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DAFTAR ISI

The Genus *Chitinophaga* Isolated from Wanggameti National Park and Their Lytic Activities
(Marga *Chitinophaga* yang diisolasi dari Taman Nasional Wanggameti dan Aktivitas Litiknya)

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ABSTRACT

The utilization of bacterial enzymes in commercial industry, agriculture, waste treatment and health is preferred over other sources like plants and animals sources because they provide many advantages for different applications. The genus *Chitinophaga* which was first described as chitinolytic Myxobacteria, known as chitin destroyer or chitin eater due to their capability to hydrolyze chitin. The present study aims to isolate, characterize, identify, and assay the indigenous bacteria from Wanggameti National Park for their lytic activity againts chitin, cellulose and protein as an initial step in bio-prospecting of Sumba Island. Eleven yellow pigmented isolates were obtained from soil and decayed wood samples using ST21 and Water Agar media. They formed halo on VY/2CX medium. Physiological charazterization showed that two isolates were able to produce catalase but none of them produced urease. The phylogenetic analysis based on 16S rRNA gene sequences indicated that all isolates belong to the genus *Chitinophaga* that consisting of *Chitinophaga filiformis*, *Chitinophaga ginsengisoli*, *Chitinophaga pinensis,* and *Chitinophaga sancti*. They were deposited in InaCC under the name InaCC B1254 to InaCC B1264. Qualitative analysis of their lytic activity exhibited that all strains were able to lyse chitin and cellulose. The strains with the highest chitinase and cellulase activity are InaCC B1260 and InaCC B1258 strains, respectively, both of them are C. *pinensis*. Hereafter, C. *filiformis* showed the highest proteolytic activity in skim milk casein amongs all strains at 1.14±0.08.

Keywords: *Chitinophaga*, chitinase, cellulase, protease, Sumba

ABSTRAK

Pemanfaatan enzim dari bakteri dalam bidang industri komersial, pertanian, pengolahan limbah dan kesehatan lebih diminati dibandingkan dengan sumber enzim lainnya, seperti tanaman dan hewan karena memberikan banyak keuntungan untuk berbagai aplikasi. Marga *Chitinophaga* yang pertama kali dipertelakan sebagai Myxobacteria kitinolitik, dikenal sebagai penghancur kitin atau pemakan kitin karena kemampuannya dalam menghidrolisis kitin. Penelitian ini bertujuan untuk mengisolasi, mengkarakterisasi, mengidentifikasi dan menguji aktivitas litik bakteri pribumi asal Taman Nasional Wanggameti terhadap kitin, selulosa, dan protein sebagai langkah awal dalam upaya bioprospeksi sumber daya hayati Pulau Sumba. Sebelas isolat bakteri berwarna kuning diisolasi dari sampel tanah dan kayu lapuk mengunakan media ST21 dan Water Agar. Isolat tersebut membentuk zona bening pada media VY/2CX. Karakterisasi fisiologis memperlihatkan bahwa sebanyak dua isolat mampu menghasilkan katalase, tetapi tidak ada satupun yang menghasilkan urease. Analisis filogenetik berdasarkan sekuen gen 16S rRNA mengindikasikan bahwa seluruh isolat termasuk dalam marga *Chitinophaga* yang terdiri dari *Chitinophaga filiformis*, *Chitinophaga ginsengisoli*, *Chitinophaga pinensis* dan *Chitinophaga sancti*. Isolat tersebut disimpan di InaCC dengan nomor InaCC B1254 sampai InaCC B1264. Analisis secara kuantitatif terhadap aktivitas litiknya menunjukkan bahwa seluruh strain mampu memecah kitin dan selulosa. Strain dengan aktivitas kitinase dan selulase tertinggi berturut-turut adalah InaCC B1260 dan InaCC B1258. Keduanya adalah C. *pinensis.* Selanjutnya, C. *filiformis* memperlihatkan aktivitas proteolitik paling tinggi di antara strain lainnya pada kasein susu skim, yakni sebesar 1.14±0.08.

Kata Kunci: *Chitinophaga*, kitinase, selulase, protease, Sumba

INTRODUCTION

The genus *Chitinophaga* was described by Sangkhobol and Skerman in 1891 with *Chitinophaga pinensis* as its type species. The genus itself was proposed as the type genus in the family Chitinophagaceae (Kampfer *et al*. 2011). *Chitinophaga* literally means chitin eater. When it was first described, the genus was called chitinolytic Myxobacteria owing to their morphological similarities excluding the ability to form fruiting bodies. Currently, the genus consists of 24

species with validly published names based on The List of Prokaryotic Names with Standing in Nomenclature (LPSN) (Euzéby, 1997). Some reports suggested that these microbes harbor hydrolytic properties so that they can be utilized as an enzyme producer (Weon *et al.* 2009; Wang *et al.* 2014).

Enzyme producing microorganisms have long been explored due to their high value for industrial purposes. Even though plants and animals are also capable of producing enzymes, those extracted from microbial sources are still preferred over other sources because most of the characteristics of enzyme producing microorganisms are suitable for biotechnological application, such as they have broad biochemical diversity, rapid in growth, only need limited space for cell cultivation, and can be genetically manipulated (Rao *et al.* 1998). Bacteria and fungi, for example *Pseudomonas* sp. (Hoshino *et al.* 1997), *Bacillus* sp. (Ellaiah *et al*. 2002, Sharmin *et al*. 2005), *Cyteromyces matritensis*, *Aspergillus dimorficus, Aspergillus ochraceus, Fusarium moniliforme, Fusarium solani, Penicillium fellutanum,* and *Fusarium waksmanii* (Rodarte *et al.* 2011) are known for their ability to produce protease. Protease is a group of enzyme that performs hydrolysis of the peptide bonds that link amino acid together in the polypeptide chain forming the protein (Srilakshmi *et al.* 2014).

In 2010, the global market for industrial enzymes is estimated at $3.3 billion. Meanwhile, technical enzymes are valued at just over $ 1 billion in the same year (Binod *et al.* 2013). These numbers are expected to increase in the following years due to the rapid development of biotechnology with some developed countries like Denmark, Germany, and Netherlands are leading as commercial enzyme producers (Li *et al.* 2012). Of the recognized commercials enzyme, more than 60% of the total enzyme market relies on protease (Aftab *et al*. 2006). They play an important role in pharmaceutical, food, feed, bio-energy, and cosmetic industry. Another microbial enzymes proficient in hydrolyze polysaccharides are cellulase and chitinase, converting cellulose and chitin into disaccharide or saccharide, respectively. Cellulose is the most abundant biopolymer on earth whereas chitin is the major source of carbon in marine ecosystem, hence degrading these polysaccharides is essential for the global earth carbon cycle, mammal nutrition, and even biofuel production (Graham *et al*. 2011; Sumerta & Kanti 2016; Talamantes *et al*. 2016). Chitinases have wide ranging applications in industry, agriculture, health, waste treatments, and biotechnology applications such as preparation of important pharmaceutical, preparation of single-cell protein, isolation of protoplasts from fungi and yeast, control of pathogenic fungi, treatment of chitinous waste, and control of malaria transmission (Dahiya *et al.* 2006).

For these reasons, in this study we explore newly collected bacteria isolated from soils and decayed wood obtained from Wanggameti National Park for their lytic activity. Wanggameti National Park is located in Sumba Island, East Nusa Tenggara Province, Indonesia. Biological resources in Sumba Island, particularly the microorganism segments, are not well recorded and studied up to now. Hence, the aims of our research are to isolate, characterize, identify, and assay the indigenous bacteria from Sumba Island for their lytic activity against chitin, cellulose, and protein as an initial step in bio-prospecting the biological resources of Sumba Island.

MATERIALS AND METHODS

The soil and decayed wood samples used in this research were collected from Wanggameti National Park area, Sumba Island, East Nusa Tenggara in April 2016. Some of the soil samples were taken from rhizosphere of local medicinal plants, including Cendana (*Santalum album*). They were air dried overnight prior isolation procedure.

Bacterial colonies were isolated using ST21CX agar media (a mix of A solution which made up in 700 mL: 1 g/L K_2HPO_4, 0.02 g/L yeast extract, 10 g/L agar; and B solution which made up in 300 mL: 1 g/L KNO_3, 1 g/L $MgSO_4.7H_2O$, 1 g/L $CaCl_2.2H_2O$, 0.2 g/L $FeCl_3$, 0.1 g/L $MnSO_4.7H_2O$) and WCX (water agar media contain 1 g/L $CaCl_2.2H_2O$ and 15 g/L agar) (Reichenbach & Dworkin 1992), both supplemented with 25 µg/mL cycloheximide to prevent fungal growth. Each of the soil and decay wood samples was put onto a Whatman

No.1 filter paper sized about 1 cm^2 that laid on ST21CX agar media. Each of the soil samples was also put on the center of *Eschericia coli* pellet that cross streaked on WCX media. These samples then were incubated at 30°C for 2-4 weeks.

The growing yellowish to yellow swarming colonies from filter paper on ST21CX agar and the previous WCX medium were transferred to a new WCX medium. This water agar medium was streaked with autoclaved *E. coli* prior application. Swarming colonies that able to produce clear zone around the dead *E. coli* were cut about 0.5 cm^2 near the edge of the swarming area. They were transfer to VY/2 agar medium (5 g/L Baker's yeast Fermipan, 1 g/L $CaCl_2.2H_2O$, 15 g/L agar, 0.5 µg/mL cyanocobalamin) supplemented with 25 µg/mL cycloheximide, designated as VY/2CX (Reichenbach & Dworkin 1992). They were incubated at 30°C for 5-7 days. This technique was repeated several times in order to obtain a pure bacterial colony. Cultivation of bacterial isolates was performed using VY/2CX medium. Pure bacterial isolates obtained were frozen in 10% glycerol and stored in -80°C for long term preservation.

The swarming colony appearance on agar plate was observed, including their pigmentation and swarming pattern using dissecting microscope Olympus SZ. The isolates were Gram stained using crystal violet, iodine, ethanol, and safranin reagents. Gram type and cell shape of the isolates were observed under a binocular microscope Olympus BX53. Physiological characteristics were determined by catalase and urease tests.

The 16S rRNA gene was amplified using a set of universal primers 27F (5'-AGAGTTTGA TCCTGGCTCAG-3') and 1492R (5'-GGTTA CCTTGTTACGACTT-3') (Brosius *et al*. 1981, Lane 1991). Composition of Polymerase Chain Reaction (PCR) consist of 12.5 µL GoTaq Green Master Mix (Promega), 0.5 µL 27F primer, 0.5 µL 1492R primer, 0.5 µL DMSO, 1.0 µL DNA genome, and 10 µL nuclease free water. PCR was performed under this following condition: 2 minutes of predenaturation at 94°C, this process was subsequently followed by 35 cycles of denaturing at 94°C for 15 seconds, annealing at 55°C for 30 seconds, elongation at 72°C for 1 minute, and final extention at 72°C for 10 minutes in Mastercycler Gradient (Eppendorf). PCR products were checked on 1% agarose gel stained with ethidium bromide solution and observed under UV transilluminator. These DNA fragments were sequenced by Macrogen Inc. (South Korea).

The nucleotide sequences obtained were analyzed using BioEdit program (Hall 1999). These sequences were aligned with validly published prokaryotic names using EzTaxon server (http://www.ezbiocloud.net/eztaxon) (Kim *et al*. 2012). Phylogenetic tree was constructed based on 16S rRNA gene sequences in MEGA 6 program (Tamura *et al.* 2013) using neighbor-joining method (Saitou & Nei 1987) and Kimura 2-parameter model (Kimura 1980) with 1,000 replicates of bootstrap. A Gram-positive bacterium *Bacillus subtilis* Acc No. AJ276351 was used as outgroup. All the identified isolates were deposited in Indonesian Culture Collection (InaCC).

All the isolates were tested for their activity to lyse macromolecules, including protein, chitin and cellulose. Basal media consist of 1 g/L glucose, 2.5 g/L yeast extract, 20 g/L agar, and supplemented with 10 g/L skim milk or 20 g/L colloidal chitin were used to assay proteolytic and chitinolytic activity, respectively (Kiran *et al.* 2015). Mineral salt media consist of 2 g/L KH_2PO_4, 1.4 g/L $(NH_4)_2SO_4$, 0.3 g/L $MgSO_4.5H_2O$, 0.3 g/L $CaCl_2$, 0.4 g/L yeast extract, 0.005 g/L $FeSO_4.7H_2O$, 0.0016 g/L $MnSO_4$, 0.0017 g/L $ZnCl_2$, 0.002 g/L $CoCl_2$, 5 g/L carboxymethyl cellulose-Na, 15 g/L agar in pH 5 were used to assay cellulolytic activity (Liang *et al*. 2014). A plug of swarming colony was inoculated onto these media. This procedure was conducted in triplicate for each tested isolate. Lytic activity was detected qualitatively by the presence of clear zone around the bacterial colony after incubation. Cellulose plates were stained with 1% Congo red for 15 minutes and destained with 1M NaCl solution prior observation. Lytic index was calculated using the following formula: Lytic index = (diameter of clear zone – diameter of colony) / diameter of colony.

RESULTS

Isolation and Preservation of Bacterial Isolates

Eleven isolates showing fairly similar morphological characteristics were obtained from soil and decayed wood samples collected

from Wanggameti National Park, East Sumba, Indonesia. These isolates swarmed away from the samples when isolated using ST21CX medium, hence produced yellowish mucoid cell masses on filter paper. They were able to grow on WCX supplemented with *E. coli* as well as on VY/2CX medium containing Baker's yeast cells. During observation, all the isolates were fast spreading on VY/2CX medium with 1% agar concentration. They consumed yeast cells, which was act as carbon and nitrogen source in VY/2CX medium, while swarming. This activity resulted in the emergence of distinct halo around the swarming colony.

Preservation of the isolates was conducted according to InaCC standard using freezing and lyophilization methods. These isolates were deposited in InaCC using the number InaCC B1254 to InaCC B1264. A proper preservation technique for microorganisms assures their viability and maintains their phenotypic characters stability for years.

Morphological and Physiological Characterization

Based on morphological observation on swarming colonies, they were pale to bright yellow pigmented. Most of the bacterial colonies showed circular shape with unique swarming patterns. Gram staining procedure revealed that these swarming bacteria were Gram negative and rod shaped (Figure 1). Six strains produced spherical resting cells in microscopic observation, including InaCC B1254, InaCC B1256, InaCC B1257, InaCC B1261, InaCC B1262, and InaCC B1264. As many as two isolates were catalase positive, but none of them were capable of producing urease. Observation on bacterial isolates morphology and physiology were summarized in Table 1.

Analysis of 16S rRNA Gene Sequences

Molecular identification revealed that these bacteria belonged to the genus *Chitinophaga*, member of family Chitinophagaceae by ≥ 98% similarity. The genus was known for their ability to hydrolyse chitin. Analysis of 16S rRNA gene sequences grouped all the 11 isolates into four groups based on their taxon name (Table 2). They are *Chitinophaga filiformis, Chitinophaga ginsengisoli, Chitinophaga pinensis,* and *Chitinophaga sancti. Chitinophaga ginsengisoli* was successfully isolated from decayed wood and soil sample. Their position among the closely related taxa was presented in Figure 2.

Lytic activity assay qualitative test on lytic activities of 11 *Chitinophaga* sp. showed that they were able to lyse chitin and cellulose. All the strains produced clear zone around the colonies, indicating chitin and cellulose degradation processes. Based on their lytic indices, strain InaCC B1260 produced the highest chitinase activity with lytic index 0.97±0.14 and strain InaCC B1258 produced the highest cellulase activity with lytic index 2.22±0.39. On the other hand, 91% of these strains were able to hydrolyse skim milk casein (Table 3). The

Table 1. Morphological and physiological characteristics of the isolates

Strain	Characteristics					
	Pigmentation	Colony shape	Cell shape	Gram staining	Catalase	Urease
InaCC B1254	Pale yellow	Irregular	Rod	Negative	-	-
InaCC B1255	Yellow	Circular	Rod	Negative	-	-
InaCC B1256	Pale yellow	Circular	Rod	Negative	-	-
InaCC B1257	Pale yellow	Circular	Rod	Negative	-	-
InaCC B1258	Pale yellow	Circular	Rod	Negative	-	-
InaCC B1259	Yellow	Circular	Rod	Negative	-	-
InaCC B1260	Yellow	Circular	Rod	Negative	+	-
InaCC B1261	Pale yellow	Irregular	Rod	Negative	+	-
InaCC B1262	Pale yellow	Circular	Rod	Negative	-	-
InaCC B1263	Pale yellow	Circular	Rod	Negative	-	-
InaCC B1264	Pale yellow	Circular	Rod	Negative	-	-

Notes: + means showing positive reaction, - means showing negative reaction

average of proteolytic indices produced by the tested strains was 0.75. Clear zones were absent on plates inoculated with strain InaCC B1259 while strain InaCC B1254 produced the highest proteolytic index at 1.14±0.08.

DISCUSSION

Several studies revealed the ability of bacterial species from the genus *Chitinophaga* to produce polysaccharides hydrolyzing enzymes such as cellulase and chitinase, whereas cellulose is obtained from plant biomass and chitin is found in wide range of organisms (Dahiya *et al*. 2006; Kishi *et al.* 2017). In this research, 11 isolates of *Chitinophaga* sp. consist of four different species were obtained from soil and decayed wood samples collected from Wanggameti National Park, East Sumba. Their morphological characteristics resemble to the genus *Chitinophaga* as described in Bergeys Manual (Kampfer 2010). They are aerobic, Gram negative, rod-shaped and yellow pigmented in the ST21CX and WCX media. The media chosen to grow *Chitinophaga* determined their colony pattern and pigmentation. This morphological observation is similar to Reichenbach (1989) that the cell mass of *Chitinophaga* is typically pale yellow on VY/2

Table 2. Molecular identification based on 16S rRNA gene sequence analysis

Strain	Source (Substrate)	Close relative	% Similarity	Sequence length (bp)
InaCC B1254	Soil (*Engelhardia spicata*)	*Chitinophaga filiformis* (AB078049)	99	1334
InaCC B1255	Soil		99	1316
InaCC B1261	Soil (*Santalum album*)		98	1282
InaCC B1262	Soil (*Santalum album*)		99	1317
InaCC B1256	Soil	*Chitinophaga ginsengisoli* (AB245374)	99	1326
InaCC B1257	Decayed wood		99	1307
InaCC B1258	Soil (*Podocarpus rhumpii*)	*Chitinophaga pinensis* (CP001699)	99	1318
InaCC B1259	Soil (*Podocarpus rhumpii*)		99	1385
InaCC B1260	Soil		99	1333
InaCC B1263	Soil (*Santalum album*)	*Chitinophaga sancti* (AB078066)	98	1318
InaCC B1264	Soil (*Santalum album*)		98	1330

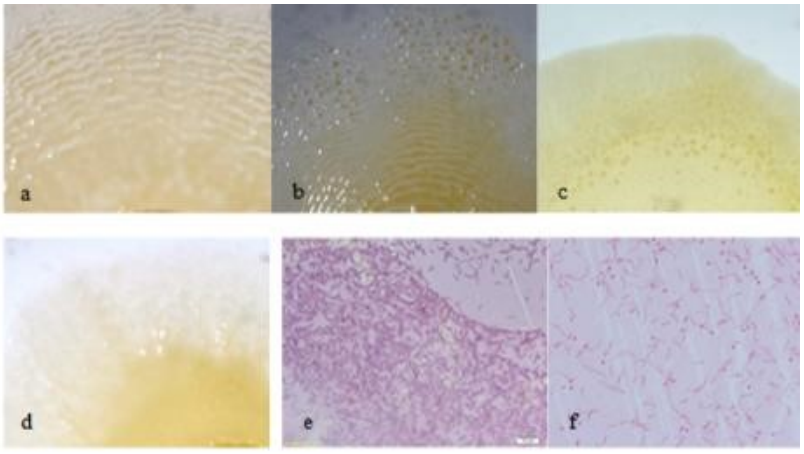

Figure 1. The pattern of swarming colony of InaCC B1254

Remarks: (a), InaCC B1256 (b), InaCC B1260 (c), and InaCC B1263 (d) grown on basal medium containing 2% colloidal chitin and incubated at 30°C for 8 days. Observation was performed in dissecting microscope Olympus SZ. Gram staining of strain InaCC B1256 showing rod shaped cells in 1000x magnification (e, the bar below indicated the size of the cells). Spherical resting cells existed in strain InaCC B1261 among their vegetative cells (f).

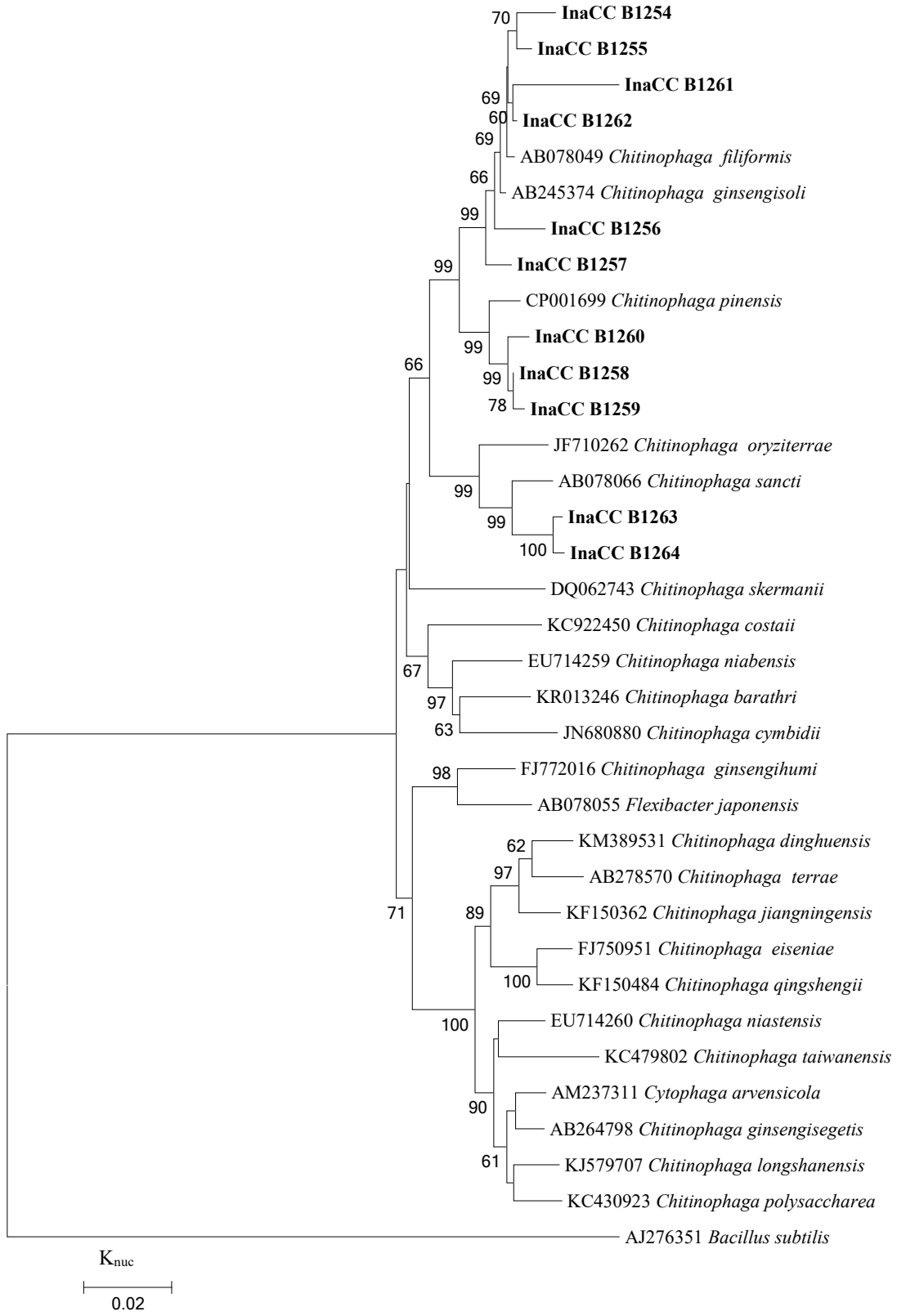

Figure 2. Phylogenetic tree constructed on the basis of 16S rRNA gene sequences using neighbor-joining method. Bar means 1 substitution per 200 nucleotides. Numeric at branch points indicate the bootstrap value as percentages derived from 1000 replications. Only values greater than 60% are shown.

medium and golden yellow on peptone media.

These isolates were also display swarming motility on VY/2CX medium, hence producing a unique colony patterns on agar plates. Swarming motility is a rapid multicellular bacterial surface movement powered by rotating flagella. In order to swarm on solid media, many swarming bacteria synthesize surfactant molecules. It is suggested that swarming motility gives some advantages for bacteria in competing with other microorganisms, bioremediation, pathogenesis and enhancing antibiotic resistance (Kearns 2010). Some of the species of *Chitinophaga* are also characterized by their gliding motility, which is defined as an active surface movement occurs along the long axis of the cell without either flagella or pili assistance. *C. filiformis, C. pinensis,* and *C. sancti* are reported to exhibit this type of motility on solid surface (Sangkhobol & Skerman 1981; Kampfer *et al.* 2006)

The isolation techniques and the media used in this research were purposed to isolate myxobacteria, a group of fruiting gliding bacteria. However, most of the species of *Chitinophaga* as well as myxobacteria are found in soil samples throughout the world and grown well on protein -based media. Therefore, they thrived on isolation media at the expense of the typically slower-growing myxobacteria. *Chitinophaga* can also be isolated from fresh water, decaying plant material and animal faces (Kampfer *et al.* 2006). In Indonesia, rarely research report related to this genus has been published.

Some of the physiological characteristics of successfully identified isolates are unequal with their type strain description. Only two out of five isolates identified as *C. pinensis* exhibit catalase activity. They are also unable to produce urease, unlike their type strain counterpart. These characters are also found in two collected *C. ginsengisoli* species. According to Lee *et al.* (2007) description, *C. ginsengisoli* are capable of producing both catalase and urease. However, it is common for physiological and metabolic characters to differ among microorganisms in one species because they correspond with the environment condition.

Lytic assay on collected *Chitinophaga* revealed that they are capable of degrading chitin and cellulose with lytic index range from 0.16 to 0.97 and from 1.36 to 2.53, respectively. It is widely reported that the utilization of *Chitinophaga* is bounded to carbohydrate-substrate related enzymes. The genome of species *C. pinensis* itself encodes nearly 200 representatives from 56 glycoside hydrolase families of carbohydrate active enzymes (McKee & Brumer 2015). *Chitinophaga pinensis* also produces some unique enzymes with mannan-degrading property (Larsbrink *et al.* 2017). A plant endophytic bacterium *Chitinophaga costaii* was also known to harbor genes that involve in cellulolytic, chitinolytic and lipolytic activities (Proenca *et al.* 2017). These reports support their potential as important bacteria to decompose biomass both in nature and industry.

Chitin degrading microbes are commonly related with their ability to inhibit the growth of pathogenic fungi, such as *Rhizoctonia solani*

Table 3. Lytic activities of *Chitinophaga* sp. strains

Strain	Lytic index		
	Chitinolytic (8 d)	**Cellulolytic (48 h)**	**Proteolytic (24 h)**
InaCC B1254	0.42 ± 0.19	1.86 ± 0.31	1.14 ± 0.08
InaCC B1255	0.69 ± 0.10	2.42 ± 0.18	0.79 ± 0.04
InaCC B1256	0.45 ± 0.04	1.36 ± 0.57	0.51 ± 0.05
InaCC B1257	0.47 ± 0.14	1.69 ± 0.10	0.89 ± 0.04
InaCC B1258	0.53 ± 0.10	2.53 ± 0.34	0.41 ± 0.06
InaCC B1259	0.75 ± 0.24	2.41 ± 0.08	-
InaCC B1260	0.97 ± 0.14	1.83 ± 0.18	0.61 ± 0.18
InaCC B1261	0.53 ± 0.11	2.22 ± 0.39	0.55 ± 0.05
InaCC B1262	0.29 ± 0.13	1.89 ± 0.45	0.82 ± 0.09
InaCC B1263	0.50 ± 0.08	1.69 ± 0.14	0.83 ± 0.15
InaCC B1264	0.16 ± 0.00	1.86 ± 0.45	0.95 ± 0.04

(Pleban *et al*. 1997). Chitin is one of the main components of fungal cell wall as well as exoskeleton of arthropods. The ability of the genus *Chitinophaga* to produce chitinase enzyme opens the possibility to utilize this microbe as an antifungal or insecticidal compounds producer.

Despite the fact that their potential in degrading carbohydrate-based molecules is undeniably promising, the interest in using this bacterial group as protease producer for industrial purposes is minor. Today microbial protease market is dominated by *Bacillus* spp. under the name Alcase, Savinase, Primatan, and Corolase 7089 (Bhunia *et al*. 2012). Yet, the chance to utilize *Chitinophaga* or other protease producing bacteria as an alternative option is still feasible. Based on our finding, the isolated *Chitinophaga* were able to lyse protein in the form of whole cells and skim milk casein. Their protease activity differs among the strains. Strain InaCC B1254 produced the highest proteolytic index at 1.14 and strain InaCC B1258 produced only 0.41 of proteolytic index. This result, indeed, is an initial step to explore Indonesian bio-resource, especially microbes from Sumba Island. Further studies need to be done to genuinely apply this bio-resource into our daily lives.

CONCLUSION

Soil and plant biomass are sources of *Chitinophaga* species which is known as chitin hydrolyzing bacteria. In this present study, 11 *Chitinophaga* species were successfully isolated from soil and decayed wood samples from Wanggameti National Park, Sumba. They were identified as *Chitinophaga* species based on morphological, physiological, phylogenetic, and lytic activities. Two strains were able to produce catalase and none produced urease, while all strains were able to hydrolize chitin and cellulose. The highest lytic index of chitinase and cellulase activity were showed by InaCC B1260 (0.97±0.14) and InaCC B1258 strains (2.22±0.39), respectively. Both of them were identified as C. *pinensis*. Ten strains exhibited protease activity with strain InaCC B1254 (C. *filiformis*) produced the highest proteolytic index at 1.14±0.08.

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PANDUAN PENULIS

Naskah dapat ditulis dalam bahasa Indonesia atau bahasa Inggris. Naskah disusun dengan urutan: JUDUL (bahasa Indonesia dan Inggris), NAMA PENULIS (yang disertai dengan alamat Lembaga/ Instansi), ABSTRAK (bahasa Inggris, dan Indonesia maksimal 250 kata), KATA KUNCI (maksimal 6 kata), PENDAHULUAN, BAHAN DAN CARA KERJA, HASIL, PEMBAHASAN, UCAPAN TERIMA KASIH (jika diperlukan) dan DAFTAR PUSTAKA. Penulisan Tabel dan Gambar ditulis di lembar terpisah dari teks.

Naskah diketik dengan spasi ganda pada kertas HVS A4 maksimum 15 halaman termasuk gambar, foto, dan tabel disertai CD atau dikirim melalui email redaksi/ web JBI. Batas dari tepi kiri 3 cm, kanan, atas, dan bawah masing-masing 2,5 cm dengan program pengolah kata *Microsoft Word* dan tipe huruf *Times New Roman* berukuran 12 point. Setiap halaman diberi nomor halaman secara berurutan. Gambar dalam bentuk grafik/diagram harus asli (bukan fotokopi) dan foto (dicetak di kertas licin atau di scan). Gambar dan Tabel di tulis dan ditempatkan di halaman terpisah di akhir naskah. Penulisan simbol a, b, c, dan lain-lain dimasukkan melalui fasilitas insert, tanpa mengubah jenis huruf. Kata dalam bahasa asing dicetak miring. Naskah dikirimkan ke alamat Redaksi sebanyak 3 eksemplar (2 eksemplar tanpa nama dan lembaga penulis).

Penggunaan nama suatu tumbuhan atau hewan dalam bahasa Indonesia/Daerah harus diikuti nama ilmiahnya (cetak miring) beserta Authornya pada pengungkapan pertama kali.

Pustaka didalam teks ditulis secara abjad.

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