Proc. KMB International Symposium
June 24-26, 2003, Muju, Korea

Global Analyses of Transcriptome and Proteome between a Parent Strain and a L-Threonine-Overproducing Mutant Strain

Jin-Ho Lee[†] , **Dong-Eun Lee**, **Bheong-Uk Lee**[1], **and Hak-Sung Kim**

Department of Biological Sciences, Korea Advanced Institute of Science & Technology
373-1, Kusung-dong, Yusung-gu, Taejon, 305-701, Korea
[1] Department of Biological Science, Kosin University, Busan, Korea
[†] Present address : R&D Center of Bioproducts, Institute of Science & Technology, CJ Corp., Kyonggi-do, Korea

We compared the transcriptome, proteome, and nucleotide sequences between the parent strain *E coli* W3110 and the L-threonine-overproducing mutant *E. coli* TF5015. DNA macroarrays were used to measure mRNA levels for the all genes of *E. coli* and two-dimensional gel electrophoresis was employed to compare protein levels. It was observed that only 54 in 4290 genes (1.3%) exhibited differential expression profiles. Typically, genes such as *aceA*, *aceB*, *icdA*, *gltA*, *glnA*, *leu* operon, *proA*, *thrA*, *thrC*, and *yigJ*, which are involved in glyoxylate shunt, TCA cycle, and amino acids biosynthesis (L-glutamine, L-leucine, proline, and L-threonine), were significantly up-regulated, while the genes *dadAX*, *hdeA*, *hdeB*, *ompF*, *oppA*, *oppB*, *oppF*, *yfiD*, and many ribosomal protein genes were down-regulated in TF5015 compared to W3110. The differential expression such as up-regulation of *thr* operon and expression of *yigJ* would result in an accumulation of L-threonine in TF5015. Furthermore, two significant mutations, *thrA345* and *ilvA97*, which are essential for overproduction of L-threonine, were identified in TF5015 through the sequence analysis. In particular, expression of the mutated *thrABC* (pATF92) in W3110 gave rise to a significant incremental effect on L-threonine production. Up-regulation of *aceBA* and down-regulation of b1795, *hdeAB*, *oppA*, and *yfiD* seem to be linked with a low accumulation of acetate in TF5015. Such comprehensive analyses provide information to understand the regulatory mechanism of L-threonine production and the physiological consequences in the mutant stain.

Introduction

In recent years, the completion of the genome project on numerous organisms has accelerated the development of very powerful tools for functional genomics such as DNA arrays (1) and two-dimensional gel electrophoresis (2-DGE) (2). Comparative analysis of the gene expression profiles has provided extensive biological information on a genome scale; response to stress/environmental change, dissection of regulatory circuitry, drug target characterization/identification, cellular response to bacterial infection, and others on many organisms including *Escherichia coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, and human cells (1, 3, 4). As well as transcription levels, proteome analysis is important in the understanding of global regulatory processes in living organisms (2, 5), since the gene expression profiles do not often directly relate to protein expression levels (6). In this sense, functional genomic techniques along with genomic information are believed to enable us to unravel the global regulatory processes or complex metabolic networks in living organisms (7), consequently offering a comprehensive blueprint of the physiology of the bacterium (4, 8).

Amino acids have been the prominent target-metabolites from microorganisms in bio-industry due to large commercial demands for flavor enhancers, animal feed, sweeteners, and therapeutic agents. Of them,

L-threonine, one of the essential amino acids, is widely used as feed- and food-additives, and various industrial strains that more efficiently produce L-threonine have been successfully developed by traditional approaches including deregulation of enzymes, elimination of competitive pathways, and amplification of genes (9, 10). In a previous work, we developed a L-threonine-producing *E. coli* TF5015 by recursive mutations (11). This strain requires both L-methionine and L-isoleucine for growth and shows resistance against various chemical analogues. It was suggested that the mechanism of L-threonine production of TF5015 was probably resulted from releasing the feedback regulation and blocking the carbon flow into undesirable byproducts. Regulation mechanism involved in L-threonine biosynthesis in *E. coli* has been relatively well characterized (12). However, to understand the overall regulatory mechanism and the physiological events in response to the accumulation of L-threonine in TF5015, detailed information including the cellular regulations, entire metabolic fluxes, and dynamic responses of the complex metabolic networks is crucial.

In this work, as an approach to get some insights into the global regulatory mechanism for L-threonine biosynthesis, we carried out the comparative analyses of transcriptome, proteome, and nucleotide sequences between the prototrophic *E. coli* W3110 and the L-threonine-producing *E. coli* TF5015. Expression patterns of the genes and proteins were investigated for both strains using DNA macroarrays containing virtually every gene of *E. coli* and 2-dimensional gel electrophoresis. The profiles were analyzed in terms of the accumulation of L-threonine and physiological consequences in the mutant strain.

References

1. DeRisi, J. L., V. R. Iyer, and P. O. Brown. 1997. Science 278:680-686.
2. Persidis, A. 1998. Nat. Biotechnol. 16:393-394.
3. Arfin, S. M., A. D. Long, E. T. Ito, L. Tolleri, M. M. Riehle, E. S. Paegle, and G. W. Hatfield. 2000. J. Biol. Chem. 275:29672-29684.
4. Tao, H., C. Bausch, C. Richmond, F. R. Blattner, and T. Conway. 1999. J. Bacteriol. 181:6425-6440.
5. Hatzimanikatis, V., L. H. Choe, and K. H. Lee. 1999. Biotechnol. Prog. 15:312-318.
6. Oh, M. K. and J. C. Liao. 2000. Biotechnol. Prog. 16:278-286.
7. Ideker, T., V. Thorsson, J. A. Ranish, R. Christmas, J. Buhler, J. K. Eng, R. Bumgarner, D. R. Goodlett, R. Aebersold, and L. Hood. 2001. Science 292:929-934.
8. Hommais, F., E. Krin, C. Laurent-Winter, O. Soutourina, A. Malpertuy, J. P. L. Caer, A. Danchin, and P. Bertin. 2001. Mol. Microbiol. 40:20-36.
9. Eggeling, L. and H. Sahm. 1999. *In* S. Y. Lee and E. T. Papoutsakis (ed), Metabolic engineering, Marcel Dekker, Inc., New York.
10. Jetten, M. S. M. and A. J. Sinskey. 1995. Crit. Rev. Biotechnol. 15:73-103.
11. Lee, J. H., J. W. Oh, H. H. Lee, and H. H. Hyun. 1991. Kor. J. Appl. Microbiol. Biotechnol. 19:583-587.
12. Patte, J. C. 1996. E. Umbarger (ed), *Escherichia coli* and *Salmonellar typhimurium*: cellular and molecular biology, 2nd ed, ASM Press, Washington, D.C.