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PERSPECTIVE

Secondary metabolism in simulated microgravity and space flight

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Space flight experiments have suggested that microgravity can affect cellular processes in microorganisms. To simulate the microgravity environment on earth, several models have been developed and applied to examine the effect of microgravity on secondary metabolism. In this paper, studies of effects of space flight on secondary metabolism are exemplified and reviewed along with the advantages and disadvantages of the current models used for simulating microgravity. This discussion is both significant and timely to researchers considering the use of simulated microgravity or space flight to explore effects of weightlessness on secondary metabolism.

KEYWORDS simulated microgravity, space flight, microorganism, secondary metabolism

INTRODUCTION

Space flight has been reported to affect microbial cellular processes, such as cell growth (Qi et al., 2011), gene expression (Wilson et al., 2007), rate of conjugation (Tixador et al., 1985), susceptibility to vacuum and UV irradiation (Spizizen et al., 1975), phage productivity and survival rate, and cell morphology, thereby potentially producing unexpected (and possibly dangerous) changes in the natural biota of the space craft. For earth based organisms, microgravity is a key factor of the space condition, since all earth based organisms have been evolving under a 1 g field for millions of years. The microgravity environment in space alters the normal physiologic course of organisms. Thus, in recent years, as space technology develops, studies concerning the influences of space flight and microgravity on microbial cells and their adaptations are attracting more and more attention.

Because of the rarity and costliness of experiments in orbit, studies of the effect of space on biology are limited to a large degree. To overcome these problems and in accordance with the hypothesis that "sensing no weight" would have comparable effects to those of weightlessness (Hejnowicz et al., 1998), several forms of ground-based setups that simulate "weightlessness" have been developed and applied to explore the effects of microgravity on microbial cells.

However, there are few reports on whether space flight and simulated microgravity affect secondary metabolism of microorganisms. The significance of exploring the effects of space flight and simulated microgravity on secondary metabolism are: (1) secondary metabolism is important for the health of astronauts, since many secondary metabolites are highly toxic, threatening the safety of the crews (Fang et al., 1997a); (2) there are many important secondary metabolites that can be utilized as medicines for both human and animals, so the effects of simulated microgravity on production of the secondary metabolites may be of considerable value to the pharmaceutical industry (Benoit et al., 2006).

SIMULATED MICROGRAVITY

Rotating wall vessel

There are several different devices to simulate microgravity, most of which however, are based on the rotating wall vessels (RWV) model. The principle of the RWV is to time-average the gravity vector to zero by rotation, and it is an optimized culture chamber creating low shear and turbulence (Hammond and Hammond, 2001; Nickerson et al., 2004). Arnold Demain was one of the scientists who pioneered the study of this field. From 1997 to 2000, he and his colleagues studied microbial secondary metabolism in simulated microgravity provided by rotating-wall bioreactors (RWBs). Four strains were investi-

gated in RWB, including *Bacillus brevis* producing gramicidin S (Fang et al., 1997b), *Streptomyces clavuligerus* producing β-lactam antibiotics (Fang et al., 1997c), *Streptomyces hygroscopicus* producing rapamycin (Fang et al., 2000), and *Escherichia coli* producing microcin B17 (Gao et al., 2001). In the four strains, only gramicidin S production was not affected by simulated microgravity; production of the other three products was decreased after the treatment of simulated microgravity. Xiao et al. (2010) studied the effects of microgravity simulated by a different kind of rotation device, rotary cell culture system, on the growth and microcystin production in *Microcystis aeruinosa*. They analyzed the growth and microcystin production profiles during a 20-day treatment and the results revealed that simulated microgravity could inhibit cell growth and improve the production of microcystin. Quick adaptation of the cells to the simulated microgravity was also observed.

Nevertheless, the fluid shear stress by utilizing rotating wall vessels to simulate microgravity may interfere with the response of cells to a randomized gravity vector (Crabbé et al., 2008). For example, in the Demain laboratory, they found that glycerol inhibited the gramicidin S production during shake flask fermentation under normal gravity condition, while in the RWV, the repressive effect of glycerol was not observed, suggesting the negative effect of glycerol on gramicidin S production was dependent on shear stress. Shear stress also affects the microcin B17 production of *Escherichia coli*, with increased shear stress enhancing the production (Gao et al., 2001). The same phenomenon in the Fang et al. (1997b) study was seen in *Bacillus brevis*, in which the cells in the RWV were much more resistant to the growth-inhibitory and production-inhibitory effects of ethanol than the cells in shaken flasks. However, for the other two *Streptomyces* strains studied, which were the major producers of active secondary metabolites, the above phenomena were not observed, by the Demain laboratory. How *Streptomyces* strains respond to the shear stress is still unclear.

Diamagnetic levitation

Just as the centrifugal force balances the gravitational force on an orbiting spacecraft, the diamagnetic force opposes the force of gravity on a levitating object. In previous works, diamagnetic force has been applied to levitate protozoan (Guevorkian and Valles, 2006), plants (Kuznetsov and Hasenstein, 1996), and mammals (Liu et al., 2010). Effects of altered gravity environments simulated by diamagnetic levitation on protein crystallization (Wakayama et al., 2006), plasmid transfer (Beuls et al., 2009), cell growth and gene expression (Coleman et al., 2007) have also been investigated. However, in common with all ground-based techniques to simulate weightlessness, there are effects introduced by diamagnetic levitation that are not present in a weightless environment. It has been demonstrated that the magnetic field that levitates the cell also induces convective stirring in the liquid, and thus enhances the oxygen availability by transporting oxygen around a liquid culture (Dijkstra et al., 2011). So the use of solid medium is necessary to avoid the magnetically driven convection of oxygen. Therefore, our lab chose *Streptomyces avermitilis* grown on solid media to study secondary metabolism in simulated microgravity (Liu et al., 2011). *S. avermitilis* is used for the industrial production of the important anthelmintic agent avermectin, and has already been proven to be a highly efficient producer of several secondary metabolites (Gao et al., 2010). Of the eight major avermectin compounds, B1a is the most efficient component against a broad range of nematodes and arthropod parasites of domestic animals (Gao et al., 2009). We assessed whether magnetic field and variations in gravity have independent effects on morphology and secondary metabolism of *S. avermitilis* in solid culture, and thus whether diamagnetic levitation is a suitable technique to simulate the altered gravity environment. In the study, we discovered that magnetic field, rather than microgravity, is the dominant factor to induce the physiological response of the strain in simulated microgravity by diamagnetic levitation, suggesting that care should be taken in the interpretation of results when using diamagnetic levitation as a technique to simulate microgravity.

Space flight

The study under simulated microgravity suggested that secondary metabolism might be affected by microgravity. More and more researchers have begun to study the effect of space flight on secondary metabolism, and try to use space flight as a way to improve the production of secondary metabolites.

Kanglemycin C producing strain, *Nocarida mediterranei* var. *kanglensis*, was carried into space by an unmanned spaceship “Shenzhou III” (Divine Vessel III, flight time: 162 h), and the biological effects of space flight on the strain was investigated (Zhou et al., 2006). One mutant strain with 200% higher kanglemycin C production was identified.

Our lab also studied the effect of space flight on avermectin production. *S. avermitilis* was carried into space by “Shenzhou VII” (Divine Vessel VII, flight time: 40 h). The results also showed that avermectin production could be improved after space flight (unpublished data).

Other researchers investigated the biological effects of longer-duration of space flight. Benoit et al. (2006) examined the actinomycin D production onboard the International Space Station during 72 days. However, only for the first two sample points (days 8 and 12), increased productivity of actinomycin D was observed, and the flight production levels were lower than the matched ground control samples after 12-day flight (Benoit et al., 2006). The results suggested that the stress increased with the duration of flight, and thus suppressed secondary metabolism.

SUMMARY

Secondary metabolism under simulated microgravity or out space condition is still a field explored inadequately. Both NASA and ESA (European Space Agency) have supported the scientific program in Space Station. However, the global economic recession forced them to cut budget (Kerr, 2011). Furthermore, the complex environment makes it difficult to clarify the effects of space on microbes. So in the near future, ground-based analogs are still the promising means to understand how secondary metabolism of microbes adapts to the space environment. But what needs more attention is that all of the analogs will introduce artifacts in simulation. How to analyze the data obtained to get rid of the artifact is the key issue. Preliminary reports on the effects of space flight on secondary metabolism suggested that short-term flight might improve the production of secondary metabolites, while long-term flight could inhibit secondary metabolism. Future work is needed to illustrate the mechanism clearly.

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