Construction of a novel gene expression system based on RNA polymerase I for large scale screening of high-nucleic acid *Saccharomyces cerevisiae* strains

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Abstract

The RNA derivatives, preferred source is *Saccharomyces cerevisiae*, are widely used as food and medicine homologous. A safe and high-throughput method for screening high RNA (especially rRNA) *S. cerevisiae* cells is urgently needed. As one of the most abundant RNAs, rRNA transcribed by Pol I has no 5' cap and 3' poly-A tail, then its genetic information cannot be transmitted to proteins. The type IV IGR IRES (intergenic region internal ribosome entry site) from CrPV (cricket paralysis virus) is an element whose secondary structure is 5' cap analog and can recruit ribosome to initiate protein translation requiring no translation initiation factors. In this work, we designed and established a Pol I-mediated protein expression system on an episomal plasmid consisting the Pol I promoter, CrPV IGR IRES, reporter genes (*URA3* or *yEGFP3*), oligo-dT and rDNA terminator for large-scale screening high-nucleic acid yeast strains. Rely on growth phenotype, this system with *URA3* worked well, and decomposition the cap-dependent translation initiation factors was unnecessary. This system expressing *yEGFP3* also enhanced fluorescence intensity. Meanwhile the fluorescence distribution drifted further after ARTP mutagenesis. Using this GFP expression system together with flow cytometry, we successfully large-scale sorted out 100 cells with higher fluorescence (>2000) after disturbance the intracellular rRNA synthesis homeostasis by ARTP. Three of twelve strains with higher fluorescence were randomly selected and showed that RNA content improved by 58% maximally without changing its Pol I promoter sequence. The system constructed in this study could be used to screen high RNA industrial yeast strain with altered rRNA biosynthesis by GFP expression levels in the future.

Brief Biography

Dr. Xiaoming Bao is a professor in School of Bioengineering, State Key Laboratory of Biobased Material and Green Papermaking, Qilu University of Technology. She is the committee member of commission on universal education in Chinese Society for Microbiology, Genetics Society of Shandong Province, the Shangdong Society of Molecular Biology and Biochemistry. She awarded Excellent Doctoral Dissertation of Shandong Province. She has been undertaking lots of national research project containing the National Natural Science Foundation of China, National High Technology Research and Development Program of China (863 Program), National Key Basic Research Program of China (973 Program) and province-level research projects. She also cooperates with well-known large enterprises for many research projects. She published about 80 papers on SCI academic journal such as Metabolic Engineering, and 15 patents including two international patents were granted.

The research activities fall in these areas: Studying and engineering metabolic pathway of *Saccharomyces cerevisiae* by rational and irrational strategies for expanding substrate range to produce bioethanol. Recombinant strains are able to utilize xylose, and also have strong tolerance to simplified cultivation. It can co-consume glucose and xylose of raw materials for ethanol production and the conversion of sugar to ethanol is more than 90%, indicating engineering strains show the well performance of C5 and C6 sugars co-utilization. These studies play an essential role in industrialization of cellulosic ethanol through lignocellulosic hydrolysates. Furthermore, the compatibility between secretory components and efficient secretion of heterologous proteins and functional study of heterologous pentose transporters are also researched in *S. cerevisiae* recently.

Brief CV

Ph.D. in microbiology,

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Education:

B.S Microbiology, Shandong University, China, 1983

Ph.D. Microbiology, Shandong University, China, 1997

Professional Career:

2000-2001: University of Gothenburg, Sweden, Postdoctoral Fellow.

1983-1985: Huazhong Agricultural University, China, Assistant Professor.

1996-1998: Shandong University, China, Associate Professor.

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Research Interests:

1. Microbial Gene Regulation
2. Microbial Systems and Synthetic Biology
3. Fermentation

Selected publications

1. Niu, Y., et al. Cellulose, 2019, 26(13): 7923-7937.
2. Tang, H. et al. Microb Cell Fact, 2018, 17: 122.
3. Wei, S. et al. Biotechnol Biofuels, 2018, 11: 112.
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