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Screening of conjugated linoleic acid producing lactic acid bacteria

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Conjugated linoleic acid(CLA), a mixture of isomers of octadecanoic acids with conjugated double bonds, has attracted considerable attention because of its potentially beneficial biological effects. Kepler and Tove(1967) showed that the first intermediate in the biohydrogenation of linoleic acid by rumen bacteria such as *Butyrivibrio fibrisolvens* is c9,t11 – 18:2. More recently, Jiang *et al*(1998) reported two strains of *Propionibacterium freudenreichii* ssp. *freudenreichii* and one strain of *P. freudenreichii* ssp. *shermanii* were found to be capable of converting free linoleic acid to extracellular CLA. There is little evidence of CLA production by lactic acid bacteria, but Dionisi(1999) showed it was possible to identify and quantify CLA in lactic acid bacteria.

Lactic acid bacteria were obtained from KCTC, silages, and feces. After subculturing twice in De Man – Rogosa – Sharpe(MRS) broth(pH 6.0)(De Man *et al.* 1960), the strains were cultured in MRS broth spiked with 9,12 linoleic acid(99% pure)(Sigma Chemicals). Linoleate isomerase activity was assayed spectrophotometrically by measuring the appearance of the conjugated diene system at 233 nm. The HPLC analysis of CLA was based on the method described by Chin *et al*(1992), sample preparation by Jiang *et al*(1998). A 1 mL culture and 2 mL isopropanol were mixed vigorously; 1.5 mL of hexane were then added and the mixture was shaken for 3min before being centrifuged at 3,000 rev min^{-1} for 5min at room temperature. The upper layer was then collected. The lower layer was extracted with 1.5 mL of hexane, twice, and the supernatant fluids were pooled with previous hexane layer in a screw – capped test tube. The extracted lipids were hydrolysed to free fatty acid by adding 2 mL of 2 mol^{-1} KOH in ethanol and heating at 50℃ for 30min. Methyl esters were prepared with 2 mL of 3N hydrochloric acid in methanol at 60℃ for 20 min. The methylated sample was mixed with 1 mL water and 1 mL hexane, and centrifuged at 3,000 rev min^{-1} for 15 min. The organic layer was dried under a stream of nitrogen at room temperature and the residue was redissolved in 1 mL methanol for HPLC analysis of total CLA. Among the 17 cultures screened, one culture showing the highest absorbance was found to have CLA methyl ester peak by HPLC analysis.

More strains will be screened by the CLA content and linoleate isomerase activity, and linoleate isomerase will be purifed and characterized for cloning and further utilization.