Construction of the metabolic engineering platform of *Bacillus licheniformis*

Shouwen Chen

State Key Laboratory of Biocatalysis and Enzyme Engineering, Environmental Microbial Technology Center of Hubei Province, College of Life Sciences Hubei University, Wuhan, Hubei, China

chenshouwen@hubu.edu.cn

摘要

地衣芽胞杆菌是公认生物安全菌株，可广泛应用于益生菌、酶制剂、抗生素、聚谷氨酸等产品生产。我们地衣芽胞杆菌为对象，系统建立了地衣芽胞杆菌代谢工程平台。（1）以特意链测序和转录组分析，分别完成地衣芽胞杆菌 WX02 和 DW2 基因组；以 GC/MS 为工具，建立了地衣芽胞杆菌代谢组分析平台和代谢通量分析方法；（2）以 T2 质粒、cas9n、dcas9 等，建立地衣芽胞杆菌基因无痕敲除、大片段基因敲除、基因插入、基因沉默等方法，开展地衣芽胞杆菌最小基因组工作；（3）系统开展信号肽、信号肽酶、信号肽肽酶、游离表达质粒启动子、5’-UTR 等改造；（4）芽孢形成、细胞分化、细胞逆境、碳氮磷等转录因子调控分析；（5）系统开展地衣芽胞杆菌糖代谢(EMP 途径、HMP 途径、TCA 循环、乙醛酸循环、碳四回补途径)、氨基酸合成和转运等系统代谢工程改造；（6）系统开展能量代谢、辅酶工程工作；（7）系统开展细胞自溶、细胞壁、细胞膜改造；（8）在上述体系基础上，分别开展聚谷氨酸、杆菌肽、乙偶姻、2，3 丁二醇、地衣素、蛋白酶、莽草酸、苯乙醇、短支链脂肪酸、中长链支链脂肪酸等代谢产物代谢工程育种。

Abstract

Bacillus licheniformis is a generally regarded as safe strain, and it have been applied in the production of probiotic, enzyme, antibiotic, poly-γ-glutamic acid etc. In our group, we have constructed metabolic engineering platform of *B. licheniformis* through more than ten years of research. (1) Through specific strand sequencing and transcriptome analysis, we have attained the genome sequences of *B. licheniformis* WX-02 and DW2; *B. licheniformis* metabolome platform and metabolic flux analysis protocol have been constructed based on GC-MS/MS. (2) With the help of plasmid T_2(2)-Ori, CRISPR/Cas9n and CRISPR/dCas9 systems, we have developed the protocols for gene-free knockout, large fragment knockout, gene insertion, gene silencing etc., and now conducting the minimal genome of *B. licheniformis*. (3) Systematically screening and modification of promoters, 5’-UTRs, signal peptide, signal peptidases and signal peptide peptidases for heterologous protein expression in *B. licheniformis*. (4) Conducting the transcription factor regulation analysis of spore formation, cell differentiation, cell stress, carbon, nitrogen and phosphorus metabolisms etc. (5) Systematically metabolic engineering of glucose metabolism (glycolytic pathway, pentose phosphate pathway, tricarboxylic acid cycle, glyoxylate cycle, carbon recovery pathway), amino acid systhesis and transportation pathways. (6) Systematically metabolic engineering of ATP supply and cofactor regeneration pathways. (7) Systematically metabolic engineering of cell autolysis, cell wall, cell membrane. (8) Based on

these above systems, we have respectively carried out metabolic engineering breeding for the production of poly-γ-glutamic acid, bacitracin, acetoin, 2,3 butanediol, licheniysin, protease, shikimic acid, phenylethyl alcohol, short-chain branched fatty acid, medium-long chain branched-chain fatty acid etc.

Speaker's biography

Prof. Chen focuses on physiology, metabolic engineering and fermentation engineering of Bacillus. He obtained a series of projects, including: 973 plan, 863 plan, national 5- year plan (10^{th},11^{th}), national natural science foundation, key science & technology projects of provinces and more than 20 items for companies. He has published about 180 articles, 10 books and obtained 25 invention patents. He obtained 3 scientific and technological achievement awards in national, provincial and ministerial level, furthermore, he is decorated for Advanced worker of Hubei province (2018), Talents Project national candidates (2015), Outstanding contributions to the national middle-aged and young experts (2015), the State Council special allowance (2016), New century talents of the Ministry of Education (2007).

Brief CV

Shouwen Chen, Ph.D.
College of Life Sciences, Hubei University

Education:

BS Biology, Huazhong Nomal University, China, 1985-1989

MS Microbiology, Huazhong Agricultural University, China, 1989-1992

Ph.D. Fermentation, Wuxi Light Industry University, China, 1995-1998

Professional Career:

1992-1998: College of Food Science and Technology, Huazhong Agricultural University, Lecturer

1999-2015: College of Life Science and Technology, Huazhong Agricultural University, Associate Professor, Professor

2014- date: College of Life Sciences, Hubei University, Professor

Research Interests:

1. Metabolic regulation
2. Metabolic engineering
3. Fermentation engineering
4. Enzyme engineering

Selected publications (Correspondence)

1. Cai D, et al. *ACS Synth Biol,* 19;8(4):866-875,2019
2. Shi J, et al. *Bioresource Technol*, 271:325-331,2019
3. Cai D, et al. *Biotechnol Bioeng*, 10:2541-2553,2018
4. Wang D, et al. *Appl Environ Microb,* 2018
5. Li K, et al. *Appl Environ Microb*, 2018, 84(6):02608-17
6. Zhu C, et al. *Appl Microb Biotechnol*, 2017,101:6375-6383
7. Zhan Y, et al. *Appl Microb Biotechnol*. 2017, 101: 7155–7164
8. Cai D, et al. *Microbial Cell Fact*, 2017, 16:70
9. Qiu Y, et al. *Biotechnol Biofeul* 2016,9:117
10. Wei X, et al. *J Agri Food Chem*, 2014, 62:12701–12706