Research Journal of Microbiology

ISSN 1816-4935

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 10 (12): 582-591, 2015
ISSN 1816-4935 / DOI: 10.3923/jm.2015.582.591

In silico Analysis of Plantaricin EF that Expressed by Plasmid-Associated Bacteriocin Production Gene of *Lactobacillus plantarum* IBL-2 for Anti-Candida Agent Potential

[1]Betty Nurhayati, [1]Marlia Singgih Wibowo, [2]Yantyati Widyastuti, [3]Pande Putu Erawijantari, [4]Wahyu Widowati, [1,5]Mohammad Rizki Fadhil Pratama and [1]Tutus Gusdinar Kartawinata

[1]School of Pharmacy, Bandung Institute of Technology, Jl. Ganesa 10, Bandung, 40132, West Java, Indonesia
[2]Research Center for Biotechnology, LIPI, Jl. Raya Bogor Km 46, Cibinong, 16911, Indonesia
[3]Biomolecular and Biomedical Research Center, Aretha Medika Utama, Jl. Babakan Jeruk 2 No. 9, Bandung, 40163, West Java, Indonesia
[4]Medical Research Center, Faculty of Medicine, Maranatha Christian University, Jl. Prof drg. Suria Sumantri No.65, Bandung, 40164, West Java, Indonesia
[5]Faculty of Medical Sciences, Muhammadiyah University of Palangkaraya, Jl. RTA. Milono Km 1.5, Central Borneo, 73111, Indonesia

Corresponding Author: Betty Nurhayati, School of Pharmacy, Bandung Institute of Technology, Jl. Ganesa 10, Bandung, 40132, West Java, Indonesia

ABSTRACT

Lactobacillus plantarum species often harbor several plasmids. These plasmids may encoded important traits such as phages or antibiotics resistances, lactose catabolism and production of proteolytic enzyme and also bacteriocins that named plantaricin. *Lactobacillus plantarum* IBL-2 that isolated from strawberry of Bali plantation have the highest anti-microorganism activity among the *L. plantarum* isolate collection of BTCC Indonesia. Only few study carried out to examine the antifungal activity of bacteriocin from *L. plantarum*. This study focus on anti-Candida activity of plasmid associated with bacteriocin production from *L. plantarum* IBL-2. The isolates were confirmed by the 16S rRNA analysis by PCR and the phylogenetic tree was built based on references sequences and one outgroups from database. The plantaricin gene screening then performed in plasmid that was isolated from *L. plantarum* IBL-2 by PCR using five pairs or plantaricin gene primer. The anti-Candida potential of plantaricin observed were analyzed by *in silico* by docking analysis between the plantaricin and receptors of apoptosis proteins. *Pln*B and *Pln*EF were observed in the *L. plantarum* plasmid. Only *Pln*EF become the focus of study. Analysis of docking study predicted that *Pln*EF have interactions with apoptosis proteins regulator in eukaryotic cells. *Pln*EF that encoded by plasmid of *L. plantarum* may exert anti-Candida potential through interactions with apoptosis proteins regulator.

Key words: Bacteriocin, plasmid, anti-candida, docking, apoptosis

INTRODUCTION

Lactic acid bacteria produce antimicrobial compounds including hydrogen peroxide, CO_2, diacetyl, acetaldehyde, D-isomers of amino acids, reuter in and bacteriocin (Cintas *et al*., 2001). Bacteriocins are ribosomally synthesized compounds that exhibit bactericidal activity either of

same species (narrow spectrum) or across genera (broad spectrum) (De Martinis and Franco, 1998; Cotter *et al*., 2005). Recently, bacteriocin producing by Lactic Acid Bacteria (LAB) have attracted significantly attention because it recognized as safe microorganisms (Yang *et al*., 2012). *Lactobacillus plantarum* is one of the most important members of LAB associated with many fermented food and known to produce bacteriocins usually called plantaricin (Sharma and Srivastana, 2014; Navarro *et al*., 2000). *Lactobacillus plantarum* is a sporophyte often associated with plant and fermenting materials and plays a major role in the fermented product such as vegetables, sausages and silage (Olasupo, 1996). *Lactobacillus plantarum* species often harbor several plasmids (Ritz-Barba *et al.,* 1991). These plasmids may encoded important traits such as phages or antibiotics resistances, lactose catabolism and production of proteolytic enzyme and also bacteriocins (Van Kranenburg *et al*., 2005).

Exploration of bacteriocin have been done by many researcher from around the world isolated from meat, fermented sausages, fish, fruits, etc (Todorov, 2009). Only few research reported about the isolation and application of bacteriocin from Indonesian isolates (Arief *et al*., 2013). Bio Technology Culture Collection (BTCC), Indonesian Science Institute has been isolating some *L. plantarum* strains from grass silage, corn, traditional fermented buffalo milk, faeces, fermented cassava and strawberry from Yogyakarta, Cibinong, Pekanbaru, Sulawesi, Bali, etc. Among them, *L. plantarum* IBL-2 that isolated from strawberry of Bali plantation have the highest anti-microorganism activity. Since, bacteriocins may be either chromosomally or plasmid-encoded (Todorov, 2009), in this study we will analyze the plasmid associated with bacteriocin production from *L. plantarum* IBL-2.

The development of new, safer and more efficacious agents to combat serious fungal infections especially *Candida* become a pressing need since the fungal infections incidence and resistance of standard antifungal was increasing (Beck-Sague *et al*., 1993). *Candida albicans* is the most important fungal opportunistic pathogen that resides as a commensal in the gastrointestinal and genitourinary tracts and in the oral and conjunctival flora (Spampinato and Leonardi, 2013). Sharma and Srivastana (2014) revealed that bacteriocins from *L. plantarum* have fungicidal activity against *Candida albicans*. Only few study carried out to examine the antifungal activity of bacteriocin from *L. plantarum* since it regarded to have narrow inhibition spectrum that just being active only on closely related bacteria (Klaenhammer, 1993). Therefore the study focus on the anti-Candida activity of the plasmid associated with bacteriocin production from *L. plantarum* IBL-2.

MATERIALS AND METHODS

Microorganisms, culture and medium: The *L. plantarum* IBL-2 was obtained from Bio Technology Culture Collection (BTCC), Indonesian Science Institute. The strains were isolated from the strawberry of Bali plantation. *Lactobacillus plantarum* IBL-2 was cultured in the Man Rogosa Sharp Broth (MRSB). *Candida albicans* were cultured in the Potato Dextrose Agar (PDA). The *L. plantarum* IBL-2in agar was inoculated into MRSB for culture activation then incubated in 30°C for 18-24 h. The *C. albicans* were inoculated in the Potato Dextrose Broth (PDB) then all were incubated in the 37°C for 18-24 h.

DNA isolation, 16S rRNA sequencing and phylogenetic tree analysis: The *L. plantarum* IBL-2 DNA was analyzed *in silico*. Genomic DNA was isolated from the *L. plantarum* culture in

broth medium using DNA extraction kit (Promega, USA) (Widyastuti *et al*., 2012). The 16S rRNA PCR then preformed using the 5'-GAGTTTGATCCTGGCTCAG-3' forward primer and 5'-AAGGAGGTGATCCAGCC-3' reverse primer. The PCR product was confirmed by electrophoresis. Sequencing then performed by Sanger sequencing in 1st BASE Pte Ltd. (Singapore). The sequence was analyze by Basic Local Alignment Search Tools Nucleotide (BLAST N), NCBI in http://blast.ncbi.nlm.nih.gov/. The references sequences was collected from the NCBI 16S Ribosomal RNA reference sequence similarity search and RDP sequence match in http://rdp.cme.msu.edu/seqmatch/. One outgroup was selected to build the phylogenetic tree. The sequence information was then imported into genetic software program for alignment. Phylogenetic trees were constructed by the Maximum Likelihood method using MEGA 4 software program with 10000 times of bootstrap after the alignment by ClustalW (Arief *et al*., 2013; Nishida *et al*., 2011).

Plasmid DNA isolation and bacteriocin gene screening: The plasmid isolation was performed based on Green and Sambrook (2012). The plasmid then confirmed by gel electrophoresis. Gene screening was performed using 5 pairs of primer *pln*A, *pln*B, *pln*EF, *pln*J and *pln*W by Polymerase Chain Reaction (PCR) (Table 1). The PCR was performed by 35 cycle of PCR. The PCR product confirmation was performed by gel electrophoresis. Sequencing then performed by Sanger sequencingin 1st BASE Pte Ltd. (Singapore). Sequence similarity searches were performed in the GenBank data library using the Basic Local Alignment Search Tools Nucleotide (BLAST N) program.

***In sillico* docking analysis:** Only *pln*EF will be focused on this *in silico* study. The Open Reading Frame (ORF) from DNA sequence was found by the NCBI ORF finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Bacteriocin gene translation from the selected ORF was predicted using proteomic program translate (http://www.expasy.org/translate). Amino acids sequence similarity searches were performed in the GenBank data library using the Basic Local Alignment Search Tools Protein (BLASTP) program. The protein structure homology analysis then performed by SWISS-MODEL analysis (http://swissmodel.expasy.org/interactive) and visualized using PyMOL.

In silico interaction of *pln* gene with receptors of apoptosis proteins were performed to analyze potential of antimicrobial activity from *plnE* and *plnF* by apoptosis mechanism. Receptors for docking analysis were chosen based on groups of apoptotic proteins regulator consists of (1) CDK (cyclin dependent kinases) enzymes group which involved in cell-cycle regulation, (2) Anti apoptotic BcL (B-cell lymphoma) protein group which involved in mitochondria-dependent apoptosis, (3) Inhibitor of caspase effectors, inhibitor of apoptosis (IAP) group and (4) Decoy receptors (DcR3) group which could interacted with Fas Ligan (Guo and Hay, 1999; Reed, 2000; Shi, 2002; Pitti *et al*., 1998). *pln*E and *pln*F from homology analysis were served as ligand for docking method. *pln*E and *pln*F were separated using AutoDockTools-1.5.6rc3 software and .pdb format file was converted with OpenBabel 2.3.2 software resulted to .hin format file. Geometry

Table 1: Primers for bacteriocin gene screening

Genes	Forward primers (5'-3')	Reverse primers (5'-3')	References
*pln*A	GTACAGTACTAATGGGAG	CTTACGCCAATCTATACG	Omar *et al.* (2008) and Diep *et al.* (2009)
*pln*B	TTCAGAGCAAGCCTAAATGAC	GCCACTGTAACACCATGAC	Omar *et al.* (2008) and Diep *et al.* (2009)
*pln*EF	GGCATAGTTAAAATTCCCCCC	CAGGTTGCCGCAAAAAAAG	Omar *et al.* (2008) and Diep *et al.* (2009)
*pln*J	TAACGACGGATTGCTCTG	AATCAAGGAATTATCACATTAGTC	Omar *et al.* (2008) and Diep *et al.* (2009)
*pln*W	GATCAGCCACGATACCAAC	CTAAAGAAAAAGCCCCTGAAAC	Saenz *et al.* (2009)

optimization was performed using HyperChem 8.0.3 software with molecular mechanics method, AMBER Force Field and Polak-Ribiere algorithm. The .hin format file then re-converted to .pdb format file using OpenBabel 2.3.2 software. Waters and other molecules of receptor protein that not included in docking method were eliminated using AutoDockTools-1.5.6rc3 software. Docking analysis was based on the free energy of binding, amino acids residue and hydrogen bonds analysis (Trott and Olson, 2010).

RESULTS

Sequences of 16S rRNA of *Lactobacillus plantarum* IBL-2 show the high similarity with several strain of *Lactobacillus plantarum* in database: *Lactobacillus plantarum* IBL-2 has the highest similarity with the *L. plantarum* Ls52 based on the 16S rRNA sequence. *Bacillus asahii* was selected as the outgroups. Figure 1 showed that the *L. plantarum* IBL-2 formed one clade with all of the *L. plantarum* strains that selected as references sequences.

***pln*B and *pln*EF were observed in *Lactobacillus plantarum* IBL2:** The PCR detection was performed in *L. plantarum* plasmid DNA of two IBL-2 isolate. The presence of *pln*B was observed in both isolate by 200 bp amplicon size. 400 bp amplicon size was also observed as *pln*EF in both isolate of *L. plantarum* IBL-2 (Fig. 2). The *pln*A as a inducer to activate the *pln*EF was not observed in both of isolate. Confirmation of the gene sequences by BLAST nucleotide showed that the *pln*B and *pln*EF observed by PCR has high identity with *pln* gene of *L. plantarum*. The *pln*EF will become the focus for the next study.

***In sillico* docking analysis:** Based on protein structure homology of *pln*E (PDB ID 2JUI) and *pln*F (PDB ID 2RLW) were chosen as ligand for docking method. Geometry optimization was performed to provided most stable molecule with lowest structure energy. *pln*E has 51.4806 kcal mol^{-1} of structure energy and *pln*F has 160.3685 kcal mol^{-1} of structure energy after geometrical optimization. Docking analysis were performed to analyze free energy of binding between ligand and active site of each receptors (Cosconati *et al*., 2010). Docking method could predict preferred orientation of one molecule ligand to a second when bound to each other to form a stable complex (Sandeep *et al*., 2011). The more negative free energy of binding shows higher affinity between ligand and receptors (Kim and Skolnick, 2008). Both *pln*E and *pln*F have free energy of binding more negative than -10 kcal mol^{-1}. That results shows that both of *pln*E and *pln*F have interaction with the apoptotic proteins regulator (Table 2, Fig. 3). Figure 3 shows the representation binding orientation of *pln*E and *pln*F to the apoptotic protein receptor.

Table 2: The interaction of *pln*E and F with apoptotic protein receptors

Receptors	PDB codes	Plantaricin E free energy of binding (kcal mol^{-1})	Plantaricin F free energy of binding (kcal mol^{-1})
CDKs			
CDK1	ILC9	-14,4	-13,5
CDK2	1HCK	-13,0	-12,7
CDK4	2W9Z	-13,9	-13,3
CDK6	4TTH	-13,5	-13,0
IAPs			
BIR2	1I3O	-12,4	-12,0
BIR3	1NW9	-12,8	-13,3
NAIP			
BIR2	2VM5	-13,0	-13,3
BIR3	2UVL	-12,1	-11,9
BcLs			
BcL-2	2W3L	-12,8	-12,8
DcR	4MSV	-12,6	-16,3

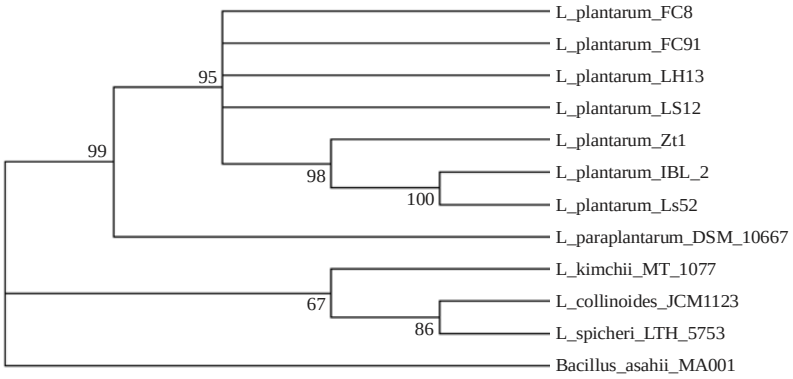

Fig. 1: Maximum likelihood phylogenetic tree of *Lactobacillus plantarum* IBL-2 among other *Lactobacillus plantarum* strains and other references sequences

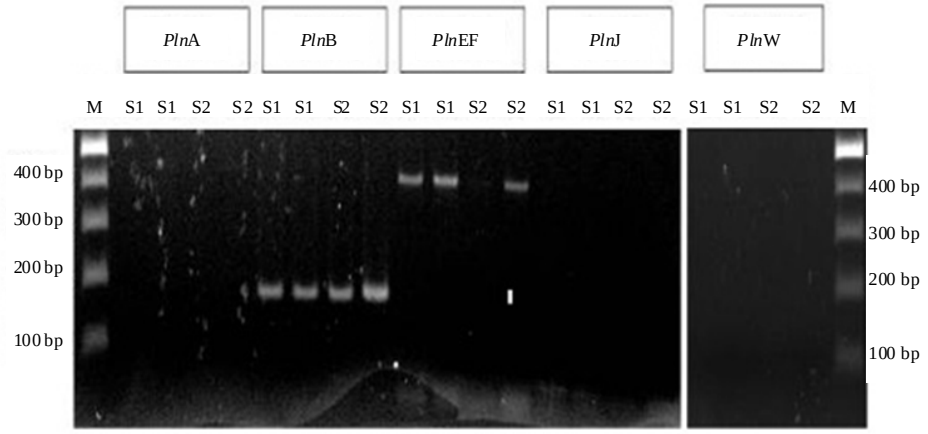

Fig. 2: Electropherogram of PCR amplicon using five primers (*pln*A, *pln*B, *pln*EF, *pln*J, *pln*W). The plasmid DNA was isolated from two isolate of *Lactobacillus plantarum* IBL-2 (S1 and S2) and both of sample was confirmed by PCR in duplicate

DISCUSSION

Among the members of LAB, the *Lactobacillus* are composed of diverse group of homofermentative and heterofermentative species and are most often cited for bacteriocin production (Klaenhammer, 1988). Many LAB produce antimicrobial peptides as bacteriocins that encoded by both of plasmid and chromosomal DNA (Maldonado *et al*., 2003). In this study, we focused on the plasmid associated bacteriocin production gene *in sillico* analysis of *L. plantarum* IBL-2 that isolated from strawberry of Bali plantation for anti-Candida potential.

Plasmid DNA in the lactic acid bacteria is not always easily detected due to the copy number and isolation procedure (Mourad, 2007). In this study two kind of bacteriocin consist of *pln*B and *pln*EF were confirmed in the *L. plantarum* IBL-2 plasmid. These results indicate that the plasmid may carry the plantaricin gene production. Plantaricin B (*pln*B) only inhibit the closely related species including *L. plantarum*, *Leuconostoc mesenteriodes* and *Pediococcus damnosus*. It was sensitive to the proteolytic enzyme and nonproteolytic enzyme could inhibit it activity (Olasupo, 1996). *pln*B along with *pln*C and *pln*D encode proteins that involved in signal transduction (Todorov, 2009). Plantaricin EF (*pln*EF) is one kind of two-peptide bacteriocins produced by *L. plantarum* along with *pln*JK, whose genes are located adjacent to each other on

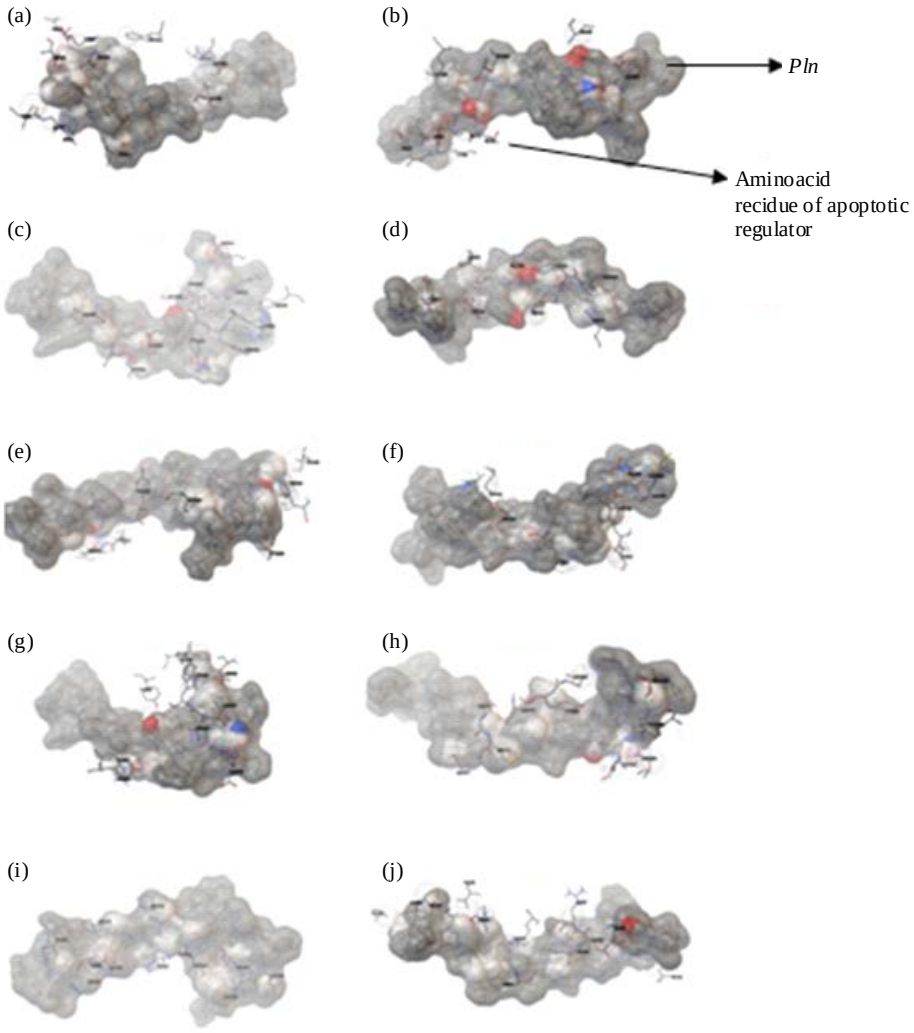

Fig. 3(a-j): Binding orientation of *pln*E with (a) CDKs, (c) IAPs, (e) NAIP, (g) BcLs, (i) DcR and *pln*F with (b) CDKs, (d) IAPs, (f) NAIP, (h) BcLs and (j) DcR

the same, *pln*EFI and *pln*JKLR, respectively. Both of that plantaricin can create pores in membranes of cells targets that can dissipate the transmembrane electrical potential and pH gradient and efficiently conduct small monovalent cations an anions respectivelly (Anderssen *et al*., 1998). In this study we focused on the *pln*EF.

In silico study was carried out to identify interactions of *pln*E and *pln*F with apoptotic protein regulator in eukaryotic cells consists of CDKs, IAPs and BcL group for anti-Candida agent potential prediction through apoptotic pathway. Both of *pln*E and *pln*F have high affinity with apoptotic protein receptors shows by negative free energy of binding that more negative than -10 kcal mol^{-1}. Free energy of binding is correlated with probability of affinity and stability of bounding between ligand and receptor. More negative free energy of binding shows higher affinity of ligand to specific binding site of receptors (Januar *et al*., 2012). The cell cycle progression and cell division in eukaryotes require activation of serine-threonine protein kinases called CDKs (Russo, 1997; Solomon *et al*., 1992). Catalytic activity of CDKs is up-regulated primarily by cyclin

binding and post-translational phosporylation of conserved threonin residues by CDK-activating kinase (CAK). CDK-cyclin complex could be inactivated by either removal of cyclin or dephosphorylation of Thr 160/161 residue. However, although these are two main ways to deactivated CDK-cyclin complex, it also could be done by phosporylation at two sites near amino terminus (Thr-14 and Tyr-15) that located hanging from the ceiling of ATP-binding site in certain position that could affect kinase activity of CDKs when phosporylated. *pln*E has more negative free energy of binding to CDKs compared to *pln*F, predicting that *pln*E have more affinity towards CDKs than *pln*F. *In sillico* docking analysis predicted that *pln*E have interaction with Thr-14 of CDK1 but kind of interaction was still undefined. However, interaction between *pln*E with Thr-14 could prevent phosphorylation of Thr-14 due to stearic barrier from *pln*E at Thr-14. Phosphorylation of Thr 14 and Tyr 15 is particularly important in CDK1 activation at mitosis (De Bondt *et al*., 1993; Chashoo and Saxena, 2014). Inhibition of CDK1 in early mitotic phase results in cell cycle arrest in G_2 and inhibition during mitosis results in exit from mitosis without cytokinesis and longer exposure lead to the apoptosis process (Vassilev *et al*., 2006).

The IAPs are family of proteins with various biological functions including regulation of innate immunity and inflammation, cell proliferation, cell migration and apoptosis. The NLR family-Apoptosis Inhibitory Protein (NAIP) and X-linked IAP (XIAP) are members of IAPs protein contains BIR domains (BIR 1-3) in the N-terminal half of the protein (Berthelet and Dubrez, 2013) *in sillico* docking analysis shows that plantaricin have interactions with NAIP and XIAP protein. There is small differences between *pln*E and *pln*F towards BIR2 and BIR3 domain of IAPs, predicting that both *pln*E and *pln*F have relative similar affinity towards IAPs. *pln*E and *pln*F binding to catalytic site of IAPs that interacted with caspase effector such as caspase-8 and caspase-9. Normally, IAPs would binds to caspase effectors and inhibits activity of caspase, resulting in delayed apoptosis process (Reed, 2000). Interaction of *pln*E and *pln*F with IAPs could prevents IAPs to inhibits caspase effectors, should that apoptosis process could occurs. The intrinsic mitochondrial pathway is result of increased mitochondrial permeability and release of pro-apoptotic molecules such as cytochrome-c into cytoplasm that regulated by a group of protein belonging to Bcl-2 family. Other apoptotic factors that are released from mitochondrial intermembrane space into cytoplasm include Apoptosis Inducing Factor (AIF), second mitochondria-derived activator of caspase (Smac), direct IAP binding protein with Low pI (DIABLO) and Omi/high temperature requirement protein A (HtrA2). On the other hand, Smac/DIABLO or OMI/HtrA2 promotes caspase activation by binding to inhibitor of apoptosis protein (IAPs) (Wong, 2011).

The Bcl-2 family of protein is comprised of pro-apoptotic and anti-apoptotic proteins that play an important role in regulation of apoptotic via the intrinsic pathway. Disruption in balance of anti-apoptotic and pro-apoptotic members of Bcl-2 family could dysregulated apoptosis in affected cells (Raffo *et al*., 1995). Docking results shows that both of *pln*E and *pln*F have relative similar interaction with Bcl-2 protein. BcL-2 protein is anti-apoptotic proteins with mechanism as inhibitor of pro-apoptotic molecules such as cytochrome-c (Reed, 2000). Interaction of BcL-2 with *pln*E and *pln*F prevents BcL-2 protein bound to cytochrome-c, resulting in increase of pro-apoptotic molecules in cytoplasm.

Decoy receptors-3 (DcR3) is a member of Tumor Necrosis Factor (TNF) family that overexpressed in some type of tumors such as lung and colon tumor. DcR-3 could disrupting balanced of Fas-FasL complex developed (Pitti *et al*., 1998). The Fas-FasL complex has important roles in apoptosis such as to mediate immune-cytotoxic killing of abnormal cells. DcR-3 would compete with Fas receptor to binds with Fas ligand, resulting in reduced number of Fas-FasL complex developed, hence reduced rate of apoptosis process in tumor cells (Reed, 2000). Both *pln*E

and *pln*F could interact with catalytic site of DcR3, resulting disability of DcR3 to compete with Fas receptor to binds FasL. Interestingly, *pln*F provide significant higher affinity towards DcR3 than *pln*E. DcR3 itself is a protein that produced outside of normal cells, where it was produced in membrane cells of tumor cells. These results indicating that *pln*F might have better potency to interacted with intercellular target.

CONCLUSION

Lactobacillus plantarum IBL-2 has *pln*E and *pln*F in its plasmid. *pln*EF that encoded by plasmid of *L. plantarum* may exert anti-Candida potential through the interaction with the apoptotic protein regulator. Further *in vitro* and *in vivo* study was still needed to confirm its mechanism of actions.

ACKNOWLEDGMENTS

We gratefully acknowledge the financial support of the Hibah Program Riset Desentralisasi Tahun 2014, Directorate of Higher Education and Politeknik Kesehatan Bandung Ministry of Health. This study was successfully done by the facilities support by LPPM ITB, Mycology and Biotechnology Laboratory of Life Sciences Center, ITB Research and Innovation Bandung; Microbiology Laboratory of Pharmacy ITB; Medical Analysis of Poltekkes Bandung; Biotechnology and Zoology, Indonesian Science Institute, Cibinong; Eijkman Laboratory, Jakarta; Molecular Biology Laboratory IPB Bogor; PT Bio Farma and Biomolecular and Biomedical Research Center, Aretha Medika Utama, Bandung, West Java, Indonesia.

REFERENCES

Anderssen, E.L., D.B. Diep, I.F. Nes, V.G.H. Eijsink and J. Nissen-Meyer, 1998. Antagonistic activity of *Lactobacillus plantarum* C11: Two new two-peptide bacteriocins, plantaricins EF and JK and the induction factor plantaricin A. Applied Environ. Microbiol., 64: 2269-2272.

Arief, I.I., J.T. Suryati, Z. Wulandari and E. Andreas, 2013. Isolation and characterization of plantaricin produced by *Lactobacillus plantarum* strains (IIA-1A5, IIA-1B1, IIA-2B2). Media Peternakan: J. Anim. Sci. Technol., 36: 91-100.

Beck-Sague, C.M., W.R. Jarvis and National Nosocomial Infections Surveillance System, 1993. Secular trends in the epidemiology of nosocomial fungal infections in the United States, 1980-1990. J. Infect. Dis., 167: 1247-1251.

Berthelet, J. and L. Dubrez, 2013. Regulation of apoptosis by inhibitors of apoptosis (IAPs). Cells, 2: 163-187.

Chashoo, G. and A.K. Saxena, 2014. Targetting Cdks in cancer: An overview and new insights. J. Cancer Sci. Ther., 6: 488-496.

Cintas, L.M., M.P. Casaus, C. Herranz, L.F. Nes and P.E. Hernandez, 2001. Review: Bacteriocins of lactic acid bacteria. Food Sci. Technol. Int., 7: 281-305.

Cosconati, S., S. Forli, A.L. Perryman, R. Harris, D.S. Goodsell and A.J. Olson, 2010. Virtual screening with AutoDock: Theory and practice. Expert Opin. Drug Discov., 5: 597-607.

Cotter, P.D., C. Hill and R.P. Ross, 2005. Bacteriocins: Developing innate immunity for food. Nat. Rev. Microbiol., 3: 777-788.

De Bondt, H.L., J. Rosenblatt, J. Jancarik, H.D. Jones, D.O. Morgant and S.H. Kim, 1993. Crystal structure of cyclin-dependent kinase 2. Nature, 363: 595-602.

De Martinis, E.C.P. and B.D.G.M. Franco, 1998. Inhibition of *Listeria monocytogenes* in a pork product by a *Lactobacillus* sake strain. Int. J. Food Microbiol., 42: 119-126.

Diep, D.B., D. Straume, M. Kjos, C. Torres and I.F. Nes, 2009. An overview of the mosaic bacteriocin *pln* loci from *Lactobacillus plantarum*. Peptides, 30: 1562-1574.

Green, M.R. and J. Sambrook, 2012. Molecular Cloning: A Laboratory Manual. 4th Edn., Cold Spring Harbor Laboratory Press, New York, ISBN-10: 1936113422, Pages: 2028.

Guo, M. and B.A. Hay, 1999. Cell proliferation and apoptosis. Curr. Opin. Cell Biol., 11: 745-752.

Januar, H.I., A.S. Dewi, E. Marraskuranto and T. Wikanta, 2012. *In silico* study of fucoxanthin as a tumor cytotoxic agent. J. Pharm. BioAllied Sci., 4: 56-59.

Kim, R. and J. Skolnick, 2008. Assessment of programs for ligand binding affinity prediction. J. Comput. Chem., 29: 1316-1331.

Klaenhammer, T.R., 1988. Bacteriocins of lactic acid bacteria. Biochimie, 70: 337-349.

Klaenhammer, T.R., 1993. Genetics of bacteriocins produced by lactic acid bacteria. FEMS Microbiol. Rev., 12: 39-85.

Maldonado, A., J.L. Ruiz-Barba and R. Jimenez-Diaz, 2003. Purification and genetic characterization of plantaricin NC8, a novel coculture-inducible two-peptide bacteriocin from *Lactobacillus plantarum* NC8. Applied Environ. Microbiol., 69: 383-389.

Mourad, K., 2007. Plasmid DNA studies in *Lactobacillus plantarum* strains isolated from olive fermentations: Production of and immunity to plantaricin OL15 is associated to a 9.6 Kb plasmid (pOL15). Grasas Aceites, 58: 136-141.

Navarro, L., M. Zarazaga, J. Saenz, F. Ruiz-Larrea and C. Torres, 2000. Bacteriocin production by lactic acid bacteria isolated from Rioja red wines. J. Applied Microbiol., 88: 44-51.

Nishida, H., T. Beppu and K. Ueda, 2011. Whole-genome comparison clarifies close phylogenetic relationships between the phyla Dictyoglomi and Thermotogae. Genomics, 98: 370-375.

Olasupo, N.A., 1996. Bacteriocins of *Lactobacillus plantarum* strains from fermented foods. Folia Microbiologica, 41: 130-136.

Omar, N.B., H. Abriouel, S. Keleke, A.S. Valenzuela and M. Martinez-Canamero *et al*., 2008. Bacteriocin-producing *Lactobacillus* strains isolated from poto poto, a Congolese fermented maize product and genetic fingerprinting of their plantaricin operons. Int. J. Food Microbiol., 127: 18-25.

Pitti, R.M., S.A. Marsters, D.A. Lawrence, M. Roy and F.C. Kischkel *et al*., 1998. Genomic amplification of a decoy receptor for Fas ligand in lung and colon cancer. Nature, 396: 699-703.

Raffo, A.J., H. Perlman, M.W. Chen, M.L. Day, J.S. Streitman and R. Buttyan, 1995. Overexpression of bcl-2 protects prostate cancer cells from apoptosis *in vitro* and confers resistance to androgen depletion *in vivo*. Cancer Res., 55: 4438-4445.

Reed, J.C., 2000. Mechanisms of apoptosis. Am. J. Pathol., 157: 1415-1430.

Ritz-Barba, J.L., J.C. Piard and R. Jimenez-Diaz, 1991. Plasmid profiles and curing of plasmids in *Lactobacillus plantarum* strains isolated from green olive fermentations. J. Applied Bacteriol., 71: 417-421.

Russo, A.A., 1997. Purification and reconstitution of cyclin-dependent kinase 2 in four states of activity. Methods Enzymol., 283: 3-12.

Saenz, Y., B. Rojo-Bezares, L. Navarro, L. Diez and S. Somalo *et al*., 2009. Genetic diversity of the *pln* locus among oenological *Lactobacillus plantarum* strains. Int. J. Food Microbiol., 134: 176-183.

Sandeep, G., K.P. Nagasree, M. Hanisha, M. Murali and M. Kumar, 2011. AUDocker LE: A GUI for virtual screening with AUTODOCK vina. BMC Res. Notes, Vol. 4. 10.1186/1756-0500-4-445

Sharma, A. and S. Srivastava, 2014. Anti-Candida activity of two-peptide bacteriocins, plantaricins (Pln E/F and J/K) and their mode of action. Fungal Biol., 118: 264-275.

Shi, Y., 2002. Mechanisms of caspase activation and inhibition during apoptosis. Mol. Cell, 9: 459-470.

Solomon, M.J., T. Lee and M.W. Kirschner, 1992. Role of phosphorylation in p34cdc2 activation: Identification of an activating kinase. Mol. Biol. Cell, 3: 13-27.

Spampinato, C. and D. Leonardi, 2013. *Candida* infections, causes, targets and resistance mechanisms: Traditional and alternative antifungal agents. BioMed Res. Int. 10.1155/2013/204237

Todorov, S.D., 2009. Bacteriocins from *Lactobacillus plantarum* production, genetic organization and mode of action. Barz. J. Microbiol., 40: 209-221.

Trott, O. and A.J. Olson, 2010. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J. Comput. Chem., 31: 455-461.

Van Kranenburg, R., N. Golic, R. Bongers, R.J. Leer, W.M. de Vos, R.J. Siezen and M. Kleerebezem, 2005. Functional analysis of three plasmids from *Lactobacillus plantarum*. Applied Environ. Microbiol., 71: 1223-1230.

Vassilev, L.T., C. Tovar, S. Chen, D. Knezevic and X. Zhao *et al*., 2006. Selective small-molecule inhibitor reveals critical mitotic functions of human CDK1. Proc. Natl. Acad. Sci. USA., 103: 10660-10665.

Widyastuti, Y., P. Lisdiyanti, S. Ratnakomala, G. Kartina and R. Ridwan *et al*., 2012. Genus diversity of actinomycetes in Cibinong science center, West Java, Indonesia. Microbiol. Indonesia, 6: 165-172.

Wong, R.S.Y., 2011. Apoptosis in cancer: From pathogenesis to treatment. J. Exp. Clin. Cancer Res., Vol. 30. 10.1186/1756-9966-30-87

Yang, E., L. Fan, Y. Jiang, C. Doucette and S. Fillmore, 2012. Antimicrobial activity of bacteriocin-producing lactic acid bacteria isolated from cheeses and yogurts. AMB Express, Vol. 2. 10.1186/2191-0855-2-48.