UNIVERSITI TEKNOLOGI MARA

ATHEROPROTECTIVE POTENTIAL OF LACTIC ACID BACTERIA AND THE UNDERLYING CHOLESTEROL-LOWERING MECHANISM(S)

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ABSTRACT

Current treatments and management against atherosclerosis rely predominantly on lipid lowering and diet modification, however it appears to be rather modest, often compromised by the lack of response in high-risk patients and adverse effects. This raises the possibility of using hypocholesterolaemic probiotics for atheroprotection. Lactic acid bacteria (LAB), the most common, non-pathogenic and safe probiotics, were reported to possess cholesterol-lowering effects. Nevertheless, the beneficial effects of LAB are believed to be strain-dependent and their mechanisms are not completely understood. As such, the present study aimed to identify a superior strain of LAB with excellent cholesterol lowering and atheroprotective properties as well as to elucidate the underlying mechanisms. The present study identified *Pediococcus pentosaceus* LAB6 and *Lactobacillus plantarum* LAB12 with promising cholesterol-lowering effects from a preliminary screening of 12 locally sourced lactic acid bacteria (LAB; 5 *lactobacilli* and 7 *pediococci*) using the new method of cholesterol quantification through high-performance thin layer chromatography (HPTLC). The LAB yielded strain-dependent cholesterol reducing activity in two different growth media (MRSC: broth containing cholesterol and MRSBC:broth containing cholesterol and bile). LAB6 (MRSC, 51.95% and MRSBC, 57.49%) and LAB12 (MRSC, 58.89%; MRSBC, 68.50%) emerged as the best cholesterol-reducing LAB. Both LAB assimilated fluorescently-tagged cholesterol at the outermost layer of cell membranes. The LAB also facilitated the deconjugation activity (LAB6:100% vs >38%; LAB12:100% vs >75% deconjugation) against glyco- and tauro-conjugated bile salts, respectively. Capitalising on these beneficial properties, this study went on to investigate the anti-atherosclerotic and atheroprotective potentials of LAB *in vitro* and *in vivo*, respectively. For the *in vitro* study, the sub-toxic concentration of 24h LAB-fermented cell-free supernatant against RAW264.7 and HUVEC were determined using the sulforhodamine B assay. The LAB-derived CFS was also assessed against the oxLDL-induced foam cell formation assays [i.e., lipid staining by Oil Red O stain, mitochondrial dysfunction using the mitochondrial membrane potential (MMP) assay and antioxidant assays (i.e., glutathione and superoxide dismutase). The effects of LAB6- and LAB12-derived CFS against oxLDL-induced endothelial dysfunction was explored in HUVEC on monocyte-endothelial adhesion and MMP assay. The LAB6- and LAB12-derived CFS not only significantly (p<0.05) reduced oxLDL uptake, reduced MMP depolarisation and increased antioxidant activity in oxLDL-induced RAW264.7 cells, but also reduced monocytes adhesion and prevented MMP depolarisation on oxLDL-induced endothelial dysfunction. For the *in vivo* study, the atheroprotective properties of freeze-dried LAB12 (LAB12-FD) was explored using C57BL/6J mice fed with high-fat diet for 20 weeks. Daily consumption of LAB12-FD significantly reduced total cholesterol content in the livers, reduced fatty streak formation and infiltration of macrophages in the aortas, as well as improved intestinal barrier (i.e., reduced serum lipopolysaccharide content and urine lactulose mannitol ratio) of C57BL/6J mice. Altogether, the present findings strongly support the possibility of using LAB12 as a dietary supplement for prevention of hypercholesterolaemia and atherosclerosis. The current data also provides important insights into the complementary use of LAB in management of metabolic diseases.

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