CLONING OF *Lab* GENE ENCODING BACTERIOCIN FROM *Pediococcus acidilactici* F11 INTO *Escherichia coli* DH5α

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ABSTRACT

***Pediococcus acidilactici* F11 is able to inhibit the growth of related species of enterobacteriaceae by secreting bacteriocin. Effort to increase bacteriocin production by transforming *lab* gene encoding bacteriocin from *P. acidilactici* F11 into *E. coli* DH5α was carried out. Plasmid pPAF11 (encoding bacteriocin from *P. acidilactici* F11) and pUC19 as vector which were double-digested with *Hind*III and *Bam*HI, ligated, and transformed into *E. coli* DH5α. White colonies, as indicator of transformant, were picked up and grown in LB-broth medium containing ampicilin. Test ability of the transformant in expressing *lab* gene was done by heating the supernatant of the transformant at 95-98° C for 15 minutes and determining its inhibition against *Enterococcus faecalis* as bacterial indicator. Location of *lab* gene was confirmed by analyzing recombinant plasmid and curing plasmid using acrydine orange. Analysis of the plasmid carried by transformant revealed that plasmid size was similar to that of *P. acidilatici*. This led to a suggestion that the plasmid was a shuttle plasmid.**

Key words : cloning, bacteriocin production, *lab* gene

INTRODUCTION

Many strains of lactic acid bacteria produce antagonistic substances identified as bacteriocins (Klaenhammer, 1988). Bacteriocins are bactericidal proteins secreted by the cells. The inhibition spectrum of bacteriocins produced by lactic acid bacteria on Gram-positive bacteria varies but it mostly confined to closely related species. Bacteriocin of lactic acid bacteria are of a great industrial importance, mainly in food fermentation process and have become a major area of study in search for new and safe food preservatives (Chassy, 1987, Gilliland, 1985, and McKay and Baldwin, 1990). These compounds can inhibit the growth of some species of *Salmonella, Pseudomonas, Yersinia, Escherichia coli, Aeromonas, Enterobacter, Enterococcus* whereas bacteriocin produced by *Pediococcus acidilactici* PAC1.0 (Cintas, *et al.*, 1995) and *P. acidilactici* H (Motlagh, *et al.*, 1992) can inhibit the growth of *Lysteria monocytogenes*.

Pediococci are a group of homofermentative lactic acid bacteria that are ecologically, morphologically, and physiologically similar to the lactic streptococci and often found as saprophytes on vegetable material. Commercially, pediococci are used in fermentation of vegetables (Pederson, 1949) and meat (Smith and Palumbo, 1983). Among the strains of *acidilactici,* some produce antimicrobial peptide or bacteriocins (Gonzales and Kunka, 1987; Bhunia, *et al.*, 1988; Hoover, *et al.*, 1988). It was shown by Gonzales and Kunka (1986) that bacteriocin production was encoded by a 9.4-kb plasmid, designated pSRQ11, in *P. acidilactici* PAC1.0.

P. acidilactici F11 isolated from fermented slice meat by Bibek Ray in Wyoming area, USA. is able to produce bacteriocin that inhibiting the growth of *Enterococcus faecalis* as bacterial indicator. For enhancing of bacteriocin production, in this research, cloning of bacteriocin gene into *Escherichia coli* DH5α which has a simple requirement of nutrition was carried out. The purpose of this research is to simplify utilization of natural substrate resources in Indonesia in order to enhance the competitiveness of bacteriocin production on scaling up of fabrication.

MATERIALS AND METHODS

Bacterial strains

P. acidilactici F11 and bacterial indicator, *Enterococcus faecalis,* from Prof. Bibek Ray, Lab. of Food Science, Dept. of Animal Science, Fac. of Agriculture, The University of Wyoming , USA. *E. coli* DH5α and plasmid pUC19 from Lab. Biochemistry, IUC Biotechnology Gadjah Mada University, Yogyakarta, Indonesia. Medium for *P. acidilactici* F11 was TGE (1% tryptone, 1% glucose, 0.2% yeast extract, 0.02%, Tween 80, 0.033 mM $MnSO_4$, 0.02 mM $MgSO_4$, pH 6.5. Incubation for *P. acidilactici* was done at temperature 30° C for 24 h.

Chemicals

IPTG (Isopropylthio-β-D-galactosidase), T4 DNA ligase, *Hind*III and *Bam*HI (Clontech, Palo Alto, CA), X-Gal (Promega, Madison, MI), and protein marker Dalton Mark VII (Sigma, Mo, USA).

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Plasmid isolation

Plasmid from *P. acidilactici* F11 (designed as pPAF11) was isolated by protocol of Ian Epperon (The University of Leicester, England) with slightly modification, pUC19 and recombinant plasmids were isolated by protocol Sambrook, *et al.* 1989.

Digestion and ligation of plasmids

Plasmids were double digested with *Hind*III and *Bam*HI for 2 h (37 °C), respectively. Ligation was done using T4 DNA ligase for overnight (15 °C).

Transformation

Transformation of the recombinant plasmids into *E. coli* DH5α was done using Mandel and Higa method (1970). Positive controls were *E. coli* transformed with pUC19, negative controls were *E. coli* transformed neither pUC19 nor recombinant plasmid. *E. coli* DH5α and both of the transformants were grown in LB (Luria Bertani) agar containing ampicilin at final concentration 125 ppm. The transformants were screened with a-complementation method (Sambrook, *et al.*, 1989) by picking up the white colonies from the selective medium, then tested their ability to express cloned *lab* gene with agar diffusion assay.

Diffusion assay

Colonies of transformant were grown in LB broth containing ampicilin (37°C, overnight) and bacterial indicator were grown in TS (tryptone soya peptone) broth at the same condition of transformants. Preparation of medium, TSA (hard) medium was poured in the petri dish and overlaid TSA (soft) medium containing bacterial indicator *Enterococcus faecalis*, and kept at cool room for one hour. The cylinder was put in the agar and fill with a certain volume of heated transformant supernatant culture (95°C, 15 minutes). Preincubation was carried out at 4°C for one hour before incubation at 37°C for 24 hours. Positive transformants, which capable in inhibiting the growth of bacterial indicator, were shown by clear zone formation around the cylinder. Confirmation was done to the *E. coli* transformants, positive controls and *P. acidilactici* F11 for inhibiting the bacterial indicator. Location of *lab* gene was confirmed by observing recombinant plasmid migration pattern and plasmid curing using acrydine orange.

RESULTS AND DISCUSSION

P. acidilactici F11 were able to inhibit the growth of *E. faecalis* which shown by the clear zone formation around the cylinder (Fig. 1). Antimicrobial substance produced by *P. acidilactici* was heat-stable and no growth of *A. faecalis* was detected in this zone. It means that this antimicrobial substance was a kind of bacteriocin eventhough other criteria should be exposed.

Plasmid from *P. acidilactici* F11 (designed as pPAF11) had size of approximately 2.6 kb. Double-digested pPAF11 inserted into *Hind*III and *Bam*HI sites of polycloning site of pUC19 was successfully transformed into *E. coli* DH5α as a host. Indication of transformant was shown by rising the white colonies in X-gal medium. Insertion of pPAF11 into polycloning site of pUC19 caused *lacZ'* gene loss its activity, it means that enzyme of b-galactosidase could not be synthesized. In contrast, almost all of positive control (*E. coli* DH5α transformed with pUC19) formed blue colonies, whereas negative control (*E. coli* DH5α) could not grow. Blue colonies formed by positive controls showed that the enzyme was synthesized and the substrates were hydrolyzed. The negative controls could not grow since they have no ampicilin resistance gene contained in pUC19.

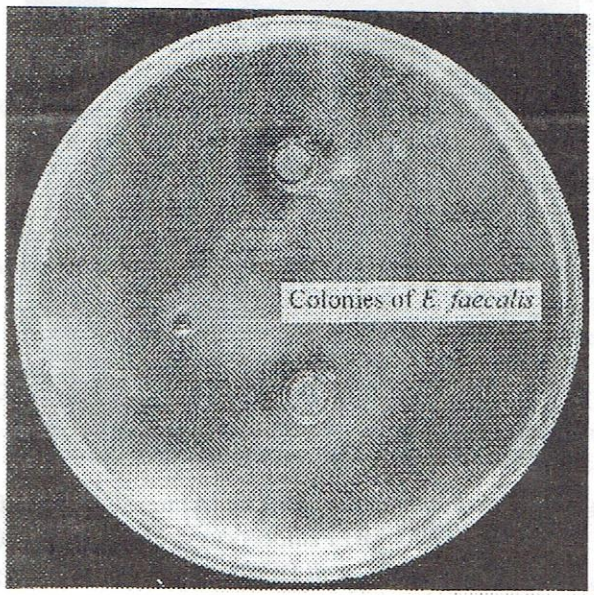

Figure 1. Inhibition of the growth of *Enterococcus faecalis* by supernatant of *Pediococcus acidilactici* F11. Clear zone : no growth of *E. faecalis.*

Table 1. Expression of *lab* gene encoding bacteriocin by transformants

No	Transformants code	Expression of *lab* gene
1	T-1	+
2	T-2	++
3	T-3	-
4	T-4	-
5	T-5	++
6	T-6	+
7	T-7	-
8	T-8	+
9	T-9	-
10	T-10	++

\- : no inhibition occurred to bacterial indicator
\+ : inhibition occurred to bacterial indicator
++ : strongly inhibition occurred to bacterial indicator

Expression of *lab* gene encoding bacteriocin was shown by the ability of transformants to inhibit the growth

of bacterial indicator. From 10 colonies tested, there were 6 (six) colonies gave positives result (Table 1), shown by the clear zone formation. In this zone, there were no indicator growth (Fig. 2). The *lab* gene expression showed that the gene was under mechanism system of control. This results were confirmed with the ability of transformant and *P. acidilactici* F11 to inhibit the growth of indicators. The contrary result was obtained on positive controls (Fig. 3). The positive transformants were isolated their recombinant plasmids. T-5 transformant was chosen to be further analyzed.

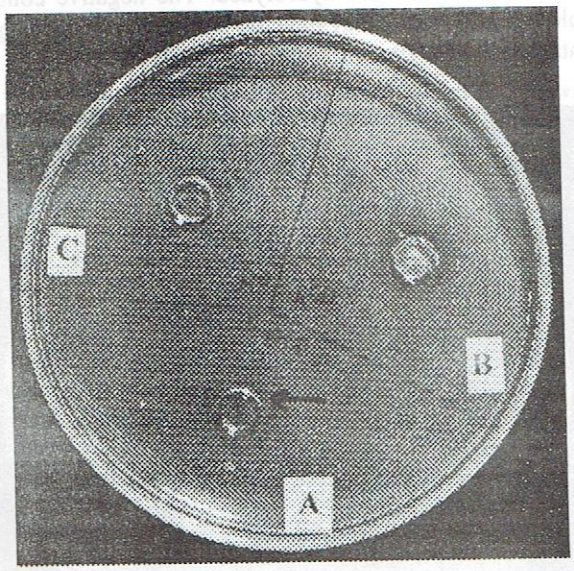

Figure 2. Expression of *lab* gene by (**A**) transformants of *E. coli* DH5α; (**B**) *Pediococcus acidilactici* F11; (**C**) wild type of *E. coli* DH5α.

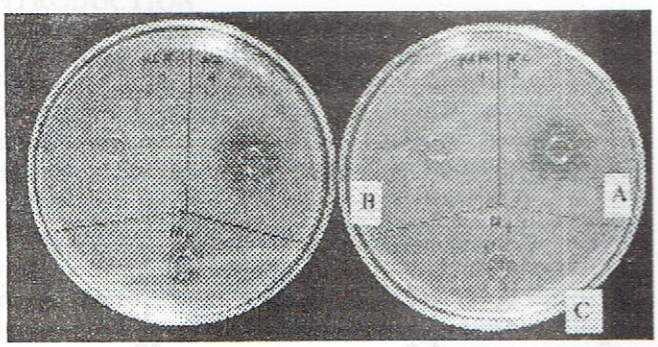

Figure 3. The ability of (**A**) transformant *E. coli* DH5α; (B) positive control - *E. coli* DH5α inserted with pUC19; and (C) *P. acidilactici* F11, to inhibit the growth of *E. faecalis*

Figure 4 showed that recombinant plasmid which was double-digested with *Hind*III and *Bam*HI had single band. Theoretically, recombinant plasmid would be two times longer than pUC19, but in fact its migration was only slightly different with pUC19. It was suggested that digested plasmid was not recombinant plasmid, but pPAF11 itself. By the time of transformation, the plasmid entering *E. coli* was still recombinant plasmids, i.e., pUC19 inserted by pPAF11, that transformant screening with a-complementation method was able to perform. By unknown mechanism(s), it was thought that recombinant plasmid turned into shuttle vectors. Shuttle vectors, according to Freifelder (1991) are those capable of replicating in various organisms. Shuttle vectors in transformants did not loose their ability expressing *lab* gene, shown by the ability to produce bacteriocin. The possibility of plasmid recombinant, with certain mechanisms, turned into pUC19 was denied after observing the inability of *E. coli* transformed with pUC19 to inhibit indicator (Figure 3).

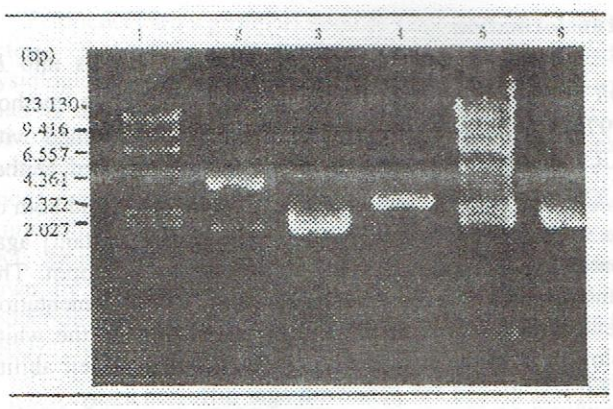

Figure 4. Digested recombinant plasmid. Lane 1 and 5, marker ; Lane 2, uncut pUC19; lane 3 and 6, recombinant plasmid/ *Hind*III-*Bam*HI; lane 4, pUC19/ *Hind*III-*Bam*HI.

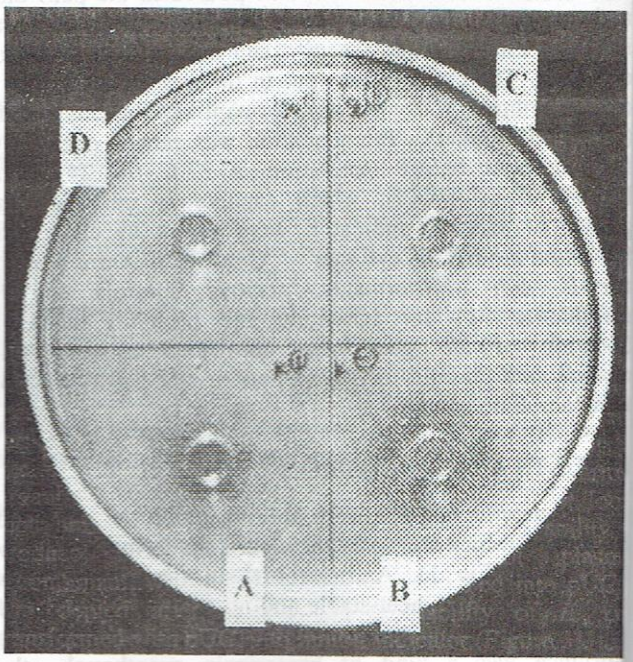

Figure 5. Curing plasmid of transformant. (A) Supernata transformant + ampicillin; (**B**) Medium LB bro + ampicillin; (**C**) Heated supernata transformant + ampicillin; (**D**) Unheate transformant + ampicillin.

Recombinant plasmid could be eliminated by 90 ppm acrydine orange. As a result, the cured transformants were no longer able to inhibit the bacterial indicators (Figure 5). The curing agent inserted into DNA and inhibited plasmid replication. In the next cell division, fewer plasmids underwent replication and then eliminated from inside the cell. Host cell without recombinant plasmid would have more rapid growth rate and soon dominated population in culture (Old and Primose, 1994). On the same concentration of curing agent, pPAF11 in *P. acidilactici* F11 could not be eliminated, and even up to 200 ppm. Based on the physical structure, intact plasmid tended to be more stable, and more resistance against treatment of curing agent than recombinant plasmid.

CONCLUSIONS

Lab gene encoding bacteriocin from *Pediococcus acidilactici* F11 has been successfully transformed into *E. coli* DH5α.

Transformants *E. coli* DH5α were able to express *lab* gene encoding bacteriocin, shown by its ability to inhibit the growth of *Enterococcus faecalis* as bacterial indicator.

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