S7-2

A Genome-Based Approach for Strain Development in *Corynebacterium glutamicum*

Masato Ikeda

Shinshu University, Japan

Based on the progress in genomics, we have developed a genome-based approach to create a minimally mutated strain for efficient amino acid production and for understanding of high-level production by using L-lysine fermentation as a model. A minimally mutated L-lysine producer was constructed in the following way.

First, all predicted genes relevant to L-lysine biosynthesis were identified from the genome and organized into a metabolic map. Next, the sequences of these same genes were determined for a classically derived L-lysine producer and were compared with the wild-type sequences to identify mutational differences. The mutations were then sequentially introduced by allelic replacement into the wild-type genome. The replacement started with mutations in the relevant terminal pathways and moved to those in central metabolic pathways, with each construct evaluated for its contribution to production. When the mutation was relevant to production, it was saved in the genome, and the resulting mutant was used as the parent to introduce and evaluate a second mutation. This iterative cycle made it possible to generate a minimally mutated strain consisting of only relevant mutations (*hom59, lysC311, pyc458, gnd361, mqo224, leuC456*).

The new L-lysine-producing strain inherits the robustness of the wild-type strain, and thus has several advantages over classically derived producers. One is its high rate of growth and sugar consumption. Since the new strain can grow and consume glucose almost as fast as the wild-type strain, the fermentation period can be shortened to nearly half of that traditionally required. This is expected to significantly benefit overall productivity as well as production capacity without the need of new fermentors. A second is improved stress-tolerance, which will allow fermentation at higher temperatures than is traditionally practiced. The new strain showed efficient L-lysine production at 40℃, whereas classical strains could not function above 35℃. This feature not only allows reduction of cooling costs, but also enables cost-effective manufacture in tropical regions that have easy access to low-cost carbon sources such as molasses and tapioca.

The genome-based approach presented here is an example of promptly demonstrating the methodology to apply the fruits of genomics to biotechnological processes. Throughout this work, it was illustrated that strain development based on the specific incorporation of biotechnologically useful mutations in a robust wild-type strain can substantially improve producer performance.

Acknowledgements

I would like to acknowledge all my coworkers, especially S. Mitsuhashi, J. Ohnishi, M. Hayashi, and H. Mizoguchi in BioFrontier Laboratories, Kyowa Hakko Kogyo Co., Ltd.

References

1. Ohnishi J, Mitsuhashi S, Hayashi M, Ando S, Yokoi H, Ochiai K, Ikeda M (2002) A novel methodology employing *Corynebacterium glutamicum* genome information to generate a new L-lysine-producing mutant. Appl Microbiol Biotechnol 58:217-223
2. Ikeda M, Nakagawa S (2003) The *Corynebacterium glutamicum* genome: Features and impacts on biotechnological processes, Appl Microbiol Biotechnol 62:99-109
3. Ohnishi J, Hayashi M, Mitsuhashi S, Ikeda M (2003) Efficient 40℃ fermentation of L-lysine by a new *Corynebacterium glutamicum* mutant developed by genome breeding. Appl Microbiol Biotechnol 62:69-75
4. Ohnishi J, Katahira R, Mitsuhashi S, Kakita S, Ikeda M (2005) A novel *gnd* mutation leading to increased L-lysine production in *Corynebacterium glutamicum.* FEMS Microbiol Lett 242:265-274
5. Hayashi M, Ohnishi J, Mitsuhashi S, Yonetani Y, Hashimoto S, Ikeda M (2006) Transcriptome analysis reveals global expression changes in an industrial L-lysine producer of *Corynebacterium glutamicum.* (2006) Biosci Biotechnol Biochem 70:546-550
6. Ohnishi J, Ikeda M (2006) Comparisons pf potentials for L-lysine production among different *Corynebacterium glutamicum* strains. Biosci Biotechnol Biochem 70:1381-1384
7. Mitsuhashi S, Hayashi M, Ohnishi J, Ikeda M (2006) Disruption of malate:quinone Oxidoreductase increases L-lysine production by *Corynebacterium glutamicum.* Biosci Biotechnol Biochem, manuscript in preparation