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CHOLESTEROL ASSIMILATION AND MODULATION OF *lactobacillus plantarum* L8 AND *lactobacillus pentosus* S1 ON ATP BINDING CASSETTE TRANSPORTER GENES AGAINST HT-29 INTESTINAL CELL LINES

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ABSTRACT

Probiotics gives a new insight in treating hypercholesterolemia. Recent research found that probiotics such as *Lactobacillus* possess multiple health benefits including cholesterol assimilation in human body. Bile salt deconjugation, cellular cell binding and ability to modulate cholesterol transporter genes were proposed as underlying mechanisms for cholesterol lowering activity. *Lactobacillus plantarum* L8 and *Lactobacillus pentosus* S1 isolated from fermented fish food known as pekasam were assessed for their ability to deconjugate bile salt such as Taurodeoxycholic acid (TDCA). To achieve this, *L. plantarum* L8 and *L pentosus* S1 were tested for bile salt deconjugation through direct plating assay on de Mann, Rogosa, Sharpe (MRS) agar supplemented with TDCA. The result showed that both *L. plantarum* L8 and *L. pentosus* S1 did not deconjugate bile salt on selective agar which might be due to species specificity factor where only certain species able to deconjugate bile salt. Different stages of cell growths (Growing, resting and dead cells) of *L. plantarum* L8 dan *L. pentosus* S1 were also tested for their ability to reduce cholesterol in MRS broth supplemented with cholesterol. It was found only *L. plantarum* L8 was able to assimilate cholesterol by 33%, 8% and 1% for growing, resting and dead cells respectively, while *L. pentosus* S1 did not show any activity which might also be due to species specificity, a condition where only certain species possess cholesterol lowering property. Bacterial growth has also been observed to identify whether incorporation of cholesterol in MRS broth would affect the growth pattern of *L. plantarum* L8 and *L. pentosus* S1 and their correlation with cholesterol reduction. As the result, *L. plantarum* L8 has higher doubling time and growth rate as compared to *L. pentosus* S1. This explains the cholesterol removal activity of *L. plantarum* L8 being higher than *L. pentosus* S1. After 18 h of incubation, *L. plantarum* L8 supplemented with cholesterol demonstrated a maintained growth rate up to 24 h as compared to its control (absence of cholesterol). For molecular approach, to evaluate probiotics effect on cholesterol transporter genes expressions in intestinal cells, HT-29 cells were treated with 24h cholesterol then incubated with *L. plantarum* L8 and *L. pentosus* S1 for 6h. Total RNA of treated HT-29 cells were extracted then converted to cDNA through reverse transcription polymerase chain reaction (RT-PCR). Subsequently, cDNA was further evaluated for transcription of *NPC1L1* and *SCARB1* gene, a key cholesterol transporters gene which involve in cholesterol metabolism by using qPCR analysis. *L. plantarum* L8 was able to downregulate *NPC1L1* and *SCARB1* while *L. pentosus* S1 was able to downregulate *NPC1L1* only. Downregulation of both genes proceed to cholesterol reduction in HT-29 cells. In conclusion. *L. plantarum* L8 and *L. pentosus* S1 can reduce cholesterol level however *L. plantarum* L8 has greater potential to act as cholesterol lowering agent. Both probiotics can be a new alternative to assist CVD prevention in future.

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CHAPTER ONE
INTRODUCTION

1.1 Research Background

Dyslipidemia or high levels of lipid in the blood contribute to detrimental cardiovascular disease (CVD) including coronary artery diseases (CAD), stroke, peripheral blockage of arteries and blockage of aorta, the largest arteries in the heart (WHO, 2021). Uncontrolled build up plaque caused severe blockage around blood vessels, limb, and arteries (NHS, 2022). It is a worrying situation where almost 96.8% patients suffered from CVD (The Star Malaysia, 2018). As early prevention steps, normally health expert would suggest a healthy lifestyle such as balance diet, exercise, no smoking, and no alcohol to be applied in daily life (Tomaro-Duchesneau et al., 2015; Kelly, 2010; American Heart Association, 2020). It was identified that exercise and controlled diet was not enough to turn down elevated cholesterol level in the body (Ballin & Nordstorm, 2021). Most common drugs used were anticoagulants, ACE inhibitors, Beta blockers and statin (O'Morain & Ramji, 2019). It was confirmed that statin was able to hijack cholesterol synthesis pathway through rate limiting enzyme in mevalonate pathway, HMG-CoA reductase (Ramkumar et al., 2016). However, this medication comes with a pricy cost and long-term aftermaths such as rhabdomyolysis, myalgia, liver and kidney damage and several drug interactions (Jia et al., 2011; Benjamin et al., 2018; Liu et al., 2019; Jansen et al., 2020).

Currently, researchers changed to probiotics as an alternative food supplement to aid in fighting hypercholesterolemia (Bhat & Bajaj, 2019). Probiotics are defined as living microorganism which provides health benefit when consumed by human body (Hill et al., 2014). Probiotics with health benefit mostly come from lactic acid bacteria (LAB) such as *Lactobacillus* spp. and Bifidobacteria spp. (Ooi & Liong, 2010; Kumar et al., 2012; de Melo Pereira et al., 2018). The exact principles for cholesterol lowering activity by LAB are still unclear, however, several mechanisms were proposed. Studies shown *Lactobacillus* spp. can reduce hypercholesterolemia by bile salt deconjugation activity (Miremadi et al., 2014; Shokryazdan et al., 2017; Singhal et al., 2021). Cholesterol undergo de novo synthesis in the liver to produce bile acid which is useful to digest lipid in duodenum (Wen & Campbell, 1977; Setchell & Kohli, 2018).