

The 16S rRNA Analysis and Enzyme Screening of *Bacillus* from Rhizosphere Soil of Lombok Island

(Analisis 16S rRNA dan Skrening Enzim *Bacillus* dari Tanah Rhizosfer Pulau Lombok)

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ABSTRACT

Bacillus are commonly found in nature, especially in soil and food. It has the ability to produce bioactive compounds as well as the enzyme. This study was aimed to isolate, identify, and screen their enzyme activities. Four soil samples from Mandalika, Lombok Island, West Nusa Tenggara (NTB), were used for isolation. *Bacillus* was isolated using the heat-shock method and characterized through Gram staining, endospore staining, and morphological phenotype. *Bacillus* identification was conducted based on 16S rRNA gene sequence. The hydrolytic enzyme activities were checked qualitatively using selective media, and the enzyme tested including amylase, galactosidase, lipase, protease, and cellulase. As many as twenty-two bacteria isolates were obtained from four soil samples and represented 15 distinct species. The member of bacteria genera successfully identified, consisted of *Bacillus* sp., *Brevibacillus* sp., and *Fictibacillus* sp. *Bacillus* sp. was the most isolated. Some of the isolated bacteria have the ability to produce lipase, protease, and cellulase that potential to be used in biotechnology processes.

Keywords: *Bacillus*, hydrolytic enzymes, identification, screening

ABSTRAK

Bacillus secara umum ditemukan di alam, terutama di tanah dan makanan. Bakteri dalam kelompok tersebut memiliki kemampuan untuk menghasilkan senyawa bioaktif seperti enzim. Penelitian ini bertujuan untuk mengisolasi, mengidentifikasi, dan menskrening aktivitas enzim yang dihasilkan oleh bakteri dari kelompok *Bacillus*. Empat sampel tanah dari Pulau Mandalika, Nusa Tenggara Barat (NTB) digunakan dalam penelitian ini. *Bacillus* diisolasi menggunakan metode *heat-shock* dan dikarakterisasi menggunakan pendekatan pewarnaan Gram, pewarnaan endospore, dan morfologi fenotipe. Identifikasi dilakukan berdasarkan urutan gen 16S rRNA. Aktivitas enzim hidrolisis dicek secara kualitatif menggunakan media selektif, dan enzim yang diuji antara lain amilase, galaktosidase, lipase, protease, dan selulase. Sebanyak dua puluh dua isolat diperoleh dari empat sampel tanah dan mewakili 15 spesies berbeda. Beberapa genus bakteri yang berhasil diisolasi antara lain *Bacillus* sp., *Brevibacillus* sp., dan *Fictibacillus* sp. *Bacillus* sp. terisolasi paling banyak dibanding dua genus lainnya. Beberapa bakteri terisolasi memiliki kemampuan untuk menghasilkan enzim lipase, protease, dan selulase yang berpotensi untuk digunakan dalam proses bioteknologi.

Kata kunci: *Bacillus*, enzim hidrolisis, identifikasi, skrening

INTRODUCTION

As part of life, microbes directly contribute to the life cycle and sustainability of materials used in the environment, food, agriculture, health, and industry. The contributions of microbes depend on their diversities. *Bacillus* are widely applied in the food, environmental, agricultural, and industrial processes. These bacteria spread in nature and are commonly found in soil, clay, rock, dust, aquatic environment, food, the digestive tract of insects and aquatic animals (Schultz *et al.* 2017). Therefore, these bacteria could

be isolated in almost all types of samples. Taxonomically, *Bacillus* belongs to Gram-positive bacteria, phylum Firmicutes, Bacillales orders, with characteristics: aerobic, rod-shaped, straight, flat, rhizoid, and white chalk. The records in January 2019, the *Bacillus* genera, consisted of 379 species, including its synonym (Parte & Road 2014).

Nowadays, *Bacillus* genera getting more attention due to their capabilities in secreting bioactive compounds, such as enzymes, antimicrobials, and other potential commercial compounds (Caulier *et al.* 2019). The other studies by Shanthi and Roymon (2018) and Powthong and Suntornthiticharoen (2017) reported that *Bacillus* sp. have the abilities to produce xylanase, chitosanase, amylase, cellulase, caseinase, esterase, and lipase. The well-known species of *Bacillus* are *Bacillus subtilis*, *Bacillus licheniformis*,

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Bacillus amiloliquefaciens (van Dijil & Hecker 2013), and *Bacillus thuringiensis* (Shrestha *et al.* 2015).

Microbial enzymes are more interesting compared to plants and animals due to their stability, availability, and cost-effectiveness. These conditions made microbial enzymes were explored more and applied in many industrial sectors that highly dependent on enzymes. Fungi, bacteria, and actinomycetes mostly produce enzymes, and the most commercial one is fungi. However, bacteria are also considered enzyme producers because they are fast-growing, stable in the extreme conditions, and sometimes have multiple enzymes. The abilities of bacteria to produce multi-enzymes will increase the benefits when used as food additives (Pereyra *et al.* 2020). Approximately 50% of total enzyme market are produced by *Bacillus*. This mainly due to these bacteria have a wide distribution, easy to cultivate, safety, and susceptibility to genetic transformations. In addition, protease from *Bacillus* is stable in wide range of temperature and pH, that suitable for industrial application (Danilova & Sharipova 2020). About more than 65% of industrial enzymes on the market are proteases (Annamalai *et al.* 2014). About 40% of the total production of protease enzyme are obtained from microbes. Besides protease, lipase also a vital enzyme in the industrial field that is applied in detergent formulations and methyl ester production.

The increasing demand for enzymes leads to doing more explorations. In this work, we investigated the diversity of *Bacillus* from soil sample and screened the enzymes activities. Through enzyme screening, we could obtain information about its ability to produce enzymes that economically prospective in the industrial field.

MATERIALS AND METHODS

Samples Source

The rhizosphere soils were collected from various locations on Mandalika, Lombok Island, in April 2019. Samples were taken from a depth of 5 cm and collected from the rhizosphere of *Peltophorum pterocarpum* (SSLO1), *Cocos nucifera* (SSLO2), *Arenga pinnata* (SSLO3), and *Theobroma cacao* (SSLO5).

Isolation and Preservation of *Bacillus*

For *Bacillus* isolation, a combination of heat-shock and serial dilution was employed. A total of one g soil sample was diluted using 9 mL of sterile distilled water, mixed properly, and heated using a water bath at 80°C for 10 min. The sample suspension was cooled for 2 min and followed with serial dilutions making the concentration to 10⁻⁴. As much as 100 µL of level 10⁻² to 10⁻⁴ dilutions were spread aseptically on the Nutrient Agar (NA) media and incubated at 30°C for 24–48 hours. The colony growth was observed and counted. The colonies showed different morphologies were taken and purified based on the quadrant method until a single colony was obtained. The selected isolates were characterized on the basis of Gram-staining,

endospore staining, and morphological phenotype i.e., form, color, margin, and elevation. The purified isolates were stored in a 10% glycerol solution and keep in a freezer at -80°C.

Identification Based on The 16S rRNA Gene Molecular Approach

DNA extraction was conducted based on the method described by Packeiser *et al.* (2013). A single colony of bacterial cells was taken using sterile toothpicks and suspended in 50 µL of free nuclease water. The cell lysis was done by incubation at 98°C for 5 min. The supernatant containing DNA and cell debris was separated using spin down. The supernatant called DNA extract was taken and stored in the new tube for PCR amplification.

PCR amplification was performed using a pair of universal primers for eubacteria, namely primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Lane 1991). 16S rDNA amplification was made in 25 µL total volume. The composition involved GoTaq Green Master Mix, primers 27F and 1492R, dimethyl sulfoxide (DMSO), DNA template, and Ultrapure water. The PCR condition was set as: initial denaturation at 95°C for 90 sec, followed by 30 cycles of denaturation at 95°C for 30 sec; annealing at 50°C, for 30 sec; elongation at 72°C, for 90 sec and a final extension at 72°C for 5 min, and finally at 4°C for 20 minutes. The amplified DNA was electrophoresed on 1% agarose gel, stained with ethidium bromide (5 mg mL⁻¹), and visualized using a UV transilluminator.

DNA Sequencing and Phylogenetic Analysis

The amplified DNA sequencing was performed using an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer) (Applied Biosystems) with a pair of primers 27F and 1492R. The sequencing data were processed using the Chromas Pro program. The closest identity was searched through the EzTaxon website (<http://www.ezbiocloud.net/>) (Yoon *et al.* 2017), and reference sequences were obtained from GeneBank/DDBJ/EMBL. The phylogenetic tree was constructed using the Neighbor-Joining method (NJT), which was implemented in the MEGA 6.0 program (Tamura *et al.* 2013). The T92+G+I (Tamura3-parameter and Gamma distributed) model was chosen as the best model for phylogenetic tree analysis and 1.000 bootstrap replication.

Screening of Enzymes-Producing *Bacillus* Strains

Bacteria isolates were streaked on NA media and incubated at 37°C for 24 h. In the 24th hour, the isolate was used in the screening of amylase, β-glucosidase, lipase, protease, and cellulase. The enzyme activity assay of bacterial isolates was performed by growing the isolates on selective media.

Screening of amylase activity was carried out using Modified Basal Medium (MM), consisting of 1 g glucose, 2,5 g yeast extract, 16 g bacto agar, 1.000 mL distilled water with the addition of 1% soluble starch

(Mazzucotelli *et al.* 2013). Bacteria isolates were spotted on selective amylase media and incubated for 24 h. After that time, the plate was flooded with Lugol solution as an indicator of starch and allowed to stand for 15 min. The clear zone formed around the bacteria colony revealed a positive amylase activity. The clear area and index diameter were measured (Mazzucotelli *et al.* 2013).

The β -galactosidase activity was evaluated using a medium with a composition of 1,8 g K_2HPO_4 , 4,0 g NH_4Cl , 0,2 g $MgSO_4 \cdot 7H_2O$, 0,1 g $NaCl$, 0,01 g $FeSO_4 \cdot 2H_2O$, 16 g bacto agar, 1.000 mL distilled water, with the addition of 1% lactose (Castro *et al.* 2014). The bacteria were spotted on the plate media and followed by incubation at 37°C for 24 h. At the 24th h, the plate was flooded with Lugol solution and allowed to stand for 15 min. A positive reaction was indicated by forming clear zones around the colony. The diameter formed was measured, and the index calculated. Measurement of the value of enzyme-activity index was carried out by the following formula: (diameter of the clear zone-diameter of the colony) / diameter of the colony (Castro *et al.* 2014).

The evaluation of protease activity was carried out using MM media with the addition of 6.2 g/L skim milk (Mazzucotelli *et al.* 2013). Bacteria isolate was spotted on the selective media surface and incubated at 37°C for 24 hours. The clear zones formed around the colonies on protease-selective media indicated protease activity. The diameter of clear zones and indexes were measured.

The lipase activity was detected using a medium containing 10 g peptone, 0,1 g $CaCl_2 \cdot 2H_2O$, 5 g $NaCl$, 10 mL Tween 80, 16 g bacto agar, and 1.000 mL distilled water. The formation of white precipitation around the colonies indicates lipase activity. White precipitation is the deposition of crystals from calcium salts formed by fatty acids from microorganisms lipase (Mazzucotelli *et al.* 2013). The diameter size is 7 mm or more, which indicates positive lipase activity. The formula index value was calculated: (diameter of white precipitation-diameter of the colony) / diameter of the colony (Mazzucotelli *et al.* 2013).

Cellulase activity was checked using MM media with the addition of 1% carboxymethyl-cellulose (CMC) as a substrate. After incubated for 48 hours, the media was flooded with 0,1% Congo red solution, allowed to stand for 15–20 min, and rinsed with 1 M $NaCl$ solution for 15–20 min. Cellulase activity was shown by forming a yellow color around the colonies. The yellow color diameter was measured and the index was calculated (Mazzucotelli *et al.* 2013).

RESULTS AND DISCUSSION

The Abundance of *Bacillus* in the Sample

Bacillus sp. is one of the bacterial groups that are most widely distributed in the natural environment, commonly found in soil, air, and food (Schultz *et al.*

2017), and able to survive in extreme environments, such as thermal treatment. The endospores forming is a specific character of *Bacillus* and makes it resistant to heat, desiccation, and UV light. Heat-shock treatment at 80°C for 10 minutes is an isolation method of *Bacillus* or spore-forming bacteria. The heating is effective in eliminating mesophilic nonspore-forming bacteria or other vegetative cells in the sample. The data presented in Figure 1 revealed that each sample had different riches of *Bacillus*. The highest abundance obtained from SSLO3 was collected from rhizosphere soil of *A. pinnata*, followed by SSLO2 from the rhizosphere soil of *C. nucifera*, SSLO1 from the rhizosphere of *P. pterocarpum*, and the last was SSLO5 from the rhizosphere of *T. cacao*. The result revealed that the abundances of microbes in the rhizosphere were influenced by the plant species and sampling locations.

The rhizosphere is one of the habitats that are rich in nutritional sources. Some studies stated that the riches of microorganisms are different up to several times between the rhizosphere and open soil (York *et al.* 2016; Bulgarelli *et al.* 2015; Schlaeppli *et al.* 2014). Further studies revealed the abundances of microbes in the rhizosphere correlated with the amount of exudate released by root cells, and it differed along with the roots (Mandic-Mulec *et al.* 2015). The tip of the oats root (*Avena fatua* L.) has the highest abundance of bacteria, followed by root hair and root base with the least population. The alteration of exudate patterns affects the microbes and greatly affects the microbial community structure and function. During chemotaxis, root exudate acts as an attractor or repellent of soil microbiota. As much as 10^5 to 10^7 bacteria for every gram of new root colonized the root surface. This condition was caused by plant-root exudate. Zhang *et al.* (2014) reported that citric acid found as the root exudates of cucumber plant and fumaric acid in banana root exudates affects the *Bacillus subtilis* N11 chemotaxis and biofilm formation. Based on the result presented in Figure 1, the result found in this study corresponds to the result reported by York *et al.* (2016), that exudates released by plants significantly affect the type and abundance of microorganisms.

The rhizosphere of *A. pinnata* showed the highest abundance of *Bacillus*. *Arenga pinata* generates

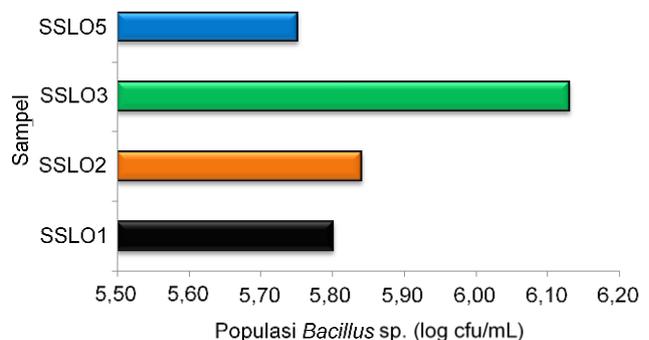


Figure 1 The abundance of *Bacillus* from rhizosphere soil in Mandalika island

essential products called palm sugar. It makes the root exudate released is secure for the rhizosphere microbes. The compounds in palm fruit are essential for microbe growth, including carbohydrates, proteins, and lipids. It was completely different from the cacao plant *T. cacao* exudate. One of the active ingredients in the cacao pod is phenol, and it was effective as an antimicrobial agent (Sabbineni 2016). Hasanuddin *et al.* (2018) also reported the cacao rind contained a candidate of an antibacterial compound. *Theobroma cacao* was recognized as a major source of antioxidants. The three main polyphenol groups found in *T. cacao* are catechins or flavan-3-ols (37%), proanthocyanidins (58%), and anthocyanins (4%) (Belščak *et al.* 2009). In the pharmaceutical, polyphenol showed an anti-carcinogenic, anti-inflammatory, antimicrobial, and analgesic agents (Hii *et al.* 2009). This compound affected the abundance of *Bacillus* sp. in the cacao plant's rhizosphere. As another part of the cocoa plant, seeds have antibacterial and antioxidant activities, perhaps involving the microbial abundance in the rhizosphere soils (Hasanuddin *et al.* 2018). The bacterial abundance in the *P. pterocarpum* and *C. nucifera* were affected by root exudate. Li *et al.* (2019) and Sukumaran *et al.* (2011) reported that the plant bark and leaves of *P. pterocarpum* have antimicrobial, antivirus, antioxidant, antifungal, antivirus, and hepatoprotective activities. The root extract of *C. nucifera*, traditionally used as an antipyretic and diarrhea treatments (Lima *et al.* 2015). Those activities were estimated to influence the microbial abundance in the rhizosphere area.

The twenty-two isolates of selected members of the *Bacillus* were successfully collected from four different rhizosphere soil samples. Observation of Gram staining, spore staining, and morphological characteristics showed that isolates were *Bacillus*. These indicated by purple after Gram-staining (Gram-positive), endospore formation, stem-shaped cells (Figure 2), catalase-positive, white-creamy color, irregular margins, and flat elevation.

The amplification results using a pair of universal primers for Eubacteria obtained an amplified area of ~1.500 bp. The 16S rRNA gene sequencing data

analysis revealed that the selected isolates were *Bacillus*. Among the twenty-two isolates represented thirteen species of *Bacillus*, one species of *Fictibacillus*, and one species of *Brevibacillus*, respectively. The abundance of *Bacillus* from each sample was not always proportional to the number of species collected. The code samples namely SSL01, SSL02, and SSL05, each was consisted of five species, whereas SSL03 only had one species (Table 1). Each sample contained different species of *Bacillus*, and it is supposed that exudates released by plant roots affecting the type of microbes found in the rhizosphere soils.

The isolation method used was selective for bacteria that able to form heat-resistant endospore at 80°C. The data presented in Table 1 and Figure 3 revealed that isolated bacteria capable of creating endospores, included in the phylum Firmicutes, class Bacilli, and the Bacillales order. The collected isolates consisted of three genera, among others *Bacillus*, *Brevibacillus*, and *Fictibacillus*. *Bacillus* and *Fictibacillus* belong to the *Bacillaceae*, while *Brevibacillus* is a member of *Paenibacillaceae* (Figure 2). The composition of the selected isolate was *Bacillus* sp. (86,36%), *Brevibacillus* sp. (9,09%), and *Fictibacillus* sp. (4,54%).

As well as *Bacillus*, *Brevibacillus* and *Fictibacillus*, taxonomically are Kingdom: Bacteria; Phylum: Firmicutes; Class: Bacilli; Order: Bacillales; Family: Bacillaceae (genus: *Bacillus*, *Fictibacillus*); Family: Paenibacillaceae (genus: *Brevibacillus*). They also form endospores and resistant to heat in isolation processes. The phenotypic characterization of the selected genus was Gram-positive, rod-shape, and cream-yellowish color. Based on the phenotypic characteristics, chemotaxonomy, genomic, and phylogenetic data, Shida *et al.* (1996) reclassified the bacteria like *Bacillus* become *Brevibacillus*, including *Brevibacillus formosus*, and Glaeser *et al.* (2013) proposed to reclassify *B. solisalsi* in the genus *Fictibacillus*.

Up to January 2021, *Bacillus* genera comprises 379 species, including synonym. To date, the molecular approach is the best strategy in bacterial grouping and identification. However, it has limitations, particularly for *Bacillus* classification. Phylogenetically, *B. cucumis*

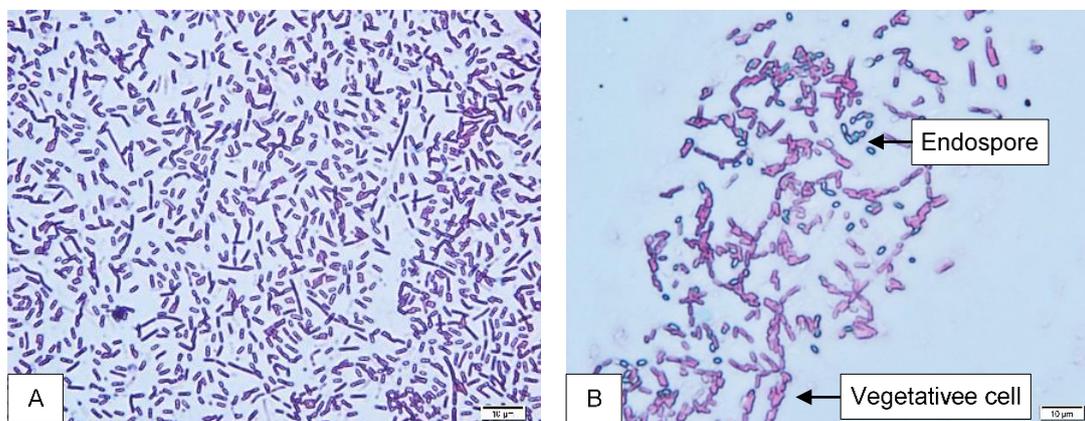


Figure 2 Gram staining (A) and spore staining (B) results of *Bacillus* sp.

Table 1 Identification result of *Bacillus* basis on molecular approach of 16S rRNA gene sequence

Isolate code	Top hit taxon	Basionym	Top hit strain	Similarity (%)
SSL01.1	<i>Bacillus wiedmannii</i>	-	FSL W8-0169	99,59
SSL01.3	<i>Bacillus cucumis</i>	-	AP-6	99,34
SSL01.4	<i>Bacillus cucumis</i>	-	AP-6	99,42
SSL01.5	<i>Bacillus drementensis</i>	-	LMG 21831	99,35
SSL01.6	<i>Bacillus pseudomycooides</i>	-	DSM 12442(T)	98,99
SSL01.7	<i>Bacillus pseudomycooides</i>	-	DSM 12442(T)	98,99
SSL01.8	<i>Bacillus acidiceler</i>	-	CBD 119(T)	99,81
SSL02.1	<i>Bacillus megaterium</i>	-	NBRC 15308	100,00
SSL02.2	<i>Bacillus deserti</i>	-	ZLD-8	99,70
SSL02.3	<i>Fictibacillus solisalsi</i>	<i>Bacillus solisalsi</i>	YC1	99,86
SSL02.4	<i>Bacillus maniponensis</i>	-	BL4-6(T)	98,92
SSL02.5	<i>Bacillus megaterium</i>	-	NBRC 15308	100,00
SSL02.7	<i>Bacillus aryabhatai</i>	-	B8W22	100,00
SSL03.4	<i>Bacillus paramycooides</i>	-	NH24A2(T)	98,91
SSL05.1	<i>Bacillus cereus</i>	-	ATCC 14579	100,00
SSL05.2	<i>Brevibacillus formosus</i>	<i>Bacillus formosus</i>	DSM 9885	99,85
SSL05.3	<i>Brevibacillus formosus</i>	<i>Bacillus formosus</i>	DSM 9885	99,78
SSL05.4	<i>Bacillus cytotoxicus</i>	-	NVH 391-98(T)	98,29
SSL05.5	<i>Bacillus ginsengisoli</i>	-	DCY53	98,97
SSL05.6	<i>Bacillus acidiceler</i>	-	CBD 119	99,93
SSL05.7	<i>Bacillus cytotoxicus</i>	-	NVH 391-98(T)	98,41
SSL05.8	<i>Bacillus acidiceler</i>	-	CBD 119	99,93

firmly connected with *B. drementensis*. It was distinguished from *B. cucumis* a century ago and recently confirmed as a different species. *B. cucumis* has some phenotypic and phylogenetic similarities. Other species are also very closed and hard to be separated utilizing the phylogenetic tree, as well *B. acidiceler* and *B. luciferensis*. *B. brevis* SSL05.2 and SSL05.3 showed 100 bootstrap values with other *Brevibacillus* groups. This case needs a particular technique to separate this species. As an alternative to 16S rDNA sequence is protein-coding genes (e.g. *rpoB*, *gyrB*, *nifD*, *recA*, *atpD*) (Ki *et al.* 2009).

Bacillus is known as potential genera that able to produce commercially valuable enzymes. Each microbial strain has the ability to produce a large amount of hydrolysis and oxidation or reduction enzymes in its metabolic system. Even though they belong to the same species, the number and type of enzymes produced by each strain are not always same. This encourages the need to do microbial exploration that can produce specific enzymes in large quantities. The ability of bacteria in the enzyme production could be analyzed through a qualitative or quantitative approach. The qualitative approach aims to prove an enzyme existence in the sample. The data obtained are positive or negative based on the color change of the selective media used. This is the initial step in the screening process of biotechnology work. The quantitative approach determines enzyme activity in the sample and produces numerical data. The screening in the enzyme production showed that isolates of *Bacillus* were able to produce lipase, protease, and cellulase. The lipid crystals formed around the colony showed positive lipase. Protease and cellulase activities are marked by the formation of

clear zones around the colony. The lipid crystals formed and color changes indicated activities of enzymes in hydrolyzing lipid polymers, proteins, and cellulose into simpler chemical compounds (Figure 4).

The results of the screening of enzyme-producing bacteria are presented in Table 2. Fourteen isolates of *Bacillus* and two isolates of *Brevibacillus* were able to produce hydrolysis enzymes lipase, protease, and cellulase. Ten isolates from 22 isolates had lipase activities, 13 isolates had protease activities, and two isolates had cellulase activities. Seven isolates were able to produce two types of enzymes i.e., lipase and protease. However, of the 22 isolates collected, none of them produced amylase and β -galactosidase enzymes.

Based on the activity, *B. formosus* signed as SSL05.2 and SSL05.3 were able to produce lipase, protease, and cellulase. Logan and Vos (2015) described that *B. formosus* able to hydrolyze casein and gelatin, but not for starch and urea. *B. formosus* BISR-1 collected from the Great Indian Desert soils can produce chitinase (Meena *et al.* 2014). *B. formosus* strains SSL05.2 and SSL05.3, and *B. cucumis* SSL01.3 had a high lipolytic index, and it revealed that those isolates could degrade lipid into fatty acids. *B. acidiceler* SSL01.8 and *B. aryabhatai* SSL02.7 had the highest proteolytic index. According to the data presented in Table 2, strains with lipase, cellulase, and protease activities are potentially used in biotechnological applications, such as detergent formulations or biodegradation of oil pollutants. Four classes of enzymes are generally used in the detergent formulation, including amylase, cellulase, lipase, and protease (Hasan *et al.* 2010). Six bacteria were *B. deserti* SSL02.2, *F. solisalsi* SSL02.3, *B. ginsengisoli*

SSL05.5, *B. acidiceler* SSL05.6 and SSL05.8, and *B. maniponensis* SSL02.4 did not produce five types of enzymes tested.

CONCLUSIONS

The identification of *Bacillus* bacteria needs a more sensitive method besides 16S rRNA gene

sequence. The protein-coding gene could be used as an alternative method to differentiate *Bacillus*. The abundances of microbes in the rhizosphere soils from Mandalika, Lombok Island are influenced by plant species and sampling locations. The root exudates released by plants significantly affect the type of microbes found in the rhizosphere soils. *Bacillus* genera isolated from Mandalika, Lombok Island, have the ability to produce hydrolytic enzymes cellulase,

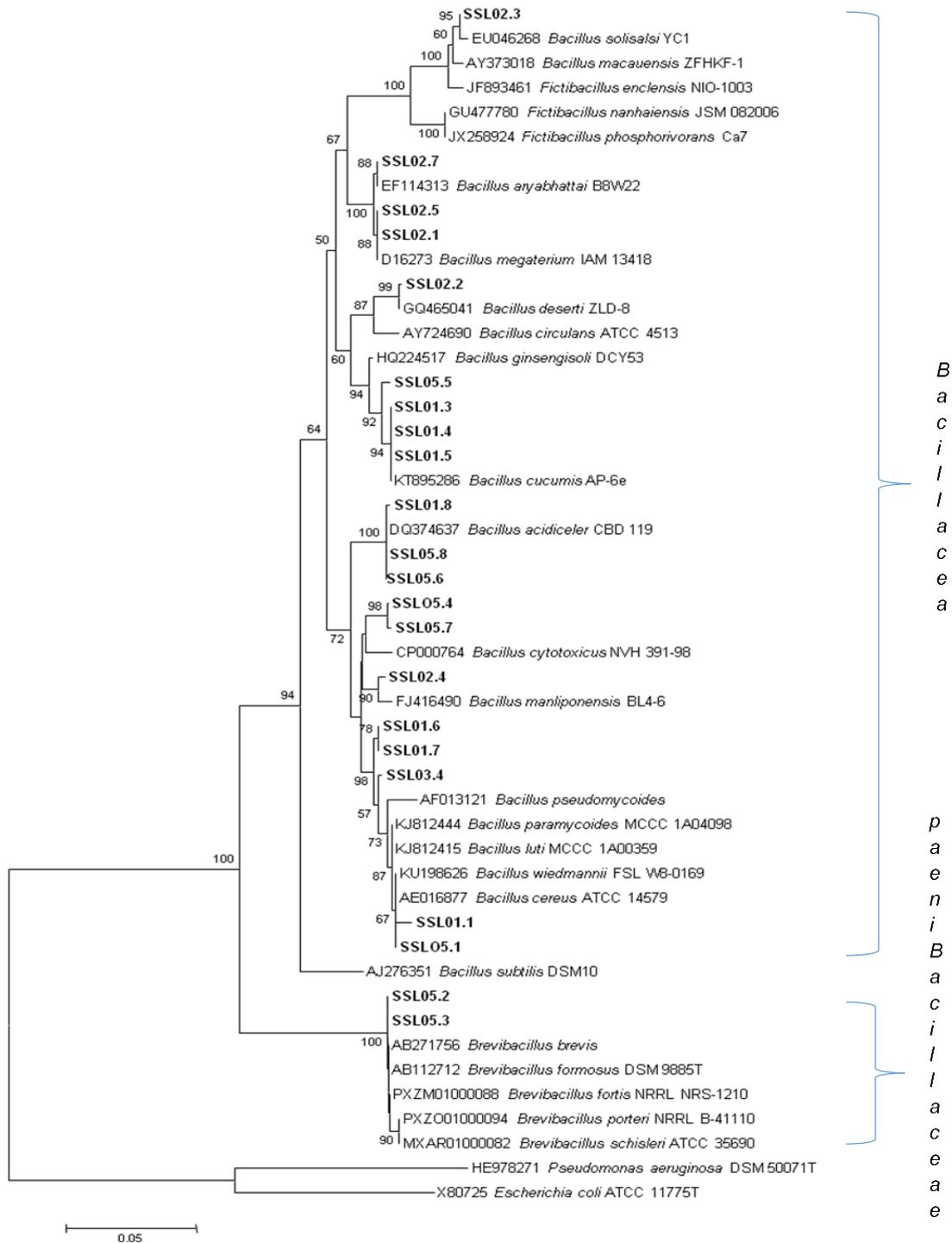


Figure 3 Phylogenetic tree of *Bacillus* isolates using Neighbor-Joining method and best model of *Kimura 2-parameter* and *Gamma* distributed with 1000 replications

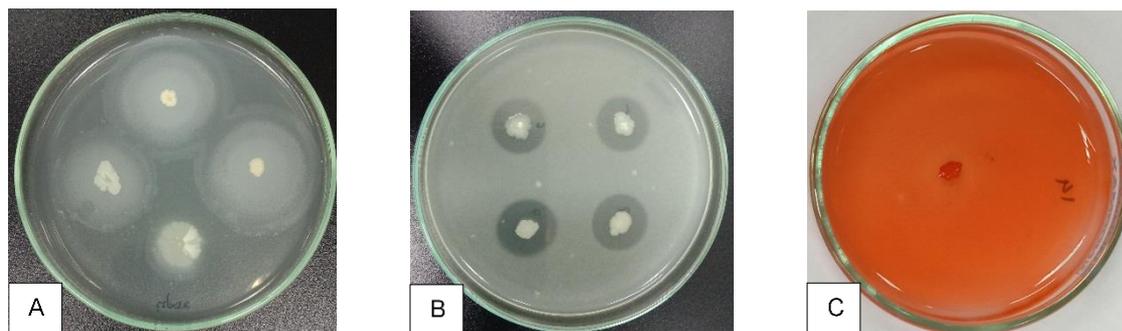


Figure 4 The screening result of enzyme activities of *Bacillus* isolates. (A: lipolytic activity; B: proteolytic activity, C: cellulolytic activity)

Table 2 The index of enzymes activity of *Bacillus* sp. collected from soil

Isolate code	Index				
	Amylase	β -galactosidase	Cellulase	Lipase	Protease
SSL01.1	-	-	-	-	1,13 \pm 0,18
SSL01.3	-	-	-	3,25 \pm 0,18	-
SSL01.4	-	-	-	2,57 \pm 0,20	-
SSL01.5	-	-	-	0,97 \pm 0,17	-
SSL01.6	-	-	-	0,95 \pm 0,06	1,29 \pm 0,40
SSL01.7	-	-	-	1,81 \pm 0,43	1,19 \pm 0,09
SSL01.8	-	-	-	-	1,57 \pm 0,20
SSL02.1	-	-	-	-	1,31 \pm 0,09
SSL02.2	-	-	-	-	-
SSL02.3	-	-	-	-	-
SSL02.5	-	-	-	-	1,25 \pm 0,00
SSL03.4	-	-	-	-	1,13 \pm 0,18
SSL05.1	-	-	-	0,32 \pm 0,14	2,00 \pm 0,00
SSL05.2	-	-	1,34 \pm 0,47	5,20 \pm 0,28	0,25 \pm 0,00
SSL05.3	-	-	1,17 \pm 0,24	5,40 \pm 0,28	0,88 \pm 0,18
SSL05.4	-	-	-	1,50 \pm 0,06	0,88 \pm 0,18
SSL05.5	-	-	-	-	-
SSL05.6	-	-	-	-	-
SSL05.7	-	-	-	1,85 \pm 0,07	1,00 \pm 0,00
SSL05.8	-	-	-	-	-
SSL02.4	-	-	-	-	-
SSL02.7	-	-	-	-	1,69 \pm 0,03

lipase, and protease. Those are potentially used in biotechnological applications as well in detergent formulations or biodegradation of oil pollutants.

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