Advances in engineered microorganisms for improving metabolic conversion via microgravity effects

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As an extreme and unique environment, microgravity has significant effects on microbial cellular processes, such as cell growth, gene expression, natural pathways and biotechnological products. Application of microgravity effects to identify the regulatory elements in reengineering microbial hosts will draw much more attention in further research. In this commentary, we discuss the microgravity effects in engineered microorganisms for improving metabolic conversion, including cell growth kinetics, antimicrobial susceptibility, resistance to stresses, secondary metabolites production, recombinant protein production and enzyme activity, as well as gene expression changes. Application of microgravity effects in engineered microorganisms could provide valuable platform for innovative approaches in bioprocessing technology to largely improve the metabolic conversion efficacy of biopharmaceutical products.

Commentary

The use of engineered microorganisms to produce primary or secondary metabolites is becoming more common in bioprocessing technology. A large of chemicals, pharmaceuticals, biofuels and agricultural compounds have been produced at high enough efficiencies through metabolically engineered microorganisms, of which Escherichia coli, S. cerevisiae and P. pastoris are widely used for expressing therapeutic proteins and industrial enzymes. However, largely obtaining amounts of products is still a major bottleneck for efficiently commercial promotion. Modern bioprocessing technology is highly dependent upon chemical and physical environmental parameters, the changes of environmental factors such as temperature, osmolality and oxygen availability can seriously affect the physiological characteristics and gene expression of engineered microorganisms.

To overcome the problems of high cost and long spaceflight duration, the specialized ground based bioreactors were designed to simulate weightlessness aspects in the laboratory, which were described as low shear modeled microgravity. Simulated microgravity (SMG) is characterized by decreased gravitational force and significant reduction in fluid shear due to absent of convection currents. The sedimentation, which leads to the different dispersion of nutrients and wastes within the vessel, is prevented under microgravity. SMG condition can be modeled by special bioreactors in ground-based experiments. One such bioreactor is Rotary Cell Culture System (RCCS, Synthecon Inc., NASA). Microgravity presents a novel condition to study metabolic conversion in engineered microorganisms. Cells and other molecules are kept suspending in a fluid medium without imparting significant shear forces that accompany stirred terrestrial systems. Previous studies showed that microgravity conditions have significant effects on numerous microbial cellular processes.

Improvement of microbial characteristics using microgravity efficacy

Most studies suggested that microgravity effects were advantageous for microbial growth. Under microgravity, microorganisms show a shortened lag phase, an increased exponential growth rate and a higher final cell count during the
stationary phase in comparison to normal gravity (NG) that are generated by the bioreactor. The growth of E. coli cultured under SMG and NG conditions were exhibited in Figure 1a. The increased optical density of microorganisms cultured under microgravity might be caused by the increased cell size and number. Microgravity effects also disturb spatially programmed budding patterns, generating strain dependent growth differences in yeast colonies on semi-solid medium. Furthermore, microgravity effects were reported to affect other distinct physiological changes including resistance to radiation, susceptibility to osmotic, thermal and acidic stress, effective substrates utilization and antibiotics resistance. The antibiotics resistance of E. coli cultured under SMG and NG conditions were exhibited in Figure 1b. The improvement of these performances is thought to be relevant to reduced extracellular mass transport that occurs in the absence of sedimentation and buoyancy-driven convection in suspended cultures. The unique microgravity effects may have greatly potential in conventional bioprocessing techniques, and ultimately can lower the industrial production cost. Microorganisms exposed to microgravity environment are significantly more resistant to the antibiotic agents due to the enhanced biofilms. The explanations can be used to construct the antibiotic producing microorganisms with improving biomass or reducing drug resistance of superbugs.

Regulation of secondary metabolites production by microgravity

Several researches revealed that microgravity effects could inhibit microbial secondary metabolism or have no effects, i.e. the β-lactam antibiotics produced by Streptomyces clavuligerus, the micromycin B17 produced by E. coli and the rapamycin produced by Streptomyces hygroscopicus. Only a few reports demonstrated that microgravity effects could enhance the accumulation of secondary metabolites. The microbial antibiotic actinomycin D produced by Streptomyces plicatus was enhanced under the space microgravity condition. The gramicidins (GS) produced by Bacillus brevis was higher when cultured in simulated microgravity bioreactor of RWV than in shaking flask. Zhang L et al. utilized the diamagnetic levitation to simulate an altered gravity environment and found that the secondary metabolite produced by Streptomyces avermitilis was increased at this culturing condition. The dramatic shift in the site of MccB17 accumulation from E. coli ZK 650 to extracellular fluid when the cell growth took place in the low shear stress characteristic of simulated microgravity bioreactors. Importantly, the question of whether microgravity effects will similarly enhance secondary metabolites production have been raised. The specific mechanisms responsible for antibiotic production under microgravity need to be elucidated.

In recent years, synthetic biology provides a significant driving force to develop complex natural products and drugs originated from engineered microorganisms. The natural biosynthetic pathways show surprising levels of built-in modularity at many levels, which can be exploited by synthetic biology approaches. However, the high-value chemicals production from microbial engineering is still much lower than that from utilizing chemical methods. The application of engineered microorganisms is constrained for the reason of intracellular biotechnological products. Microgravity effects can be introduced to improve metabolic conversion efficacy in bioprocessing technology.

Tuning recombinant protein expression by microgravity

It is widely used for expressing prokaryotic and eukaryotic origin proteins using engineered microorganisms. However, there is evidence of major bottlenecks to primarily obtain large amounts of functional proteins. Some studies demonstrated that the expression of recombinant proteins from engineered microorganisms was changed when the cells were cultured under microgravity, compared with normal gravity. The microgravity effects could promote protein expression, such as the expression of the recombinant β-galactosidase and the
glycodelin in human cells, the recombinant $\beta$-glucuronidase in E. coli (Fig. 1c) and P. pastoris and the human monoclonal antibody in Sp2/0 myeloma mouse cell line. These results suggested that microgravity effects could be used for efficient production of recombinant proteins from engineered microorganisms. Interestingly, the enzyme catalysis process was also changed under microgravity. The activities of intracellular antioxidant enzymes, superoxide dismutase and catalase, were higher under space microgravity condition. Qi et al. revealed the significant alterations in catalytic properties of the recombinant $\beta$-glucuronidase in response to low-shear modeled microgravity effect. The catalytic efficiency (kcat/Km) of this enzyme expressed by E. coli was 3.7 times higher under microgravity than under which of normal gravity (Table 1).

The further investigation of mechanisms through which microbial cells sense microgravity effects signals can give rise to advanced knowledge about microbial process. The effects of microgravity can potentially be used in catalytic bioprocess. Additionally, the research can also elevate the amount of recombinant proteins produced by engineered microorganisms.

Table 1. Kinetic constants of the recombinant PGUS expressed by E. coli under SMG and NG conditions

| PGUS expression | Vmax (umol min$^{-1}$ mg$^{-1}$) | Km (mM) | Kcat (s$^{-1}$) | Kcat/Km (mM$^{-1}$s$^{-1}$) |
|-----------------|---------------------------------|--------|----------------|-------------------------|
| SMG             | 147.20                          | 104.61 | 415.4          | 3.97                    |
| NG              | 7.29                            | 12.01  | 21.5           | 1.79                    |
regulated genes of methanol metabolism and carbohydrate metabolic process, cell redox homeostasis and oxidative stress, translation and protein folding-related, and protein transportation were contributed to enhancing production and secretion of the recombinant protein from *P. pastoris* under SMG condition.\(^{32}\) It was observed that SMG had a significant effect on *P. pastoris* molecular progress (Fig. 2). Bradamante et al. demonstrated the cells developed a "multiple ways oxidative stress response" to prevent oxidative damage created by the microgravity effects. The regulation of the pathways appeared to be differentially controlled during spaceflight and SMG environment, compared to conventional culture.\(^{33}\) The present researches are only at the very beginning of clarifying the role of microgravity effects at the molecular levels. The important mechanisms have not been unraveled.

The analysis of omic-data can provide important platforms to define the molecular mechanisms of response to environmental changes and help to investigate potential synergistic actions of individual helper targets for cell engineering. Notably, microgravity effects can help to obtain more accurate data needed for the improvement of certain ground-based processes and products. Huangfu et al. examined the proteomic profiling of the recombinant *P. pastoris* grew under simulated microgravity and identified some potential helper genes which could be used for strain improvement. Overexpressing the gene encoding detoxifying enzyme of thiol peroxidase was proved to efficiently counteract oxidative stress arising from heterologous protein production and improve the productivity of recombinant proteins in methylotrophic *P. pastoris*.\(^{34}\) The utilization of deeply "omics" analyzing platforms for engineered microorganism under microgravity condition to improve the metabolic conversion efficacy of biopharmaceutical products is an attractive field (Fig. 3).

**Conclusion**

Studies concerning the influence of the microgravity effects on microbial cells may receive much attention. Utilizing microgravity effects combined with systems biotechnology based strategy, and synthetic biology techniques to find novel target genes will boost metabolic conversion in bioprocessing technology.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest was disclosed.

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