Biological production of xylitol with high-yield and high-productivity

Executive Summary

Xylitol, the sweetest of the polyols, is a sugar alcohol, which is produced from hardwood sources such as birch. Xylitol can be synthesized either by chemical hydrogenation of xylose or by fermentation. Biological production of xylitol form hemicellulosic material such as agricultural byproduct and waste stream is cost-effective and environment-friendly method compared to the current chemical process. *Candida* strains are considered as the best xylitol producing microorganisms.

We developed an advanced technology for manufacturing xylitol with high-yield and high-productivity using a xylitol dehydrogenase-deficient mutant of *Candida tropicalis*. We obtained a final xylitol concentration of 250g/l after 60h fermentation. The volumetric productivity of xylitol during the fermentation was 5g $liter^{-1}$ h^{-1} and the xylitol yield was 98%.

We also have developed various improved xylitol dehydrogenase-deficient *C. tropicalis* strains by metabolic engineering.

- The codon-optimized *Neurospora crassa* xylose reductase gene expressing strain.
- The L-arabinose pathway engineered strain for the arabitol-free xylitol production from biomass hydrolysate.
- The engineered strain co-expressing two genes involved in pentose phosphate pathway.

Inventor's information

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- Dr. Ing. Thesis: *Growth Kinetics of Penicillium cyclopium and Rheological properties of its Culture Broth* (under the supervision of Professor J. M. Lebeault).
- M. S. Thesis: *Production of Single Cell Protein from Methanol; Optimization of Medium and Maximization of Biomass Productivity* (under the supervision of Professor D. Y. Ryu).

Key Technology Highlights

- Xylitol production by the xylitol dehydrogenase gene-disrupted mutant of *C. tropicalis*.
  - We have cloned the xylitol dehydrogenase gene (XYL2) of *C. tropicalis* and constructed an XYL2-disrupted mutant.
  - The final xylitol concentration was 250g/l after 60h fermentation. The volumetric productivity of xylitol during the fermentation was 5g $liter^{-1}$ h^{-1} and the xylitol yield was up to 98%.
- Xylitol production by the codon-optimized *Neurospora crassa* xylose reductase gene expressing strain.
  - The codon-optimized *Neurospora crassa* xylose reductase gene was expressed in the xylitol dehydrogenase gene-disrupted *C. tropicalis* mutant using the GAPDH promoter. In xylitol fermentation using glucose as a co-substrate with xylose, this strain showed higher production rate than our previously developed strain.
  - Fed-batch culture was performed in fermentation medium with glucose as a co-substrate for cell growth and co-factor (NADPH) regeneration. The resulting recombinant strain showed a higher xylitol production rate and xylitol yield than corresponding values for the parent strain.
- Arabitol-free xylitol production from biomass hydrolysate using L-arabinose pathway engineered *C. tropicalis*.
  - L-arabinose-preferring endogenous XR was replaced by D-xylose-preferring heterologous XR to minimize the flux from L-arabinose to arbitol in C. tropicalis. In addition, the bacterial L-arabinose utilization pathway was introduced into *C. tropicalis*.
  - The resulting recombinant strain successfully assimilated L-arabinose for cell growth without arabitol formation, and xylitol production was enhanced.

Current Progress

A pilot scale fermentation using corn cob hydrolysate was successfully performed.

Commercial Prospect

Xylitol is a popular sugar substitute used in confectionery, food, oral-health, pharmaceutical and supplement applications. The Xylitol market is rapidly increasing as more people become aware of its unique health benefits such as its anticariogenic effect, safety for diabetics and cooling effect.

According to Frost & Sullivan, the US xylitol market is expected to grow to $159.3 million in 2012 and is expected to record a compound annual growth rate (CAGR) of 12.3 percent for revenues for the period 2005-2012.

Patent information

Seven patents covering the technology and specific strains described above have been either granted, filed or are in the process of being filed as the time schedule demands.

1	**Patent Number** **Patent title**	KR 10-0730315 Method for manufacturing xylitol with high-yield
2	**Publication Number** **Patent title**	KR 2009-0013617 Xylitol Dehygrogenase-inactivated and arabinose reductase-inhibited mutant of candida tropicalis, method of producing high-yield of xylitol using the same and hydrolyzate, and xylitol produced thereby
3	**Publication Number** **Patent title**	AU 2007203093 Xylitol dehydrogenase-inactivated and arabinose reductase-inhibited mutant of Candida Tropicalis, method of producing high-yield of xylitol using the same, and xylitol produced thereby
4	**Patent Number** **Patent title**	US 7820414 Xylitol dehydrogenase-inactivated and arabinose reductase-inhibited mutant of candida tropicalis
5	**Patent Number** **Patent title**	US 7745177 Method for manufacturing xylitol with high-yield and high-productivity
6	**Publication Number** **Patent title**	KR 2011-0097157 Xylitol producing-strain using the glucose as the co-substrate and high-producing fermentation methods using the same
7	**Publication Number** **Patent title**	KR 2011-0080138 Xylitol production method with high productivity using constitutive expression of xylose reductase

Related Publication

- Ahmad, W. Y. Shim, W. Y. Jeon, B. H. Yoon, J. H. Kim, "Enhancement of xylitol production in Candida tropicalis by co-expression of two genes involved in pentose phosphate pathway.", *Bioprocess Biosyst Eng*, 35, 199-204 (2012)
- W. Y. Jeon, B. H. Yoon, W. Y. Shim, J. H. Kim, "Xylitol production is increased by expression of codon-optimized Neurospora crassa xylose reductase gene in Candida tropicalis.", *Bioprocess.Biosyst.Eng*, 35, 191-198 (2012)
- Yoon BH, Jeon WY, Shim WY, Kim JH, L-Arabinose pathway engineering for arabitol-free xylitol production in Candida tropicalis, *Biotechnol. Lett.,* 33(4), 747-53 (2011)
- B. S. Ko, H. C. Jung, J. H. Kim, "Molecular cloning and characterization of NAD+-dependent xylitol dehydrogenase from Candida tropicalis ATCC 20913.", *Biotechnol. Prog*., 22(6), 1708-1714 (2006)
- B. S. Ko, C. H. Rhee, J. H. Kim, "Enhancement of xylitol productivity and yield using a xylitol dehydrogenase gene-disrupted mutant of Candida tropicalis under fully aerobic conditions.", *Biotechnol. Lett.,* 28(15), 1159-1162 (2006)
- B. S. Ko, J. Kim, J. H. Kim, "Production of xylitol from D-xylose by a xylitol dehydrogenase gene-disrupted mutant of Candida tropicalis." *Appl. Environ. Microbiol.,* 72(6), 4207-4213 (2006)
- Y. S. Kim, S. Y. Kim, S. C. Kim, and J. H. Kim, "Xylitol production using recombinant Saccharomyces cerevisiae containing multiple xylose reductase genes at cromosomal δ-sequence.", *J. Biotechnol.,* 67, 159-171 (1999)
- S. Y. Kim, D. K. Oh and J. H. Kim, "Improvement of xylitol production by controlling oxygen supply in Candida parapsilosis." *J. Ferm. Bioeng.,* 83(3), 267-270(1997)

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