Transcriptional analysis of recombinant coryneform bacteria in ethanol production process from wood biomass; Effect of fermentation inhibitors

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Bioethanol is recognized as an environmentally friendly and acceptable substitute for petroleum and an additive to petrol [1]. Wood biomass, which is renewable and non-competitive with food supplies, is attractive feedstock for bioethanol production [2].

Microorganisms such as recombinant *E. coli* and several yeast species have been utilized as ethanol producers. In such cases, ethanol production was accompanied by cell-growth; therefore the production rate was low and the energy loss for growth was considerable. RITE, Research Institute of Innovative Technology for the Earth, reported that coryneform bacteria produce valuable materials without growth under oxygen deprivation conditions. The recombinant coryneform bacteria producing ethanol from carbohydrates were constructed by introduction of two heterologous genes.

A problem associated with efficient conversion of wood biomass to ethanol is that a broad range of compounds which inhibit the fermenting microorganisms are liberated or formed along with the saccharides during wood hydrolysis. Inhibitors of fermentation include furan derivatives, such as furfural and 5-hydroxymethylfurfural (5-HMF), low-molecular-mass aliphatic acids, such as acetic acid and levulinic acid, and phenolic compounds [3]. The mechanism of fermentation inhibition has not been elucidated.

Although various studies to improve the methods of hydrolysis have been carried out, formation of fermentation inhibitors is inevitable in any case to a greater or lesser extent. Several papers described detoxification systems for wood hydrolysates, such as use of ion-exchange resins, treatment with activated charcoal, and over-liming [4][5][6]. Any detoxification system increases the cost of the whole process, therefore genetic manipulation of ethanol producers to improve their robustness to such inhibitors is expected. For the first step to construct efficient ethanol producers from wood biomass, it is important to investigate the inhibitory mechanism at the gene expression level.

In the present study, first ethanol producing coryneform bacteria were constructed, and the ethanol production rates were determined to select the best producer. Beech wood chips were pretreated with ethanolysis and hydrolyzed with Meicelase. The resulting soluble fraction (SF) was added to the ethanol producing reaction and the aerobic culture to evaluate the effects of fermentation inhibitors. The ethanol production rates decreased as the concentrations of SF increased. The effects of the inhibitors were also analyzed at the gene expression level with DNA microarray. Secondly potential fermentation inhibitors in SF were identified. Six potential inhibitors were detected by GC/MS. Thirdly each identified potential inhibitor was added to the ethanol producing reaction and the aerobic culture to evaluate the effects. DNA microarray analysis was also carried out.

This master thesis reports the effects of fermentation inhibitors derived from wood on ethanol producers at the whole genome expression level.

REFERENCES

[1] D. J. Schell et al. (2004) Bioresour. Technol. 91: 179-188
[2] Z. L. Liu et al. (2004) J. Ind. Microbiol. Biotechnol. 31: 345-352
[3] S. Larsson et al. (2001) Appl. Environ. Microbiol. 67: 1163-1170
[4] Horvath I.S. et al. (2004) Appl. Biochem. Biotechnol. 113-116: 525-538
[5] Lee W.G. et al. (1999) Appl. Biochem. Biotechnol. 77-79: 547-560
[6] Martinez A. et al (2001) Biotechnol. Prog. 17: 287-293