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Isoptericola sp. A10-1, Chitinase Producing Actinobacterium Isolated from Indonesian Tropical Shrimp Pond Waste Water

Amrih Prasetyo[1]*, Lies Mira Yusiati[2], Yuny Erwanto[2], Wihandoyo[2], Nanung Agus Fitriyanto[2], Tomoyuki Nakagawa[3] and Takashi Hayakawa[3]

[1]Indonesian Agency for Agricultural Research and Development Jl. Ragunan 29 Pasar Minggu Jakarta Selatan 12540, Indonesia.
[2]Faculty of Animal Science, Gadjah Mada University, Bulaksumur, Yogyakarta 55281, Indonesia,
[3]Applied of Biological Sciences, Gifu University, Yanagido 1-1, Gifu 901-1135, Japan.
Corresponding E-mail: *amrihprasetyo@yahoo.co.id

ABSTRACT: The purpose of this study was to obtain superior chitinolytic bacteria capable of degrading chitin from the waste water of tropical shrimp pond. The bacterium characterization was conducted to yield high-activity chitinase isolates, while bacterium identification was carried out based on morphology, physiology, biochemistry and molecular. The 16SrRNA method was used to identify strain *Isoptericola sp*. A10-1, similarity (99%). This strain was able to produce of extracellular chitinase in media containing colloidal chitin a carbon source. Results of morphological identification, biochemical and molecular identified as *Isoptericola sp*. A10-1 including the genus *Isoptericola* showed chitinase activity in medium containing chitin as a carbon source. Chitinolytic bacteria *Isoptericola sp.* A10-1 are able to degrade chitin specifically into the monomer in the form of glucosamine. They are widely used in the fields of agriculture, food and health industries.

Keywords: Isolation, *Isoptericola sp.*, Chitinase, Shrimp Waste

INTRODUCTION

The growth of shrimp farms in marine areas of Java and Lampung, Indonesia is growing rapidly. Small-scale shrimp farms also grown in Bantul, Yogyakarta. So that the potential of shrimp waste very much, which is a material for producing chitin.

Chitinase (EC 3.2.11.14) enzyme can hydrolyze insoluble chitin from oligomers and monomer. Chitin can be found in a variety of organisms including bacteria, fungi, insects, higher plants, and animals. They play important physiological roles depending on their origin (Gooday, 1990). Chitinase has a wide-range of applications such as preparation of pharmaceutically relevant chitooligosaccharides. Chitin waste can be altered into N-acetyl-D-glucosamine, treatment of chitinase waste, and functional food (Dahiya *et al*., 2006). Chito-oligomers produced by enzymatic hydrolysis of chitin are used in various function for agricultural and industrial applications, such as antibacterial, antifungal, and as a food quality enhancer (Bhattacharya *et al*., 2007).

The first step in the degradation of chitin, which is mainly carried out by microbes, is the hydrolysis of the glycosidic bond between N-acetylglucosamine residues by chitinase (EC 3.2.1.14) (Cottrell *et al*. 1999). Chitinase hydrolyze chitin polymer into oligosaccharides, particularly kitobiosa (GlcNAc), a dimer of subunit N-acetylglucosamine. β-N-asetilase (EC 3.2.1.52) hydrolyze (GlcNAc) to produced the final product, N-acetylglucosamine (GlcNAc). Hydrolysis β-(1,4)-glukosidik chitin bond can resume activity endokitinase and eksokitinase (Cabib, 1987)

Actinomycetes are a group of microorganisms that have morphology and growth properties are located between fungi and bacteria. In the book Bergey's Manual, Actinomycetes are grouped into groups of Gram-positive bacteria with filament yarns in the form of short branched mecelium with a diameter of 0.05 to 2 μm (Holt *et al*., 1994).

This study aimed to gain superior isolates chitinolytic bacteria capable of degrading chitin

from shrimp waste, especially in the tropical shrimp pond. Characterize isolates with the highest chitinase activity based on morphology, physiology, biochemistry and molecular identified isolates.

MATERIALS AND METHODS

The isolates were analyzed for species identity using the 16S rRNA gene sequencing method according to Rochelle *et al*., (1995). The gene sequencing was performed at Genomics Research (Gifu Univ). DNA sequences were aligned using DNA star & Data Collection v3.1 Communication Patch1. Bacterial 16S rRNAs were amplified by using the following universal bacterial 16S rRNA primers. Forward primer 27 F (5'-AGAGTTTGATCMTGGCTCAG-3') and reverse primer 1792 R (5'-TACGGYTACCTTGTTACGACTT-3') (Gomaa, 2012).

RESULTS AND DISCUSSION

Identification of the isolates A10-1 based on biochemical, morphological and molecular. Cells are Gram-positive-staining, coccoid- or rod-shaped, non-motile and have no spores. Colonies on TSA are orange, circular, convex, smooth and 1.0–2.0 mm in diameter after 48 h incubation at 30 ºC. Optimum growth occurs at 35 ºC, at pH 8.0 and with 2% (w/v) NaCl. In addition to the characteristics presented in Table 1. The Gram staining showed Gram positive on (Figure 1). Oxidase positive biochemical test results, using the substrate D-trehalosa, sucrosa and maltosa. Enzym test β-N-Acetyl-Glucosamine positive and oxygen requirements are aerobic and facultative anaerobic.

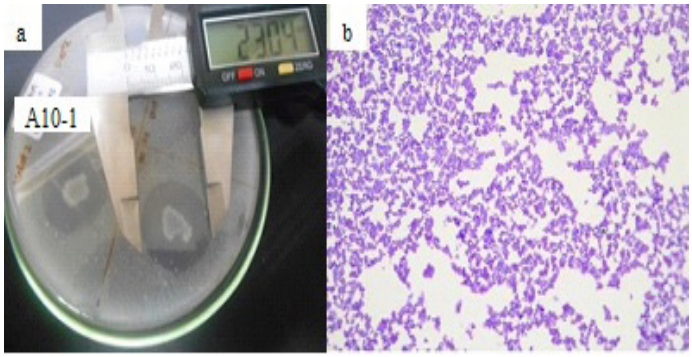

Figure 1. a). Isolate A10-1 grown on chitin agar plates 1% and showed clear zones. The culture was incubated at 30°C for 5 days
b). Gram staining of bacteria showed coccoid and gram-positive

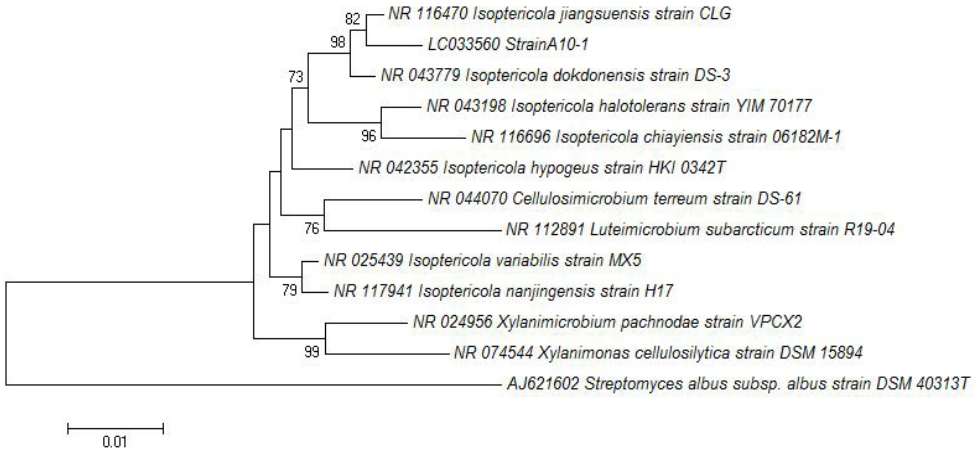

Figure 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain LC033560 A10-1 *Isoptericola sp.* Bootstrap values (expressed as presentages of 1000 replications).of >70% are shown at branch points. The Scale bar corresponds to 0.01 subscriptions per nucleotide position.

The phylogenetic tree showed that A10-1 is closely related to members of the species *Isoptericola sp* A total of 1177 bp of the 6S rRNA gene sequence was sequenced. Comparative 16S rRNA gene sequence analysis showed that strain A10-1 was most closely related to members of the genus *Isoptericola*.

Similarities between the 16S rRNA gene sequences of strain A10-1 and *I. jiangsuensis* strain CLG, *I. dokdonensis* strain DS-3, *I. nanjingensis* strain H17 and *I. hypogeus* strain HKI 0342 were 99, 99, 98 and 98 %, respectively. In a phylogenetic tree based on the neighbour-joining algorithm (Saitou & Nei, 1987), strain A10-1 and *I. dokdonensis* strain DS-3 formed an independent cluster at a bootstrap value of 98% (Figure 2).

Table 1. Morphological, biochemical, and physiological characteristics of isolates A10-1

Shaped	Coccoid or rod	Utilization of:	
Growth :		D-Fructosa	-
10°C	+	D-Trehalosa	+
42°C	-	Sucrosa	+
Gram stain	+	Maltosa	+
Spora	-	Acetat	-
Motilitity	-	L-Glutamat	-
Colonies morphology:	Circular, orange	API ZYM test:	
Aerob, anaerob facultatif	+	β-N-Acetyl-glucosamine	+
NaCl 2% (w/v)	-	α-galactosidase	+
Oxidase	+	Acid phospatase	-
Hidrolysis:		Alkaline phospatase	+
Urea	+	Trypsine	-
Gelatin	+	Valine arylamidase	-
Casein	-	Cystine arylamidase	-
Starch	-	Lipase (C14)	+
Hypoxanthine	-	Esterase (C4)	+
Xanthine	-		
Tyrosine	-	Type peptidoglycan	L-Lys-D-Asp

VITEX system was used; +, positive; –, negative,

The genus *Isoptericola* was proposed by Stackebrandt *et al.* (2004) for the misclassified species *Cellulosimicrobium* variabile. The genus *Isoptericola* currently comprises seven species with validly published names: *Isoptericola variabilis* (Stackebrandt *et al.*, 2004), *Isoptericola hypogeus* (Groth *et al.*, 2005), *Isoptericola halotolerans* (Zhang *et al.*, 2005), *Isoptericola dokdonensis* (Yoon *et al.*, 2006), *Isoptericola jiangsuensis sp.* nov. (Wu *et al.*, 2010), *Isoptericola chiayiensis* (Tseng *et al.*, 2011), and *Isoptericola nanjingensis* (Huang *et al.*, 2012),

CONCLUSION

The strain A10-1 was selected among those giving maximum enzyme production in the shortest time. It was further identified as *Isoptericola sp.* A10-1. The chitinase enzyme that was produced by this strain has chitinase activities.

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