Isolation of thermotolerant yeasts for a non-cooling fermentation system in tropical areas

Ambient temperatures in tropical areas are high, therefore ethanol fermenters also show a rise in temperature (up to around 40 °C) during fermentation. *Saccharomyces cerevisiae*, the typical fermenting yeast, cannot carry out fermentation under high temperatures because it does not have thermotolerance.

We isolated the thermotolerant yeasts, *Kluyveromyces marxianus* (Y2) and *Issatochenkia orientalis* (C19). These yeasts, which have shown greater thermotolerance than *S. cerevisiae*, can produce ethanol under high temperature conditions (more than 40 °C) and can tolerate the fermentation inhibitors contained in saccharified lignocellulosic biomass. Consequently, ethanol fermentation without cooling can be achieved using these yeasts. It is expected to save energy through reduced cooling costs.

These thermotolerant yeasts can grow at higher temperatures than *S. cerevisiae.* Y2 and C19 strains can grow and ferment at 45 °C and 42 °C, respectively (Figs.1A and B). Y2 strain has shown tolerance to furfural, a fural compound, and is relatively insulated from the inhibition by the furfural induced from biomass hydrolysis (Fig. 2A); C19 strain has displayed tolerance to weak acids, hence it is relatively insulated from the inhibition by acetic acid induced from hydrolysis of lignocellulose biomass (Fig. 2B). The kinds of inhibitors to fermentation depend on the type of lignocellulose, therefore, we can select these yeasts as adequate for fermentation in biomass hydrolysis.

In terms of productivity, it can be pointed out that ethanol yield of C19 (73%) is lower than that of Y2 (90%). Additionally, Y2 often produces glycerol as a byproduct when it is incubated under stress conditions.

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Fig. 1. Ethanol fermentation by thermotolerant yeasts, Y2(A) and C19(B) in 10%YPD (10% glucose, 2% peptone, 1% yeast extract) at different temperature conditions. These yeasts have the thermotolerance to produce ethanol under 42 °C.



Fig. 2. The influence of inhibitors on yeast growth. (A) Y2 shows tolerance to 40mM furfural at 42 $^{\circ}$ C. (B) C19 shows tolerance to 60mM acetic acid at 42 $^{\circ}$ C.

Reference :

Y. Mori, A. Kosugi, Y. Murata, and T. Arai, Ethanol Production from Sap of Old Oil Palm Trunks Felled for Replanting (2010)Journal of the Japan Institute of Energy 89, 1147-1152