# RADIOLABELING OF PLANTARICIN F AS A NATURAL ANTIBIOTIC USING IODINE-131

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## ABSTRACT

**Aim:** Plantaricin F is bacteriocins are mostly applied in food to prevent microbial contamination called as biopreservation. As a new compound, effectiveness of plantaricin F as antibiotic is not yet been fully established due to the lack of scientific information, therefore its efficacy and safety should be evaluated scientifically. One method of testing bioavailability is use nuclear techniques using labeled compounds as radiotracers. This study is aimed to find the labeled condition of plantaricin F using iodine-131 radioisotope. [$^{131}$I]I-plantaricin F labeled compound will be used as radiotracer to study bioavailability of plantaricin F in infection animal model in the next research.

**Methods:** Labeling plantaricin F with iodine-131 radioisotope using direct labeling method conducted through chloramine-T as oxidizing agent. Varying some influential parameters such as oxidizing amount, ligand amount and reaction time to get the high labeling yield.

**Results:** $^{131}$I-plantaricin F with radiochemical purity 95.27 ±0.69 % was prepared using 60 µg plantaricin F, 10 µl [$^{131}$I]NaI (100 µCi), 150 µg chloramine-T, 300 µg sodium metabisulfite in a Tris HCl buffer solution (pH 7.5) and incubated in room temperature for 60 seconds. The radiochemical purity was determined by using Whatmann 1 chromatography paper with methanol 90 % and chloroform as mobile phase.

**Conclusion:** [$^{131}$I]I-plantaricin F labeled compound may potentially use as a radiotracer for in-vivo study in the next research, therefore the effectiveness of plantaricin F as a natural antibiotic could be proven.

**Keywords**: $^{131}$I-plantaricin F, labeled compound, radiotracer, bacteriocin

## INTRODUCTION

Infection is disease causing by pathogenic microorganism such as bacteria, viruses, fungi or parasites. Based on the published data in 2012, infectious disease responsible for the death of more than 8.7 million people worldwide in 2008 [1]. Infectious disease treatment generally uses antibiotics but cases of antibiotic resistance are also high. Therefore many studies regarding the discovery of new drugs for the treatment of infections [2–5].

Lactic acid bacteria are gram-positive bacteria that commonly used in food fermentation [6–9]. Bacteriocins are antimicrobial proteins or peptides that kill other strains.

Plantaricin F (pln F) is bacteriocins produce by Lactobacillus plantarum S34 isolated from Indonesia traditional-fermented meat (Bekasam) [10,11]. Pln F is bacteriocins are mostly applied in food to prevent microbial contamination called as biopreservation. Preservatives carried out by lactic acid bacteria caused by lactic acid produced by these bacteria during food fermentation will reduce the pH value of the growth environment that inhibits the growth of some other microorganisms [12]. Therefore plantaricin F is potential to be developed from antimicrobial in biopreservatives food industries to antibiotic as a therapeutic agent in medical applications [13].

As a new compound, its effectiveness is not yet been fully established due to the lack of scientific information, therefore its efficacy and safety should be evaluated scientifically. One method of testing bioavailability is the use of nuclear techniques using labeled compounds as radiotracers. Labeled compounds is a chemical substance that contains radioactive atom within its structure and are suitable for diagnosis or treatment of disease. Labeled compounds can to monitors the in vivo behaviour of a functional molecule, and can be used to provide biological information in a living system. This is caused by gamma radiation that emitted from this labeled compounds readily escapes from the body and permitting external detection and measurement [14]. Therefore, the radiolabeled pln F as radiotracer will be useful to understanding its antibiotic activity and determine their effectiveness as an antibiotic. The purpose of this study is to optimize the preparation of [$^{131}$-I]pln F labeled compounds with high radiochemical yield for the development of [$^{131}$-I]pln F as a radiotracer.

Iodine-131 radioisotope has an 8-day half-life and 364-keV and used extensively for labeling the compounds of medical and biological purpose. In a protein, the phenolic ring of tyrosine or imidazole ring of histidine is the primary site of iodination. Some parameter plays an important role in protein iodination such as oxidizing amount, pH, temperature, and reaction time. The optimum pH for protein iodination is 7-9 [15].

## MATERIALS AND METHODS

### Materials

Materials that used in this research were plantaricin F (Thermo, USA), chloramines T hydrate (Sigma Aldrich, America), sodium metabisulphite (Merck, Germany), methanol lichrosolv (Merck, Germany), chloroform (Merck, Germany), Na$^{131}$I (PRSG-BATAN), sterile aquabidest (IPHA, Indonesia), pH indicator (Merck, Germany), and whattman 1 (Whattman). The equipment that used in this experiment were dose calibrator (Victoreen), vortex mixer, single channel analyzer (Ortec), electrophoresis, and paper chromatography apparatus.

### Labeling of $^{131}$I-pln F

Labeling of pln with radioisotope iodine-131 was performed using direct labeling method using chloramines T as an oxidizing agent. Some parameters were varying to obtain the optimum conditions such as the amount of chloramines T, amount of pln F, and the incubation time. The labeling process was carried out by adding of chloramines T solution (1 mg/100 µL Tris-HCl pH 7.5) into the vial that contains of pln F solution in Tris-HCl pH 7.5 and Na$^{131}$I, then incubated in room temperature. After incubation time reached, sodium metabisulphite in Tris-HCl pH 0.75 was added.

### Determination of Radiochemical Purity (RCP) of $^{99m}$Tc-rutin

The determination of RCP of $^{131}$I-pln F was done using ascending paper chromatography method with whattman 1 (10 × 1 cm) as the stationary phase and methanol 90% as the mobile

phase to separate the impurities of $^{131}I^-$ in Rf 1.0 and chloroform as the mobile phase to separate $I_2$ impurities. The chromatograms were dried in the oven at 80 $^oC$, and then every 1 cm piece of paper was cut and measured using Single Channel Analayzer (SCA) with detector NaI(Tl).

**Optimization of Chloramines T**

Into five vials containing 5 µL pln F n Tris-HCl pH 7.5 (3 mg/mL) were added varying amount (45, 35, 25, 15, and 5 µL) of Tris-HCl pH 7.5 and 10 µL of $Na^{131}I$ (100 µCi) solution. Then, a varying amount (5, 10, 15, 20, and 25 µL) of chloramines T in Tris-HCl pH 7.5 (1 mg/100 µL) was added, stirred for 60 seconds and added a varying amount (5, 10, 15, 20, and 25 µL) µl of sodium metabisulphite in Tris-HCl pH 7.5 ( 2 mg/ 100 µL ). The optimum amount of oxidizing agent was determined from the radiochemical purity of $^{131}I$-pln F using paper chromatography method as described above.

**Optimization of pln F amount**

Into each vial, containing varying amount (5, 10, 15, 20, and 25 µL) of pln F (3 mg/mL) were ad until 30 µl (to get same volume) using Tris-HCl pH 7.5, added 10 µL of $Na^{131}I$ (100 µCi) solution and 15 µl (150 µg) of chloramines T. After stirred for 60 seconds, into each vial added 15 µl (300 µg) of sodium metabisulphite in Tris-HCl pH 7.5. The optimum amount of pln F was determined from the radiochemical purity of $^{131}I$-pln F using paper chromatography method.

**Optimization of incubation time**

Into five vials containing 20 µL (60 µg) of pln F were added 10 µL of $Na^{131}I$ (100 µCi) solution and 15 µl (150 µg) of chloramines T solution. The solution were stirred with varying time (30, 60, 90, 120 and 150 second) in room temperature and then added 15 µl (300 µg) of sodium metabisulphite in Tris-HCl pH 7.5. The optimum incubation time will be the time that gives a high radiochemical purity of $^{131}I$-pln F and was determined using paper chromatography method.

**Stability of [$^{131}I$]I-pln F in HCl/PBS/Serum**

The labelled compounds [$^{131}I$]I-pln F that obtained from the optimum conditions are as follows :

Into a vial containing 60 µg/20 µL of pln F solution was added 10 µL of $Na^{131}I$ (100 mCi) solution and 150 µg/15 µL of chloramineT. The mixture was stirred for 60 seconds in room temperature and then added with 300 µg/15 µL of sodium metabisulphite solution. Stability testing was performed at 0, 1, 2, 3, and 24 hour after adding sodium metabisulphite solution to see the stability of $^{131}I$-pln F.

## RESULTS AND DISCUSSION

Several methods of iodination have been used to label many compounds, one method that is often used is the chloramine-T method. Chloramine-T method use the sodium salt of N-chloro-4-methyl benzene sulfonamide as the iodinating agent. At pH 7-8 chloramin-T will be hydrolyzed to sodium hypochlorite [14], which oxidizes radioiodide to hypoiodous acid according to the following reactions :

$$CH_3\text{-}C_6H_4SO_2NaNCl + H_2O \longleftrightarrow CH_3\text{-}C_6H_4SO_2NH_2 + NaOCl \quad (1)$$

$$NaOCl + HI^* \longleftrightarrow HOI^* + NaCl \quad (2)$$

According to previous research the amino acid sequences of pln F are VFHAYSARGVRNNYKSAVGPADWIISAVRGFIHG [10,11,16]. The presence of amino acids tyrosine and histidine in the pln F structure will facilitate iodination reaction in this protein. Iodination reaction in tyrosine and histidine show ini Figure 1 and 2, the electrophilic substitution reaction is facilitated by strong electron donating groups such as –OH and $–NH_2$ in the aromatic ring.

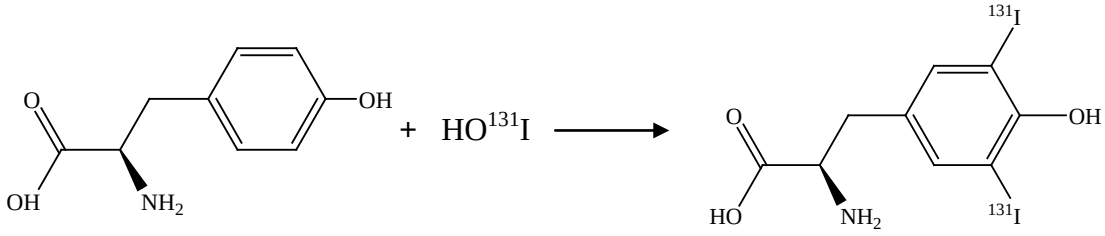


**Figure 1**: Radioiodination reaction of tyrosine

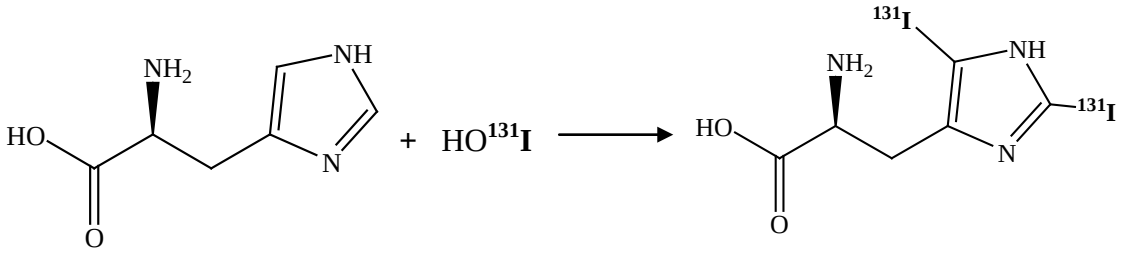


**Figure 2**: Radioiodination reaction of histidine

In order to optimize the radioiodination conditions, we studied the influence of chloramine-T amount, ligand (pln F) amount and reaction time on the radiochemical purity of [$^{131}$I]I-pln F. In this reaction, the fresh chloramine-T solution added to the mixture of pln F in Tris-HCl buffer pH 7.5 and [$^{131}$I]NaI. Chloramine-T is oxidizing agent so amount of chloramine-T influential on radiochemichal purity of [$^{131}$I]I-pln F. The powerful of chloramine-T as oxidizing agent must be controlled because may damage proteins [17]. The effect of oxidizing amount has been examined by varying chloramine-T amount (Fig 3). The optimum chloramine-T amount was 150 µg and will be decreased at amount above it. These result are in accordance with those obtained Salacinski et al [18], which suggested that the decrease of RCP with higher chloramine-T amount can be attributed to the destructive side reaction and the damage of the ligand.

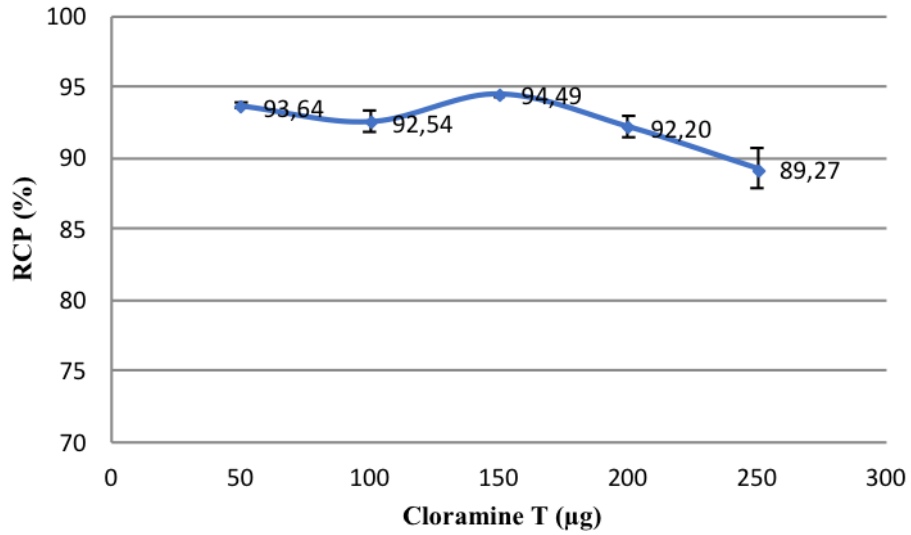


**Figure 3**: Effect of the chloramine-T amount on the RCP of [$^{131}$I]I-pln F

To obtain the maximum RCP the amount of pln F as ligand were varied. Pln F is a white crystalline solid which is soluble in water. In this study pln F was dissolved in Tris-HCl buffer pH 7.5. The optimum pln F amount in the labeling process was shown in Fig. 4. This chart shows that amount of ligand do not significantly affect to RCP of [$^{131}$I]I-pln F. The RCP value from 30-75 µg is similar and we choose 60 µg as an optimum ligand amount because the smallest SD value. This shows that almost all iodine-131 present in the system has bonded to the ligand so that the addition of ligands has no effect.

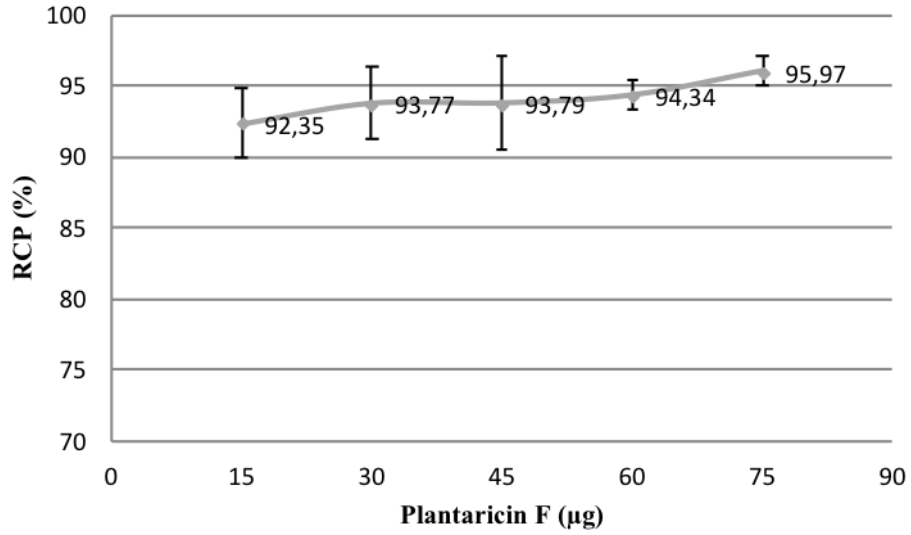


**Figure 4**: Effect of pln F amount on the RCP of [$^{131}$I]I-pln F

Further improvement of labeling pln F is associated with the reaction time. Fig. 5 illustrates the relationship between the reaction time and RCP of [$^{131}$I]I-pln F. It is clear that RCP of [$^{131}$I]I-pln F stable from 30-120 seconds reaction time but decrease in 150 seconds. This caused by too long exposure of the ligand (pln F) to the oxidizing agent which causes oxidative side reaction like chlorination, polymerization and denaturation of the ligand [19]. Therefore, for this reaction, we choose 60 seconds for the optimum reaction time.

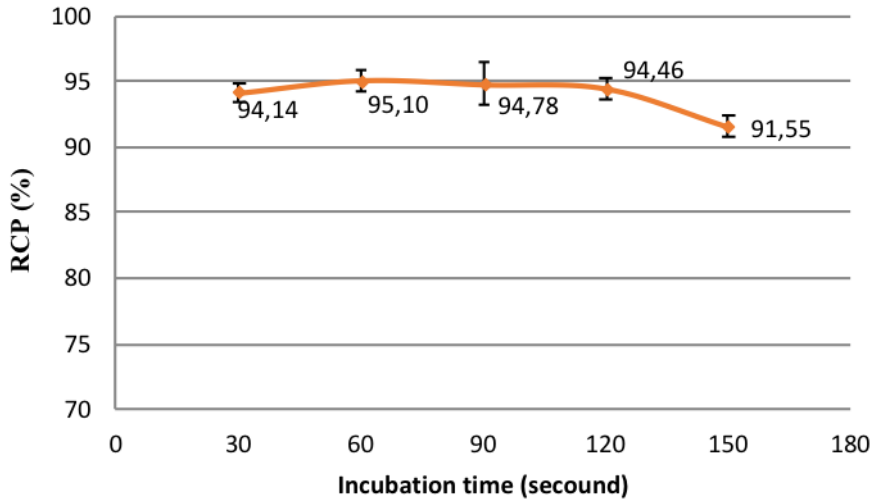


**Figure 5**: Effect of the reaction time on the RCP of [$^{131}$I]I-pln F

In this research we evaluated stability of [$^{131}$I]I-pln F in PBS, HCl 0,1 N pH 1,2 and human serum to know the best way to the delivery of [$^{131}$I]I-pln F in preclinical study in the next research. Fig. 6. shows that RCP of [$^{131}$I]I-pln F in human serum decreased more faster than in PBS but RCP of [$^{131}$I]I-pln F in HCl 0,1 N pH 1,2 decreased slowly. Until 24 h RCP of [$^{131}$I]I-pln F in HCl 0,1 N pH 1,2 still 86.02 %. From these results, drug delivery of [$^{131}$I]I-pln F will be given orally to obtain detailed information concerning the organ distribution and in vivo behaviour of [$^{131}$I]I-pln F. The [$^{131}$I]I-pln F as radiotracer will be useful to understanding its antibiotic activity and determine their efficacy and effectiveness as antibiotic.

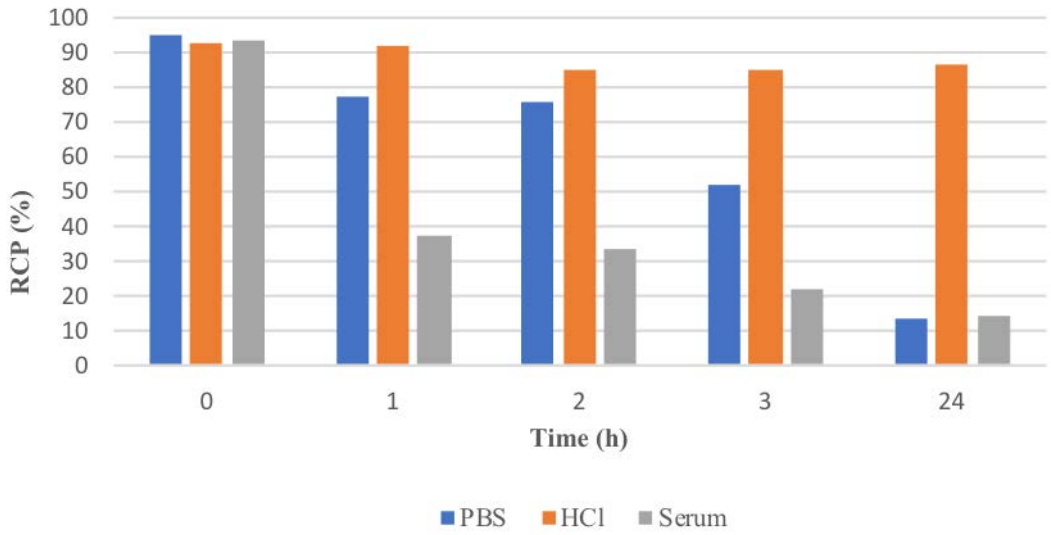


**Figure. 6**: In vitro stability of [$^{131}$I]I-pln F in PBS; HCl 0,1 N pH 1,2; human serum

## CONCLUSION

The feasibility of labeling pln F using iodine-131 is confirmed. The optimum labeling conditions of [$^{131}$I]I-pln F with maximum radiochemical purity are found to be one minute of reaction time, 60 µg of plantaricin F, 10 µl of [$^{131}$I]NaI (100 µCi), 150 µg of chloramine-T, 300 µg of sodium metabisulfite in a Tris HCl buffer solution (pH 7.50. The [$^{131}$I]I-pln F complex more stable in HCl 0,1 N pH 1,2 compared to in PBS and human serum. With this successfully to preparation of [$^{131}$I]I-pln F, there is an opportunity to continue to in-vitro and in-vivo study.

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