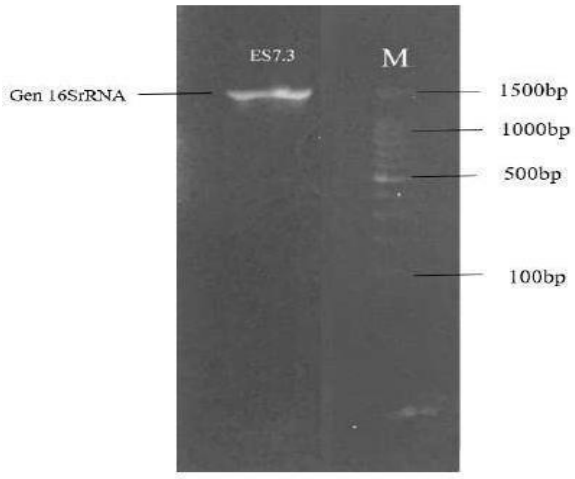


Figure 1. DNA visualization results of *Bacillus* sp. ES7.3 isolate

Table 1. *Bacillus* sp. ES7.3 nucleotide analysis using BLASTn

Species name	Accession number	% Homology	Query cover
Bacillus velezensis strain CBMB205	NR 116240.1	99.31%	100%
Bacillus velezensis strain FZB42	NR 075005.2	99.30%	99%
Bacillus amyloliquefaciens strain NBRC 15535	NR 112685.1	99.30%	99%

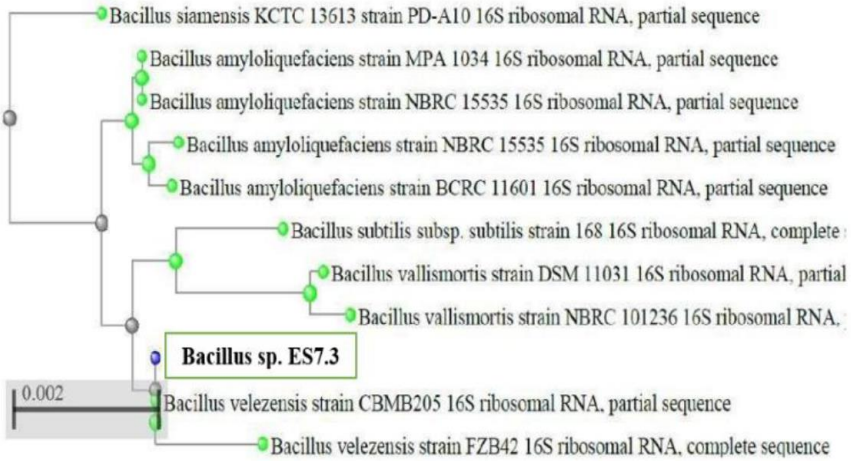


Figure 2. Results of phylogenetic tree analysis of *Bacillus velezensis* ES7.3 isolate with other types of *Bacillus* spp. isolates

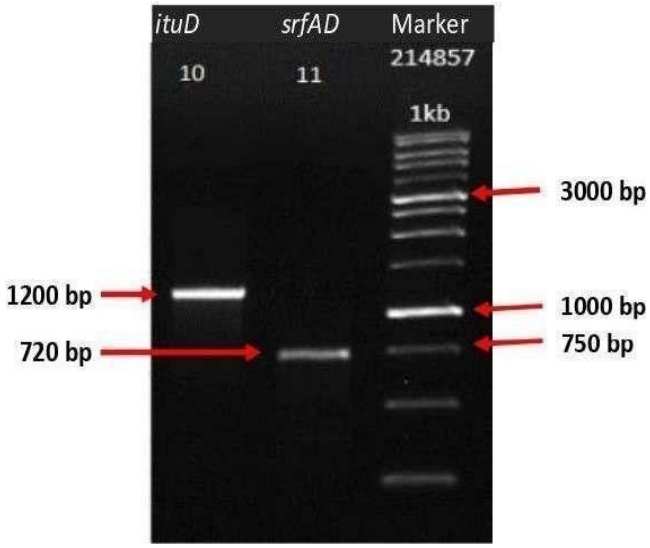


Figure 3. Electrophoretic visualization of *Bacillus velezensis* ES7.3 amplified srfA-D and ituD

Analysis of biosurfactant encoding-genes in B. velezensis ES7.3

The results of srfAD and ituD gene amplification in sequences were analyzed using BioEdit and aligned using BLASTn and BLASTx on the NCBI for Biotechnology Information website. Visualization of the srfAD and ituD gene amplification results for *B. velezensis* ES7.3 isolates is shown in Figure 3.

The srfAD and ituD gene DNA bands showed a size of 720 and 1200 bp, respectively, indicating that there are DNA sequences on primers that are complementary or homologous

to the sequences of srfAD and ituD gene samples that were successfully amplified (Figure 3). The results of BLASTn srfAD and ituD analyses showed that the srfAD and ituD gene sequences of *Bacillus* sp. ES7.3 exhibited more than 99% homology with those of *B. velezensis* (Table 2).

Amino acid sequence analysis using BLASTx NCBI showed that the srfAD gene sequence is the same as the "surfactin biosynthesis thioesterase srfAD" gene, with a percent homology of 99.98% with *B. amyloliquefaciens*. Meanwhile, according to BLASTx NCBI results, the sequence of the gene ituD from *B. velezensis* ES7.3 is the same gene as "Bacillomycin D biosynthesis malonyl-CoA transacylase BamD," with a percent homology of 99.72% with *B. velezensis* (Table 3).

Screening of biosurfactant activity by hemolytic test

A hemolytic activity test of the *B. velezensis* ES7.3 isolate was conducted using blood agar media (Figure 4). The test results showed that the isolate *B. velezensis* ES7.3 effectively hemolyzed blood, indicating that the bacterium produced biosurfactants, which effectively maintained a clear area around the bacterial colony in the medium. Furthermore, the amount of biosurfactant produced is reportedly proportional to the clear area on the blood agar.

Production of crude biosurfactant B. velezensis ES7.3 on coconut meal medium

The growth response of *B. velezensis* ES7.3 in the coconut meal medium was measured based on the values of bacterial biomass and cell density (CD). These measurements were performed from 0 to 144 h of incubation time. The biomass and OD values for *B. velezensis* ES7.3 cultured on the coconut meal medium are shown in Figure 5.

Table 2. Analysis of the percent homology of nucleotide sequences of the srfAD and ituD genes of *B. velezensis* ES7.3 using BLASTn

Target gene	Bacterium	Suitability length sequences	% Homology	Accession number
srfAD	*Bacillus velezensis* strain Htg6	100%	99.86	CP050462.1
ItuD	*Bacillus velezensis* strain KD1	100%	99.72	CP014990.2

Table 3. Analysis of the percent homology of amino acid sequences of the srfAD and ituD genes of *B. velezensis* ES7.3 using BLASTx

Target gene	Gene description of amino acid sequence	Length of sequence concordance	% Homology	Accession number
srfAD	Surfactin biosynthesis thioesterase srfAD (*Bacillus amyloliquefaciens* group)	99%	99.49%	WP0031563831
ItuD	Bacillomycin D biosynthesis of CoA transacylase BamD (*Bacillus velezensis*)	99%	99.72%	WP0700819041

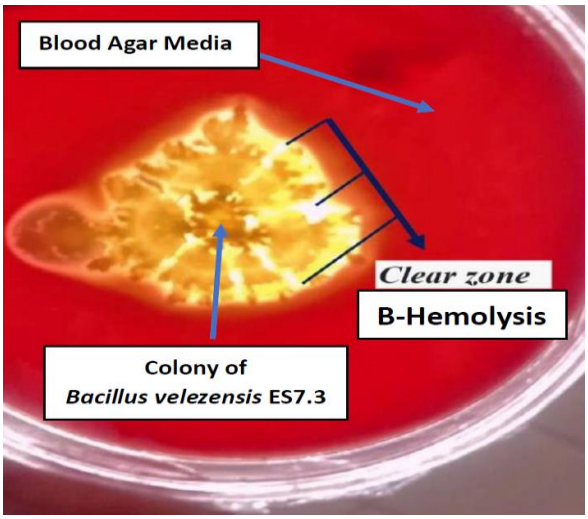


Figure 4. *β*-Hemolysis activity of *Bacillus velezensis* ES7.3 on blood agar media

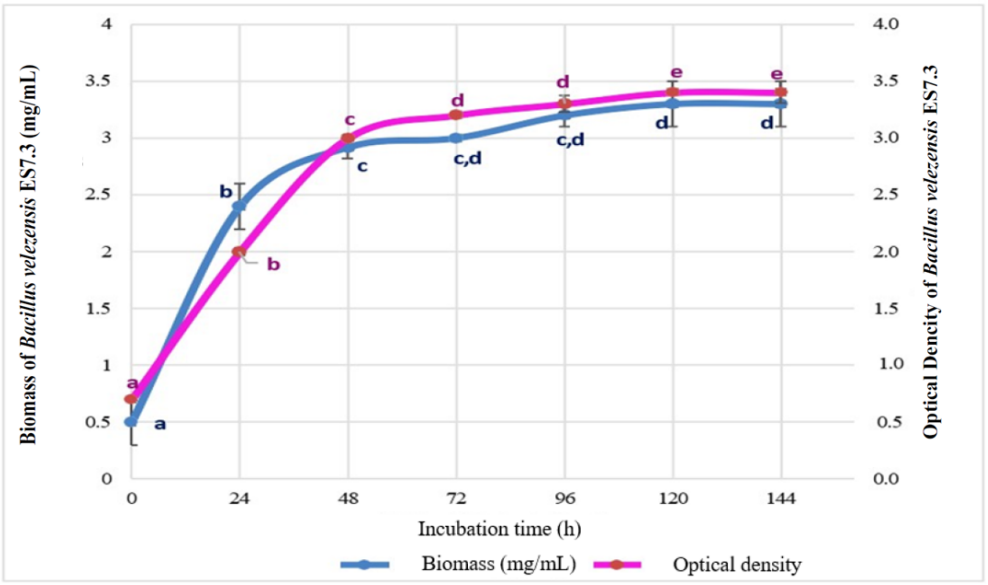


Figure 5. Growth curve of *Bacillus velezensis* ES7.3 on coconut meal medium in terms of biomass and OD values at 0, 24, 48, 72, 96, 120, and 144 h after incubation initiation. Different lowercase letters on the growth OD curves at the different sampling time points indicate a significant difference

The growth of *B. velezensis* ES7.3 began to slow down after 48 h incubation, presumably due to limited nutrient availability in the medium.

Consistently, as Figure 6 shows, the total amount of sugar in the medium significantly decreased after 48 h incubation. Figure 7 shows that the stationary phase of *B. velezensis* ES7.3 growth occurred over the 120–144 h period of the incubation time. The emulsification activity of *B. velezensis* ES7.3 reached a maximum value (80%) at 144 h. This high emulsification activity at 144 h indicated increased crude biosurfactant production until the last day of incubation. Different lowercase letters on the biomass curve

and emulsification bars at different sampling time points indicate a significant difference. The increase in the percent emulsification activity of crude biosurfactant in coconut meal medium resulted in a high emulsion layer produced by *B. velezensis* ES7.3 (Figure 8).

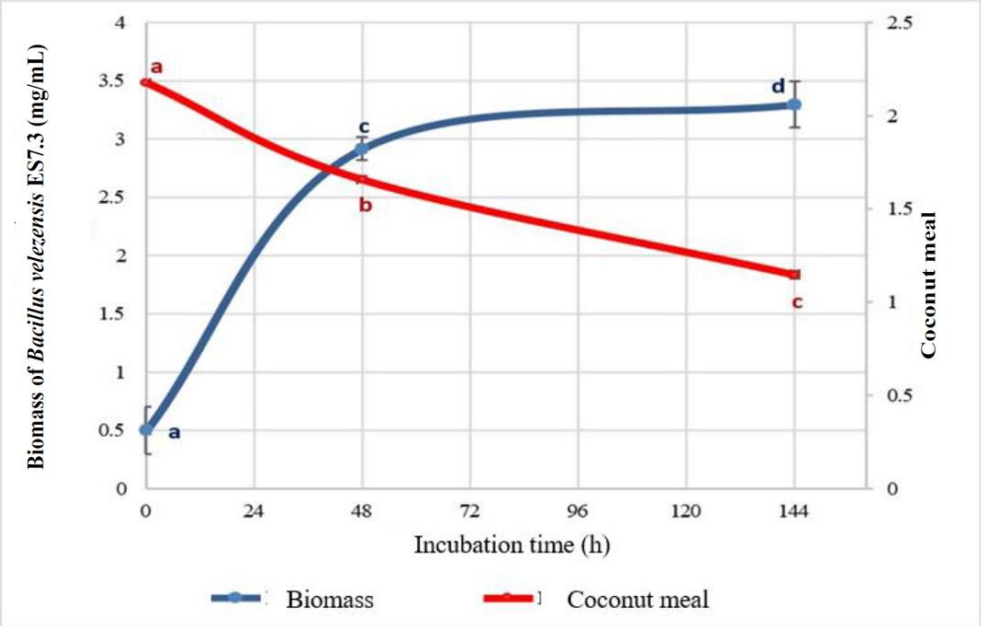


Figure 6. Growth curve of *Bacillus velezensis* ES7.3 on coconut meal medium and total sugar concentration changes over the 144 h incubation period. Different lowercase letters on the growth and sugar concentration curves at different sampling time points indicate a significant difference

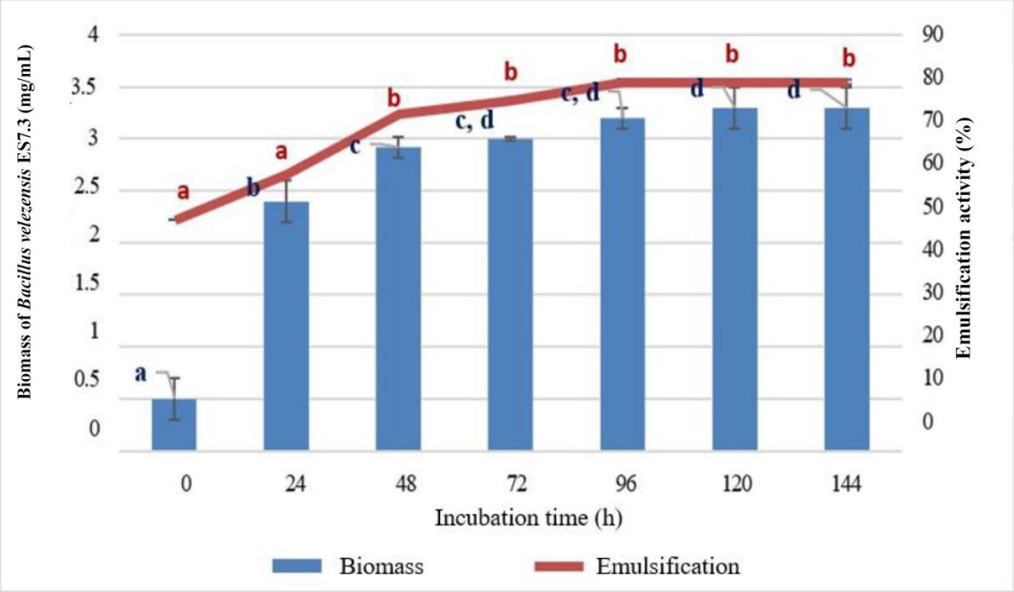


Figure 7. Relationship between the biomass of *Bacillus velezensis* ES7.3 grown on coconut meal medium and percent emulsification activity of crude biosurfactants

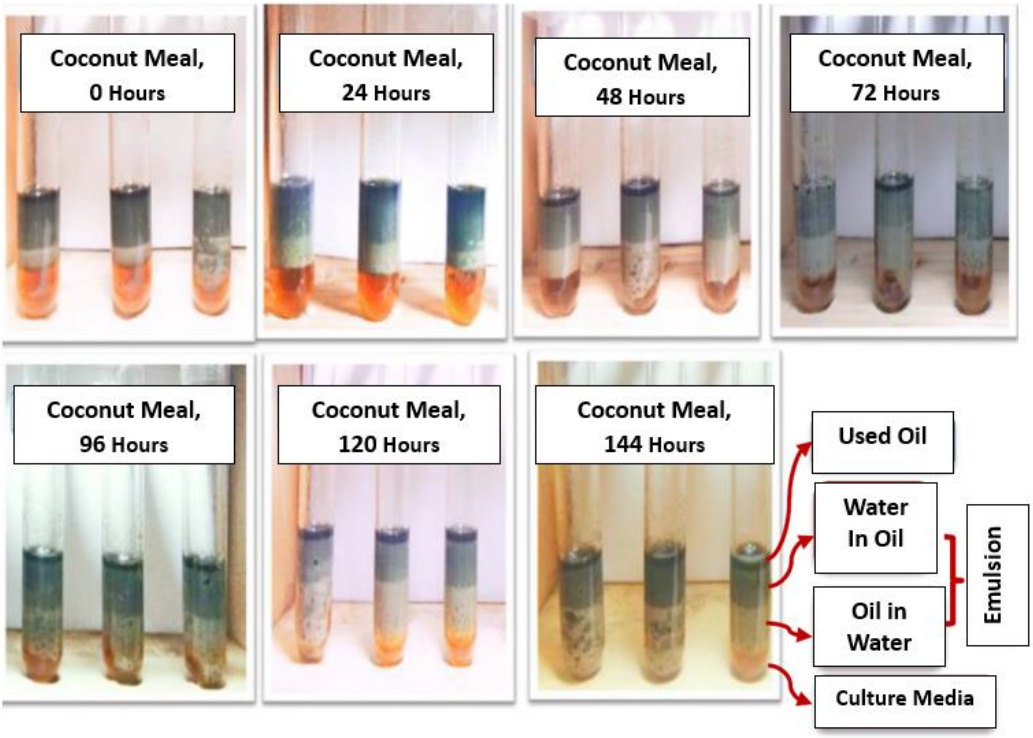


Figure 8. Emulsification activity of crude biosurfactant produced by *Bacillus* velezensis ES7.3 on coconut meal media using used oil

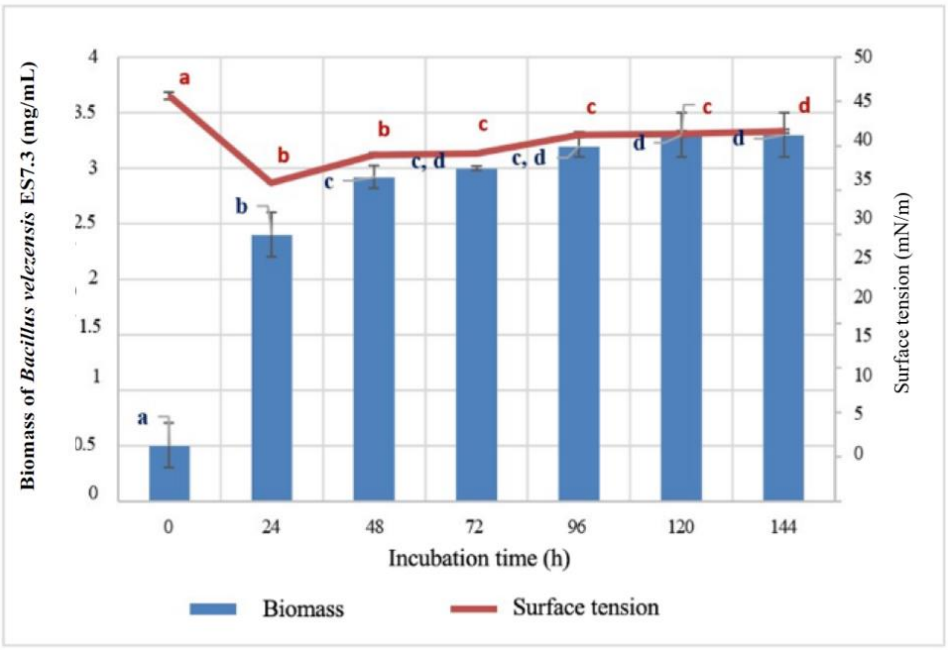


Figure 9. The biomass and surface tension of *Bacillus velezensis* ES7.3 in coconut meal medium. Different lowercase letters on the biomass curve and the surface tension bars at different sampling time points indicate a significant difference

The surface tension of crude biosurfactant in coconut meal medium was lowest (35.9 mN/m) at 24 h of incubation (Figure 9), but tended to increase after 48 h of incubation and continued to show this trend until the end of the 144-h incubation period. The decrease in surface tension at 24 h of incubation time may be due to the presence of *B. velezensis* ES7.3, which released low molecular weight biosurfactants, such as surfactin and iturin D, that act as surface tension-reducing agents in the culture medium.

However, during the interval from 48 to 144 h incubation time, *B. velezensis* ES7.3 released high molecular weight biosurfactants in greater quantities, increasing the surface tension over that time interval. Concomitantly, emulsification activity increased until the 144-h incubation period. A comparison of the effectiveness of crude biosurfactant production by *B. velezensis* ES7.3 grown on coconut meal medium against that observed on SMW glucose substrate medium as shown in Figure 10, the highest percentage of emulsification activity in the coconut meal and SMW + glucose media occurred at 144 h of incubation.

The coconut meal medium showed a higher emulsification rate (80%) than that observed in the SMW + 2% glucose medium. Additionally, a significant difference was observed between the surface tension of the crude biosurfactant in coconut meal and that in SMW + 2% glucose medium (Figure 10).

The surface tension was lower in coconut meal than in SMW + 2% glucose medium. The lowest surface tension in the coconut meal medium was 35.9 mN/m, whereas that in the SMW + glucose medium was 55.1 mN/m (Figure 11).

Antifungal activity of B. velezensis ES7.3 against F. oxysporum

Biosurfactants are known to be applied in various fields, including agriculture. The ability of *B. velezensis* ES7.3 to suppress the growth of the plant pathogenic fungus, *F. oxysporum*, was evaluated for 7 d (Figure 12).

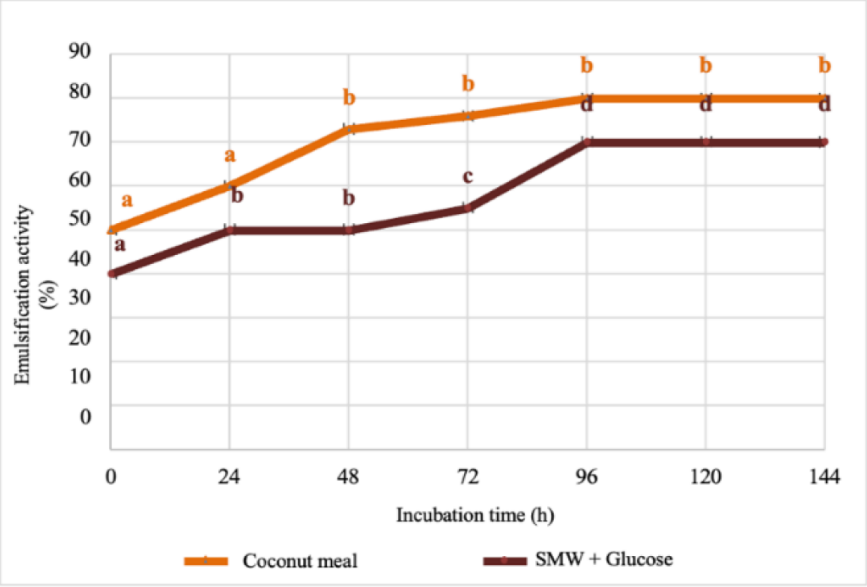


Figure 10. Comparison of the effectiveness of crude biosurfactant production by *B. velezensis* ES7.3 grown on coconut meal media with that observed on SMW glucose substrate media

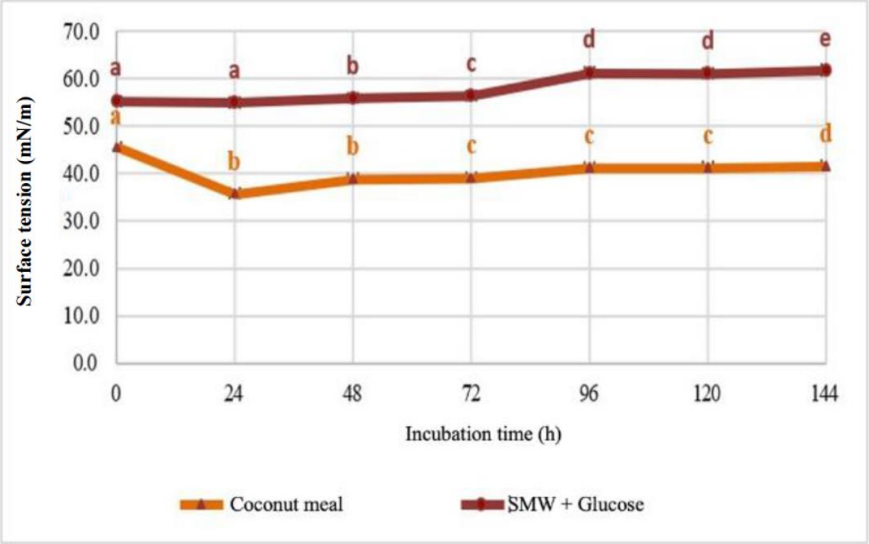


Figure 11. Surface tension of crude biosurfactant produced by *B. velezensis* ES7.3 in coconut meal and SMW (Steril Mineral Water) + 2% glucose medium

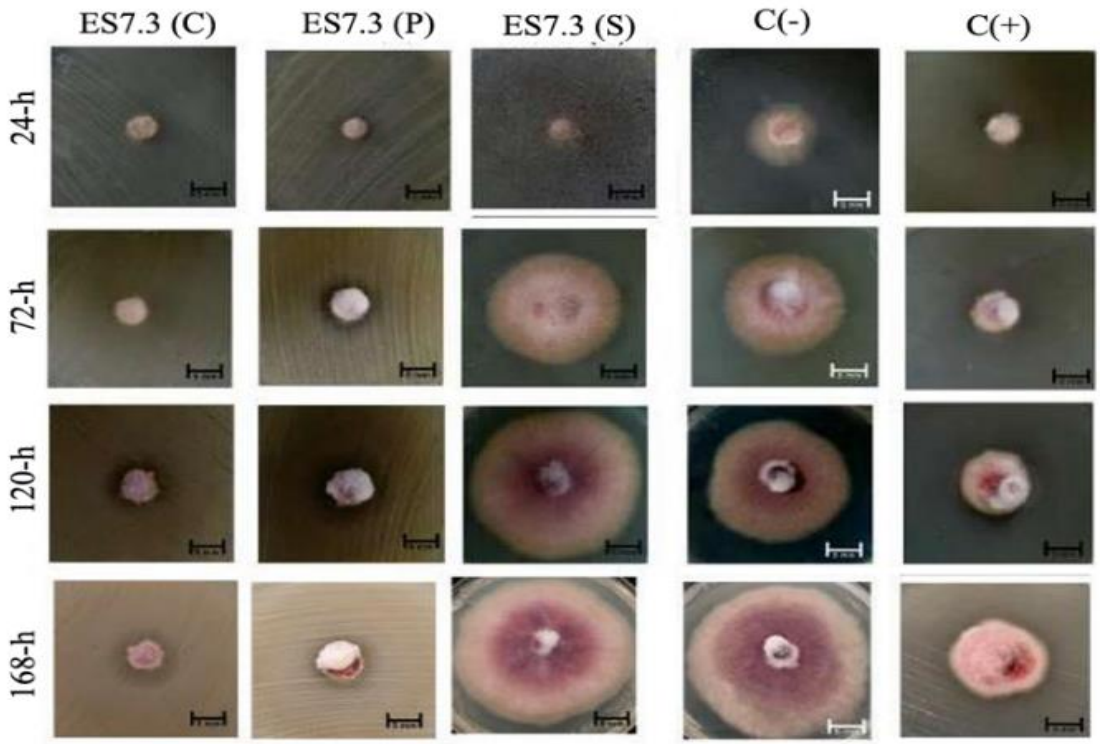


Figure 12. Growth of the pathogenic fungus *F. oxysporum* in the culture (C), pellet (P), and supernatant (S) fractions of *B. velezensis* ES7-3 at different incubation times. positive control (C+), negative control (C-); incubation for 24, 72, 120, and 168 h

Table 4. Antifungal activity *of B. velezensis* ES7.3 against *F. oxysporum* based on the diameter of fungal colonies

Fraction of the *B. velezensis* ES7.3 culture	*F. oxysporum* growth in colony diameter (mm)	Barrier levels of *F. oxysporum* (%)
Sonication culture	10.9	82.53
Sonication pellet	11.2	82.04
Supernatant free-cell	65.2	0
Positive control	24.35	-
Negative control	62.38	-

The fractions of the *B. velezensis* ES7.3 isolate used for this test included the culture fraction, pellet, and supernatant. Among these, the lowest diameter of *F. oxysporum* growth was observed for the culture fraction, which showed the best results compared to the positive control. The growth of *F. oxysporum* and the level of inhibition are shown in Table 4.

The sonicated culture fraction had the greatest (82.53%) inhibitory effect on *F. oxysporum*, reducing fungal colony diameter to 10.9 mm. *Bacillus* sp. ES7.3 isolate was successfully isolated from the sediment of *Aedes aegypti* larvae in the study reported [23]. *Bacillus* sp. ES7.3 bacteria are moderately sized and have an irregular shape, white color, flat elevation, and serrated edges. Furthermore, *Bacillus* sp. ES7.3 is known to bear oval endospores located at the subterminus; it is a gram-positive bacterium with rod-shaped cells and excellent larvicidal activity, causing up to 96.7% larval mortality within 48 h of exposure [23]. In this study, *Bacillus* sp. ES7.3 was identified to the species level through 16S rRNA gene detection, as this gene is a molecular marker commonly used to identify and classify bacterial species. The 16S rRNA gene DNA band of *Bacillus* sp. ES7.3 was 1500 bp, in accordance with the DNA ladder, and was very close (99.31% homology) to that of *B. velezensis* CBMB205. Bacteria are considered to belong to the same species if they have a homology rate greater than 97% [1]. This result differed from the identification through conventional macroscopic, microscopic, and physiological characterization performed by [23], who reported that *Bacillus* sp. ES7.3 similarity index (Ss) with *B. thuringiensis* was 71.4%.

The phylogenetic tree illustrating the relationship between *B. velezensis* ES7.3 and other *Bacillus* species revealed that *Bacillus* sp. ES7.3 is closely related to *B. velezensis* strains CBMB205 and FZB42, as can be appreciated from the branching locations of the three types of bacteria (Figure 2). According to reports [6], another cause for the close kinship between *Bacillus* sp. ES7.3 and *B. velezensis* CBMB205 might be the low nucleotide variation between *Bacillus* sp. ES7.3 and *B. velezensis* CBMB205, according to [24], which was successfully isolated from the rice (*Oryza sativa* L. cv. O-dae) and has a gene encoding a phosphate solubilizer that is a critical growth factor, allowing plants to easily use it. In addition, *B. velezensis* CBMB205 has a gene that is part of the biosynthesis pathway of thiamine and vitamin B [25,26]. Furthermore, catalase, oxidase, pectinase, and protease activities have been observed in *B. velezensis* CBMB205 [24].

Bacillus sp. produces a type of lipopeptide biosurfactant that is classified as the most powerful biosurfactant with therapeutic properties, including broad-spectrum antimicrobial and antiviral activities and antitumor effects [27]. *Bacillus* spp. produce highly diverse lipopeptides with more than 100

different structures [28]. Based on their constituent amino acids, lipopeptides are classified into three families: fengycin (fengycin and plipastatin), iturin (mycosubtilin, iturin, and bacillomycin), and surfactin (surfactin, pumilacidin, and lichenysin) [29]. Based on the DNA banding pattern relative to the markers used herein, *B. velezensis* ES7.3 seemingly contains the srfAD and ituD genes (Figure 3), which were shown to be similar in nucleotide sequence to their counterparts present in *B. velezensis* strain Htg6 (99.86% homology) and those in *B. velezensis* strain KD1 (99.72% homology). Based on the detection results of srfAD and ituD genes (Table 3), srfAD sequence differences between *B. velezensis* ES7.3 and the database (gene polymorphism) occurred for two amino acids, namely serine and methionine, as per the sequence alignment. *B. velezensis* is a member of the *B. amyloliquefaciens* group with similar morphological, physiological, biochemical, phenotypic, and phylogenetic characteristics. Therefore, from the analyses reported herein, it is difficult to separate these two taxa [30]. The difference in amino acid sequence between Bacillomicin D from *B. velezensis* ES7.3 and the database (polymorphism gene) occurred at one amino acid during the sequence alignment process. In *B. velezensis* ES7.3, Bacillomicyn D, synthesized by the bamD gene, is a member of the iturin family; thus, it shows a similar structure.

B. velezensis ES7.3 exhibited beta (β) hemolysis for hemolytic activity (Figure 4). *β*-hemolysis is the complete hemolysis of red blood cells, as seen from the clear zone produced, which is particularly notable due to the complete lysis of all red blood cells and the release of hemoglobin from them. Bacteria that cause *β*-hemolysis have the greatest potential to produce biosurfactants because biosurfactants act as hemolytic substances. Hemolysins act as antibodies against erythrocyte membrane antigens that cause hemolysis [31]. Based on research conducted by [32], *B. cereus* forms a clear zone (*β*-hemolysis), which indicates the potential of biosurfactants formed due to contact between bacterial cells and erythrocytes causing lysis and forming a clear zone. The hemolytic activity of biosurfactants can occur through one of two mechanisms: normal membrane dissolution at high biosurfactant concentrations or increased membrane permeability to small solutes at low biosurfactant concentrations due to osmotic lysis [33]. Thus, the inhibition zone formed by observing hemolytic activity indicates biosurfactant production. The larger the diameter of the blood agar lysis, the higher the concentration of biosurfactants [34].

The clear zone formed around the colony in the hemolytic activity test indicated the production of a biosurfactant. The ability of *B. velezensis* ES7.3 to produce biosurfactants was continuous in small-scale production on coconut meal medium. The growth response of *B. velezensis* ES7.3 showed increasing growth starting from 0 to 144 h of incubation, as seen from the bacterial cell biomass accumulation and OD measurements (Figure 5).

Based on the growth curve 24–48 h of incubation, the growth of *B. velezensis* ES7.3 was very fast until a biomass of 2.9 mg/mL was attained, with an OD value of 3. The rapid growth response resulted from faster bacterial cell division in the growth medium with a complete nutrient supply [35]. This indicates that the composition of coconut meal, especially with respect to carbon and nitrogen sources, is a suitable growth medium for *B. velezensis* ES7.3. Coconut meal is a waste material that contains excellent energy sources and the most important elements needed for the biosynthesis processes necessary for growth: (1) Anaerobic materials: Ca 0.04%, Cl 0.37%, K 1.83%, Mg 0.31%, Na 0.04%, P 0.52%, S 0.31%, Cu 25 ppm, Fe 486

ppm, Mn 69 ppm, and Zn 49 ppm, organic matter: Crude protein 22% [36].

Based on laboratory tests, 10 g of coconut meal dissolved in 100 mL contains 2.18% total sugar, 1.69% fat, 2.18% protein, 2.11% C, 0.21% N, and 0.13% P. Coconut meal is an excellent source of C and N, which are the main nutrient components of the culture medium. However, along with incubation time, the growth rate of *B. velezensis* ES7.3 decreased during a 120 h incubation, presumably because, in this phase, the amount of nutrients in the growth medium decreased and the pH value increased to close to 9. Based on the analysis, the growth performance of *B. velezensis* ES7.3 grown on coconut meal medium was very good in the presence of nutritional components and suitable culture medium-environmental conditions for the growth of *B. velezensis* ES7.3.

In this study (Figures 9 and 11), *B. velezensis* ES7.3 seemingly began to produce biosurfactants at 24 to 96 h of incubation, as can be seen from the 9.8 mN/m decrease in surface tension of the coconut meal medium at 24 h of incubation and the presence of emulsification activity at 60%. The 24 h incubation time was in the middle of the exponential growth phase (Figure 4), in agreement with a report by [37], which revealed that biosurfactants are secondary metabolites produced in the middle of the exponential phase.

In this phase, *B. velezensis* ES7.3 experiences rapid growth, and a large amount of nutrients is consumed, decreasing nutrient concentrations in the culture medium. In turn, a significant change in the amount of nutrients in the medium likely caused metabolic changes in response to changes in the chemical environment of the media, and the induction or repression of gene expression occurred in bacteria within a few minutes, allowing cells to synthesize secondary metabolites in the form of biosurfactants [38]. Biosurfactants can form a thin film on the surface of microorganisms and facilitate the release or attachment of cells to other cell surfaces, thus playing a role in regulating bacterial motility and quorum sensing [38].

Other studies have shown that when *B. subtilis* enters the stationary growth phase, cells differentiate into several subpopulations, including cannibal, biofilm or protease-producing, spore-producing, and competent cells. Approximately 10–20% of competent cells can take up extracellular DNA and produce surfactin [39]. Based on Figures 6 and 8 illustrating the emulsification activity and surface tension of *B. velezensis* ES7.3, crude biosurfactants are more likely to act as emulsifiers than surface tension reducers. This observation indicates that the biosurfactant molecules produced by *B. velezensis* ES7.3 in coconut meal medium have high molecular weights. Biosurfactants with high molecular weight include lipoproteins, lipopolysaccharides, and amphiphatic polysaccharides [40]. High-molecular-weight biosurfactants affect different structures and influence emulsion formation and stability, but do not reduce surface tension [41].

The difference in biosurfactant activity produced by *B. velezensis* ES7.3 grown on coconut meal medium relative to that observed in SMW + glucose might be caused by differences in the amount of biosurfactant produced. The differences in biosurfactant production were due to the differences in components of the culture medium used for biosurfactant production. The coconut meal medium is composed of fats and proteins. The difference between these compounds in the two media can be a single material for the direct synthesis of lipid and protein structures of biosurfactants. Further, [42] reported that there are several kinds of biosurfactant synthesis pathways, namely, de novo synthesis of hydrophilic and hydrophobic groups by two independent

pathways, followed by a process for combining these groups to form molecules. Complete biosurfactant: 1) de novo synthesis of the hydrophilic moiety and substrate-dependent synthesis of the hydrophobic moiety, followed by a combination process to form a complete biosurfactant molecule; 2) de novo synthesis of the hydrophobic moiety and substrate-dependent synthesis of the hydrophilic moiety, followed by a combination process to form a complete biosurfactant molecule. The biosynthesis of both hydrophobic and hydrophilic components depends on the type of substrate used to produce the biosurfactant. This is why the coconut meal medium produced more biosurfactants than the SMW+2% glucose medium. The significant difference in results makes coconut meal a more effective biosurfactant production medium than SMW + 2% glucose for *B. velezensis* ES7.3.

Three important elements support the effectiveness of biosurfactant production in coconut meal medium by *B. velezensis* ES7.3: (1) the presence of nutrients that are qualitatively and quantitatively suitable for the growth of *B. velezensis* ES7.3; (2) the appropriate environment, namely pH, temperature, agitation, and sugar concentration; and (3) the genetic potential of *B. velezensis* ES7.3 to produce biosurfactants.

In this study, *B. velezensis* ES7.3 was shown to inhibit the growth of *F. oxysporum* (Figure 12). The greatest inhibition was observed in the complete culture and pellet fractions, whereas the inhibition in the supernatant fraction was not significant compared with the negative control. According to [43], *B. velezensis* has shown antifungal activity against fungal pathogens, and the volatile organic compounds produced by *B. velezensis* NKG-2 can affect fungal growth. Furthermore, *B. velezensis* NKG-2 has shown antifungal activity against fungal pathogens in plants and has produced biomolecules that show good prospects for development through genetic engineering and which can be useful in the agricultural sector [44]. *B. velezensis* produces secondary metabolites, including cyclic lipopeptides, surfactin, iturin, and fengysine [45]. These lipopeptides are composed of glutamic acid, aspartate, lysine, arginine, and fatty acids, which primarily inhibit the growth of pathogenic fungi. Another method involves disrupting the integrity of the fungal cell membrane by damaging it and inhibiting hyphal growth [45]. The antibiotic compounds bacillaene, macrolactin, and dificidin inhibit cell wall synthesis, metabolism, and growth of *F. oxysporum* [45]. Thus, the isolate can be developed as a potential bacterium to create environmentally friendly biofungicides for the biocontrol of *F. oxysporum.*

Conclusion

This study demonstrated that *B. velezensis* ES7.3 is a potent biosurfactant-producing bacterium capable of utilizing coconut meal as an efficient agro-industrial substrate. Molecular identification confirmed 99.31% similarity to *B. velezensis* strain CBMB205, and the presence of biosurfactant biosynthesis genes (*srfAD* and *ituD*) further supports its strong genetic capacity for lipopeptide production. Coconut meal medium significantly enhanced biosurfactant synthesis compared with SMW + 2% glucose, as indicated by higher emulsification activity and lower surface tension. In addition to its biosurfactant-producing potential, *B. velezensis* ES7.3 exhibited notable antifungal activity against *F. oxysporum*, with the highest inhibition observed in the complete culture and pellet fractions. This isolate can be further developed for environmentally friendly applications in agriculture, including biofungicides and plant-protective formulations. Future work may focus on optimizing fermentation conditions, purifying

the active compounds, and evaluating field-level efficacy for integrated disease management.

Acknowledgment

The authors would like to thank the Dean of the Faculty of Science and Technology, the Head of the Institute for Research and Community Service, and the Rector of Universitas Airlangga, Surabaya, Indonesia, who have provided laboratory facilities. This research funded by Ministry of Higher Education, Science, and Technology of the Republic of Indonesia (Kemendiktisaintek), which is Fundamental Research of Doctoral Dissertation Research scheme (PDD) 2024, Contract Number: 1756/b/un3./lppm/pt.01.03/2024.

Disclosure Statement

The authors declared that no conflicts of interest exist regarding the publication of this work.

ORCID

Farah Aisyah Nafidiastri
https://orcid.org/0009-0002-5445-7199
Salamun
https://orcid.org/0000-0002-3535-5915
Ninik Fadhillah
https://orcid.org/0009-0003-4813-2272
Ni'matuzahroh
https://orcid.org/0000-0002-4631-1096
Fatimah
https://orcid.org/0000-0002-9831-2154
Almando Geraldi
https://orcid.org/0000-0003-4178-0819
Amalia Rizky Febriyanti
https://orcid.org/0009-0004-1396-4316

References

[1] Johnson, J.S., Spakowicz, D.J., Hong, B.-Y., Petersen, L.M., Demkowicz, P., Chen, L., Leopold, S.R., Hanson, B.M., Agresta, H.O., Gerstein, M. Evaluation of 16s rRNA gene sequencing for species and strain-level microbiome analysis. *Nature Communications*, **2019**, 10(1), 5029.

[2] Vieira, I.M.M., Santos, B.L.P., Silva, L.S., Ramos, L.C., de Souza, R.R., Ruzene, D.S., Silva, D.P. Potential of pineapple peel in the alternative composition of culture media for biosurfactant production. *Environmental Science and Pollution Research*, **2021**, 28(48), 68957–68971.

[3] Zhao, X., Kuipers, O.P. Identification and classification of known and putative antimicrobial compounds produced by a wide variety of bacillales species. *BMC Genomics*, **2016**, 17(1), 882.

[4] Kim, Y.T., Park, B.K., Kim, S.E., Lee, W.J., Moon, J.S., Cho, M.S., Park, I.H., Kim, S.U. Organization and characterization of genetic regions in Bacillus subtilis subsp. krictiensis ATCC55079 associated with the biosynthesis of iturin and surfactin compounds. *PLoS ONE*, **2017**, 12(1), e0188179.

[5] Sreedharan, S.M., Rishi, N., Singh, R. Microbial lipopeptides: Properties, mechanics and engineering for novel lipopeptides. *Microbiological Research*, **2023**, 271, 127363.

[6] NAFIDIASTR, F.A., Susetyo, R.D., Nurhariyati, T., Supriyanto, A., Geraldi, A. Biosurfactant activity of indigenous bacillus sp. ES4.3 isolated from endemic breeding sites of dengue hemorrhagic fever vector in surabaya, east java, indonesia. *Biodiversitas*, **2021**, 22(12), 5375–5381.

[7] Meena, K.R., Tandon, T., Sharma, A., Kanwar, S.S. Lipopeptide antibiotic production by Bacillus velezensis KLP2016. *Journal of Applied Pharmaceutical Science,* **2018**, 8(03), 91–98

[8] Susetyo, R.D., Nafidiastri, F.A., Zain, R.A., Sari, R.P., Geraldi, A. Potential biocontrol agent of indigenous bacillus sp. EG6.4: Molecular identification, larvicidal toxicity, and mechanism of actions. *Biodiversitas*, **2022**, 23(10), 5431–5438.

[9] Susetyo, R.D., Geraldi, A., Supriyanto, A., Nurhariyati, T., Nafidiastri, F.A. Biosurfactant production of entomopathogenic bacillus subtilis bK7.1, as potential biocontrol bacteria, isolated from baluran national park, east java, indonesia. *Biodiversitas*, **2023**, 24(3), 1785–1792.

[10] Rharma, R. Agro-industrial waste to energy-sustainable management. *Sustainable Materials and Technologies*, **2024**, 41, e01117

[11] Prado, A.A.O.S., Santos, B.L.P., Vieira, I.M.M., Ramos, L.C., de Souza, R.R., Silva, D.P., Ruzene, D.S.

Evaluation of a new strategy in the elaboration of culture media to produce surfactin from hemicellulosic corncob liquor. *Biotechnology Reports*, **2019**, 24, e00364

[12] Cortés-Camargo, S., Perez-Rodriguez, N., de Souza Oliveira, R.P., Huerta, B.E.B., Domínguez, J.M. Production of biosurfactants from vine-trimming shoots using the halotolerant strain bacillus tequilensis ZSB10. *Industrial Crops and Products*, **2016**, 79, 258–266.

[13] Das, A.J., Kumar, R. Production of biosurfactant from agro-industrial waste by bacillus safensis J2 and exploring its oil recovery efficiency and role in restoration of diesel contaminated soil. *Environmental Technology & Innovation*, **2019**, 16, 100450.

[14] Rane, A.N., Baikar, V.V., Kumar, V.R., Deopurkar, L.R., Alejandra, M., De Rienzo, D. Agro-industrial wastes for production of biosurfactant by Bacillus subtilis ANR 88 and its application in synthesis of silver and gold nanoparticles. *Frontiers in Microbiology*, **2017**, 8, 492.

[15] Al-Dhabi, N.A., Esmail, G.A., Arasu, M.V. Enhanced production of biosurfactant from *bacillus subtilis* strain al-dhabi-130 under solid-state fermentation using date molasses from saudi arabia for bioremediation of crude-oil-contaminated soils. *International Journal of Environmental Research and Public Health*, **2020**, 17(22), 8446.

[16] Dhiman, R., Meena, K.R., Sharma, A., Kanwar, S.S. Biosurfactants and their screening methods. *Research Journal of Recent Sciences*, **2016**, 5(10), 1–6.

[17] Gurkok, S., Ozdal, M. Screening methods for biosurfactant-producing microorganisms. *Multifunctional Microbial Biosurfactants*. **2023**, 1-24

[18] Trindade, M., Sithole, N., Kubicki, S., Thies, S., Burger, A. Screening strategies for biosurfactant discovery. Advances in Biochemical Engineering/Biotechnology, **2022**, 181, 17–52.

[19] Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., Struhl, K. Preparation of genomic DNA from bacteria. Current *Protocols in Molecular Biology*, John Wiley & Sons, Inc., **2003**.

[20] Kuznetsov, A., Bollin, C.J. Multiple Sequence Alignment: Methods and Protocols, *Methods in Molecular Biology*, **2021**, 2231, 261–295.

[21] Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., Kumar, S. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, **2011**, 28(10), 2731–2739.

[22] Kumar, S., Tamura, K., Nei, M. MEGA: Molecular evolutionary genetics analysis software for microcomputers, *Bioinformatics*, **1994**, 10(2), 189-191.

[23] Salamun, Ni'matuzahroh, Fatimah, Maswantari, M.I.F., Rizka, M.U., Nurhariyati, T., Supriyanto, A. Diversity of indigenous entomopathogenic bacilli from domestics breeding sites of dengue hemorrhagic fever vector based on the toxicity against Aedes aegypti larvae. *Ecology, Environment and Conservation*, **2020**, 26, S21–S26.

[24] Shaikh, I.A., Turakani, B., Malpani, J., Goudar, S.V., Mahnashi, M.H., Al-Serwi, R.H., Iqubal, S.S. Extracellular protease production, optimization, and partial purification from Bacillus nakamurai PL4 and its applications. *Journal of King Saud University – Science*, **2023**, 35, 102429

[25] Rodríguez, H., Fraga, R. Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances*, **1999**, 17(4-5), 319–339.

[26] Zhang, Y., Begley, T.P. Cloning, sequencing and regulation of thiA, a thiamin biosynthesis gene from bacillus subtilis. *Gene*, **1997**, 198(1-2), 73–82.

[27] Felix, A.K.N., Martins, J.J., Almeida, J.G.L., Giro, M.E.A., Cavalcante, K.F., Melo, V.M.M., Pessoa, O.D.L., Rocha, M.V.P., Gonçalves, L.R.B., de Santiago Aguiar, R.S. Purification and characterization of a biosurfactant produced by bacillus subtilis in cashew apple juice and its application in the remediation of oil-contaminated soil. *Colloids and Surfaces B: Biointerfaces*, **2019**, 175, 256–263.

[28] Jacques, P. Biosurfactants: From genes to applications. Springer-Verlag Berlin Heidelberg, **2010**, pp. 57–91.

[29] Ali, N., Pang, Z., Wang, F., Xu, B., El-Seedi, H.R. Lipopeptide biosurfactants from bacillus spp.: Types, production, biological activities, and applications in food. *Journal of Food Quality*, **2022**, 2022(1), 3930112.

[30] Huynh, T., Vörös, M., Kedves, O., Turbat, A., Sipos, G., Leitgeb, B., Kredics, L., Vágvölgyi, C., Szekeres, A. Discrimination between the two closely related species of the operational group b. Amyloliquefaciens based on whole-cell fatty acid profiling. *Microorganisms*, **2022**, 10(2), 418.

[31] Al-Marri, S., Eldos, H.I., Ashfaq, M.Y., Saeed, S., Skariah, S., Varghese, L., Raja, M.M. Isolation, identification, and screening of biosurfactant-producing and hydrocarbon-degrading bacteria from oil and gas industrial waste. *Biotechnology Rep*orts, **2023**, 39, e00804.

[32] Setiati, N., Hidayah, N. The use of two pairs primer for CO1 gene amplification on traded stingray at fish auction tasik agung rembang. *Journal of Physics: Conference Series*, **2020**.

[33] Zaragoza, A., Aranda, F.J., Espuny, M.J., Teruel, J.A., Marqués, A., Manresa, A., Ortiz, A. Hemolytic

activity of a bacterial trehalose lipid biosurfactant produced by *Rhodococcus* sp.: Evidence for a colloid-osmotic mechanism. *Langmuir*, **2010**, 26, 8567–8572.
[34] Singh, V. Biosurfactant-isolation, production, purification & significance. *International Journal of Scientific and Research Publications*, **2012**, 2(7).
[35] Senez, J.C. Some considerations on the energetics of bacterial growth. *Bacteriological Reviews*, **1962**, 26(2 Pt 1-2), 95–107.
[36] Stein, H.H., Casas, G.A., Abelilla, J.J., Liu, Y., Sulabo, R.C. Nutritional value of high fiber co-products from the copra, palm kernel, and rice industries in diets fed to pigs. *Journal of Animal Science and Biotechnology*, **2015**, 6, 1-9.
[37] Sari, S.K., Trikurniadewi, N., Ibrahim, S.N.M.M., Khiftiyah, A.M., Abidin, A.Z., Nurhariyati, T. Bioconversion of agricultural waste hydrolysate from lignocellulolytic mold into biosurfactant by achromobacter sp. BP (1)5. *Biocatalysis and Agricultural Biotechnology*, **2020**, 24, 101534.
[38] Sharma, J., Sundar, D., Srivastava, P. Biosurfactants: Potential agents for controlling cellular communication, motility, and antagonism. *Frontiers in Molecular Biosciences*, **2021**, 8, 727070.
[39] Qiao, J., Borriss, R., Sun, K., Zhang, R., Chen, X., Liu, Y., Liu, Y. Research advances in the identification of regulatory mechanisms of surfactin production by bacillus: A review. *Microbial Cell Factories*, **2024**, 23(1), 100.
[40] Ron, E.Z., Rosenberg, E. Natural roles of biosurfactants. *Environmental Microbiology*, **2001**, 3(4), 229–236.
[41] Zhou, H., Chen, J., Yang, Z., Qin, B., Li, Y., Kong, X. Biosurfactant production and characterization of bacillus sp. ZG0427 isolated from oil-contaminated soil. *Annals of Microbiology*, **2015**, 65(4), 2255–2264.
[42] Maťátková, O., Michailidu, J., Ježdík, R., Jarošová Kolouchová, I., Řezanka, T., Jirků, V., Masák, J. Production and characterization of rhamnolipids produced by pseudomonas aeruginosa DBM 3774: Response surface methodology approach. *Microorganisms*, **2022**, 10(7), 1272.
[43] Myo, E.M., Liu, B., Ma, J., Shi, L., Jiang, M., Zhang, K., Ge, B. Evaluation of bacillus velezensis NKG-2 for bio-control activities against fungal diseases and potential plant growth promotion. *Biological Control*, **2019**, 134, 23–31.
[44] Adeniji, A.A., Loots, D.T., Babalola, O.O. Bacillus velezensis: Phylogeny, useful applications, and avenues for exploitation. *Applied Microbiology and Biotechnology*, **2019**, 103(9), 3669–3682.
[45] Barale, S., Ghane, S., Sonawane, K. Purification and characterization of antibacterial surfactin isoforms produced by bacillus velezensis SK. Amb *Express*, **2022**. 12 (1), 1-20..

HOW TO CITE THIS MANUSCRIPT
F.A. Nafidiastri, Salamun, N. Fadhillah, Ni'matuzahroh, Fatimah, A. Geraldi, A.R. Febriyanti. Biosurfactant Production Using Coconut Meal by Indigenous *Bacillus Velezensis* ES7.3: Genetic Characterization and Antifungal Activity against *Fusarium Oxysporum*. *Asian Journal of Green Chemistry*, 10 (3) 2026, 464-482.
DOI: https://doi.org/10.48309/ajgc.2026.556599.1859
URL: https://www.ajgreenchem.com/article_236767.html