学位第３号様式）

# 学 位 論 文 要 旨

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| 題　目 | Studies on yeast strains from Coconut (*Cocos nucifera*) Toddy in Sri Lanka: the tolerance to high temperature, salt, and inhibitors derived from lignocellulosic hydrolysates and mechanism of thermotolerance |


First, I isolated 27 yeast strains from coconut toddy in Sri Lanka. Three different genera were identified; *Saccharomyces cerevisiae* (18 strains), *Pichia manshurica* (8 strains), and *Saccharomycodes ludwigii* (one strain) by using the sequence data of the 26S rDNA gene D1/D2 region. All isolated strains of *S. cerevisiae* grew in the presence of 10.0% (w/v) NaCl in yeast extract-peptone-dextrose (YPD) and one of the isolates (SLY-10) grew even with 13.0% (w/v) NaCl. All the isolates grew well at 40°C, and all the 18 *S. cerevisiae* strains grew even at 42°C. Moreover, they still showed better growth than the reference strains (Laboratory yeast S288C strain and Awamori yeast 101-18 strain) in the presence of 7.5% NaCl (w/v) at 40°C. I found that all *S. cerevisiae* isolates produced higher alcohol concentrations than reference strains when growing on batch-fermented media with 100 g· $L^{-1}$glucose at 40°C. The five strains (SLY-3, SLY-4, SLY-8, SLY-9 and SLY-10) produced significant amounts of ethanol even at 45°C, and in fact, the amount of produced alcohol was higher with 160 g· $L^{-1}$ glucose than with 100 g· $L^{-1}$glucose, significantly at 45°C.

Second, I checked aerobic growth of 18 *Saccharomyces* strains (SLY-1 to SLY-18) in the presence of vanillin, which is known as one of the most potent inhibitors for alcohol fermentation, and all of them could grow up to 18 mM vanillin in YPD medium. The five yeast strains (above mentioned) produced alcohol even in the presence of 24 mM vanillin in the range of 11.4–33.1 g· $L^{-1}$ (72 h). Interestingly, the only one strain (SLY-10) showed significant growth with 21 mM vanillin, and showed the highest alcohol production with 24 mM vanillin. I found that all five strains showed the conversion of vanillin to vanillyl alcohol. They also tolerated other strong inhibitors: 4-hydroxybenzoic acid (PHBA, 24 mM), furfural (30 mM), 5-hydroxymethyl-2-furaldehyde (5-HMF, 36 mM) and acetic acid (75 mM). All these five strains showed significant growth and alcohol production (16.2–32.7 g· $L^{-1}$) with inhibitor mixture (0.81 mM vanillin, 9 mM furfural, 9 mM 5-HMF, 22.5 mM acetic acid and 22.5 mM formic acid). However, the four strains (SLY-3, SLY-4, SLY-8 and SLY-9) were shown to tolerate the inhibitor mixture to a similar extent and better than SLY-10, while SLY-10 produced ethanol was the best in the presence of vanillin alone.

Finally, I investigated the sequence of *ERG3* gene, which is essential for ergosterol biosynthesis, and also ergosterol content of the cells grown at 30°C, 37°C, and 40°C in five yeast strains. All the five strains showed the same single nucleotide variants (SNVs) in *ERG3* gene, which is C279T with no change of amino acid residue (phenylalanine), and T461C with change of amino acid residue from valine to alanine, suggesting that *ERG3* genes in the five strains are normally working. Accordingly, the contents of ergosterol in five strains are comparable to the reference strains. As growth temperature increases, the content of ergosterol increases. Although I observed that two different peaks (unknown compounds) from the five strains in addition to the ergosterol at high temperatures, it is difficult to say the relationship between the contents of these unknown sterols and thermotolerance. Therefore, the mechanism of thermotolerance of the five strains seems different from the mechanism involved with *ERG3* gene and ergosterol content, and it is not yet concluded and further analyses will be required.