1. INTRODUCTION

1.1. Background

Food safety issues have become a major concern globally due to foodborne illnesses that spread rapidly. Hence, the utilization of bacteriocin as food preservatives will satisfy the increasing demand for safe natural antimicrobial agents to inhibit pathogenic microbes in food (Soltani et al., 2021; Vijay Simha et al., 2012). In this case, pediocin will be the main focus of bacteriocin which could be utilized as a natural antimicrobial agent to inhibit pathogen-causing foodborne illnesses namely *Listeria monocytogenes* (Tanih et al., 2015). A preliminary antimicrobial assay was conducted and showed that spray drying had no significant effect with *P. acidilactici* still showing antimicrobial activity against *L. monocytogenes*. However, the SDS-PAGE result of the cation-exchange eluent of both spray-dried and non-spray-dried *P. acidilactici* did not reveal any peptide band with a size of 4.6 kDa which represents pediocin. Due to this, metabolites other than pediocin need to be eliminated to concentrate and determine whether pediocin is responsible for the antimicrobial activity of *P. acidilactici*. Efficient downstream processing of *P. acidilactici* fermentation broth is desired for the purpose of analysis of pediocin. Filtration methods typically performed usually consisted of $(NH_4)_2SO_4$, precipitation, ion-exchange chromatography, and reversed-phase HPLC (RP-HPLC). Nevertheless, these purification techniques are complex, time-inefficient, and generate a low yield of bacteriocin (Elegado et al., 1997). This research paper aims to evaluate the antimicrobial activity of pediocin against *L. monocytogenes*, concentrating pediocin in the cell-free supernatant (CFS), and also develop a standardized method to purify pediocin from fermentation broth using heat denaturation followed by adsorption-desorption method. By concentrating pediocin in the CFS, the antimicrobial activity of pediocin could be studied.

1.2. Research Questions

Based on the introduction, several research questions were devised:

1. What is the difference in the antimicrobial activity between three different *Pediococcus acidilactici* fermentation duration i.e. 24h, 36h, and 48h against *Listeria monocytogenes*?

2. What is the difference in the antimicrobial activity of *Pediococcus acidilactici* CFS treated at five different temperatures during the heat denaturation method against *Listeria monocytogenes* and which one is the optimum temperature for pediocin-concentrating

3. What is the difference in the antimicrobial activity of *Pediococcus acidilactici* fermentation before and after the adsorption-desorption method against *Listeria monocytogenes*

1.3. Hypothesis

These are the hypotheses related to the research questions:

1. H_0: There will be no significant difference in the antimicrobial activity of the cell-free supernatant containing pediocin derived from *P. acidilactici* fermentation against *L. monocytogenes* between three different fermentation duration

H_1: There will be a significant difference in the antimicrobial activity of the cell-free supernatant containing pediocin derived from *P. acidilactici* fermentation against *L. monocytogenes* between three different fermentation duration

2. H_0: There will be no significant difference in the antimicrobial activity of the cell-free supernatant containing pediocin derived from *P. acidilactici* fermentation against *L. monocytogenes* after heat denaturation treatment

H_1: There will be a significant difference in the antimicrobial activity of the cell-free supernatant containing pediocin derived from *P. acidilactici* fermentation against *L. monocytogenes* after heat denaturation treatment

3. H_0: There will be no significant difference in the antimicrobial activity of the cell-free supernatant containing pediocin derived from *P. acidilactici* fermentation against *L. monocytogenes* after adsorption-desorption treatment

H_1: There will be a significant difference in the antimicrobial activity of the cell-free supernatant containing pediocin derived from *P. acidilactici* fermentation against *L. monocytogenes* after adsorption-desorption treatment

1.4. Research Scope

The research scopes of this study were:

1. Culture preparation and growth curve of wild-type *P. acidilactici*
2. Collection of cell-free supernatant from *P. acidilactici* fermentation followed by pediocin-concentrating method with heat denaturation and adsorption-desorption
3. Antimicrobial testing with agar-well diffusion assay and measurement of inhibition zone
4. Quantification of protein for comparison before and after pediocin-concentrating treatment.