

Introduction of the *lft* gene-carrying plasmid into a number of levan-producing *Bacillus subtilis* strains and its possible effects on DFA IV production

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[Introduction and objectives] Di-D-fructofuranosyl 2,6':2',6 anhydride (DFA IV) is a new prebiotic nondigestible oligosaccharide. It promotes absorption of calcium, magnesium and zinc in small and large intestine of rats. DFA IV can be produced from levan by levanfructotransferase (*lft*) of *Arthrobacter nicotinovorans* GS-9. Levan is a part of natto mucilage, therefore *Bacillus subtilis* strains are the candidates for the DFA IV producer, by using as a host to express *lft* gene, because DFA IV could be directly produced from sucrose in a single culture. The best levan producer may have a chance to produce higher amount of DFA IV. However, transformation efficiency in *B. subtilis* strains are very low in general. The aims of this study were to compare levan production from 24 strains of *B. subtilis* and to compare their transformation efficiencies using pLFT-SD36, which contains the genes for DFA IV production.

[Methods] Levan measurement Levan production medium contained 20% sucrose. Amount of levan produced were measured after it was precipitated by 2-propanol, digested by HCl and then reacted with carbazole and cysteine-sulfuric acid. The purple reaction mixture was measured for the absorbance at 560 nm.

Transformation Competent cells and plasmid DNA containing *lft* were exposed to a single electrical pulse and were plated on medium containing chloramphenicol. Transformants were confirmed for the existence of plasmid by colony PCR.

DFA IV production Transformants were cultured in the levan production medium and produced DFA IV was measured by HPLC with a refractive index detector.

[Results] Levan production All *B. subtilis* isolated from natto could produce levan, while four *B. subtilis* AHU1031, AHU1033, AHU1036 and AHU1232 could not produce levan in the production medium. The best levan producer was AHU1886 which produced levan 3.92 ± 0.75 g/L.

Transformation efficiency *B. subtilis* transformation efficiencies were much lower compared with of *E. coli*. The highest transformation efficiency was $1.13 \pm 0.08 \times 10^3$ transformants/ μ g DNA, which was obtained by *B. subtilis* 168. The secondly highest efficiency was achieved by the strain N2.1, isolated from a commercial natto, with $4.0 \pm 0.01 \times 10^2$ transformants/ μ g DNA. On the other hand, AHU1031, AHU1032, AHU1033, AHU1035, AHU1233, AHU1722, AHU1889 and N1.1 did not succeed in transformation.

DFA IV production DFA IV productions of the transformants were observed in 3 strains, *B. subtilis* 168/pLFT-SD36, AHU1391/pLFT-SD36 and AHU1892/pLFT-SD36. All the strains were able to produce DFA IV 45.1 ± 2.0 g/L, 2.5 ± 0.2 g/L and 2.3 ± 0.05 g/L. Any relationship between levan production and DFA IV production was not found, might be due to the difference of gene expression efficiency among the strains.