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ORIGINAL ARTICLE

Comparative growth, cross stress resistance, transcriptomics of *Streptococcus pyogenes* cultured under low shear modeled microgravity and normal gravity

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Abstract *Streptococcus pyogenes* is commonly found on pharynx, mouth and rarely on skin, lower gastrointestinal tract. It is a potential pathogen causing tonsillitis, pneumonia, endocarditis. The present study was undertaken to study the effects of low shear modeled microgravity on growth, morphology, antibiotic resistance, cross-stress resistance to various stresses and alteration in gene expression of *S. pyogenes*. The growth analysis performed using UV–Visible spectroscopy indicated decrease in growth of *S. pyogenes* under low shear modeled microgravity. Morphological analysis by Bio-transmission electron microscopy (TEM), Bio-scanning electron microscopy (SEM) did not reveal much difference between normal and low shear modeled microgravity grown *S. pyogenes*. The sensitivity of *S. pyogenes* to antibiotics ampicillin, penicillin, streptomycin, kanamycin, hygromycin, rifampicin indicates that the bacterium is resistant to hygromycin. Further *S. pyogenes* cultured under low shear modeled microgravity was found to be more sensitive to ampicillin and rifampicin as compared with normal gravity grown *S. pyogenes*. The bacteria were tested for the acid, osmotic, temperature and oxidative cross stress resistances. The gene expression of *S. pyogenes* under low shear modeled microgravity analyzed by microarray revealed upregulation of 26 genes and down regulation of 22 genes by a fold change of 1.5.

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1. Introduction

Microgravity is a condition found to exist, when the small collision accelerations are not exceeding 10^{-5} to $10^{-4} \times g$ of background level (Albrecht-Buehler, 1992). The bacterial cells experience microgravity when force of gravity acting upon them

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is opposed by another force. Microgravity is one of the conditions existing in space. Space flight was found to suppress the immune system in humans and animals, (Nefedov et al., 1978; Pellis et al., 1997; Taylor, 1974) suggesting that risk of infectious diseases during spaceflight is high. Studies on changes experienced by bacteria under microgravity conditions during space flight are important in order to understand the behavior of microorganisms under microgravity. Spaceflight effects on the process of infectious diseases were investigated early at the level of host susceptibility, so present researches were carried to find the ability of bacteria to change their physiology and virulence which is important to decrease the risks to crew members during space flight (Nickerson et al., 2000). It is difficult to study each and every bacterium during the space flights because of constraints faced in space crews. So high aspect rotating vessel (HARV), a ground based model had been utilized for performing experiments by mimicking microgravity condition.

Alterations in composition of oral and cutaneous microflora were observed in Vostok crew members after space flight. Also decrease in useful microorganisms and increase in pathogens were observed during space flight on Salyut and Mir orbital stations (Alekseeva, 1965; Ilyin, 2005). A variety of bacterial species were found to exhibit large population changes under the reduced gravity conditions. Growth patterns of bacteria were analyzed in various studies (Baker et al., 2005; Brown et al., 2002; Kacena and Todd, 1999; Kacena et al., 1999; Klaus et al., 1997; Mennigmann and Lange, 1986; Thevenet et al., 1996) and the bacteria were found to gain resistance to various other stresses like acid, thermal, osmotic and ethanol (Nickerson et al., 2000; Wilson et al., 2002; Gao et al., 2001). The microorganisms were found to become resistant to antibiotics, and *in vitro* studies revealed that higher concentrations of antibiotics were required to inhibit the growth of microorganisms in space when compared with the microorganism cultured on ground (Leys et al., 2004).

Streptococcus pyogenes is a normal human flora present commonly in mouth, pharynx and rarely on the skin, conjunctiva, lower gastrointestinal tract and vagina. *S. pyogenes* is spherical, Gram positive, facultative anaerobe, nonmotile and non spore producing bacterium occurring in chains. *S. pyogenes* is considered as an opportunistic pathogen of human beings, residing in the respiratory tract of many people. It usually does not cause complications if the immune system of host is functioning properly but when the host becomes compromised to natural immune defences, it causes infections. *S. pyogenes* causes mild superficial skin infections, tonsillitis, pneumonia and endocarditis. *S. pyogenes* is unique in producing strep throat, impetigo, necrotic fasciitis and streptococcal toxic shock syndrome. As the immune system was found to be suppressed in space, *S. pyogenes* unexplored for their change under low shear modeled microgravity is selected for the present study. Previous studies compared low shear modeled microgravity cultured cells with the cells grown in normal gravity using HARV. Another research compared the phenotypic, transcriptomic and proteomic changes in B*acillus cereus* after a short term flight with the ground controls grown in incubators (Su et al., 2014). Our aim was to analyze the changes of *S. pyogenes* under low shear modeled microgravity with incubator culture which is commonly used for the microbiological studies rather with normal gravity cultures cultured in HARV vessel. So we have attempted to compare the low shear modeled microgravity cultured bacteria with the normal gravity grown bacteria in incubators.

This study had been undertaken to analyze the growth and morphology of *S. pyogenes* under low shear modeled microgravity. Growth studies were performed by turbidity measurement with UV–Vis spectrophotometer and the morphological studies were done by Bio-transmission electron microscopy (TEM) and Bio-scanning electron microscopy (SEM). The changes of bacterial resistance in response to low shear modeled microgravity for various antibiotics were measured using antibiotic disc diffusion assay. The modulation of virulence in response to environmental stress is commonly seen with the pathogenic bacteria. Various parameters like osmolarity, temperature, pH, growth medium of bacteria, starvation have been found to affect the expression of virulence factors in a wide range of pathogens (Foster and Spector, 1995; Mahan et al., 1996). So the cross resistance of *S. pyogenes* grown under low shear modeled microgravity to acidic, osmotic, and temperature stresses was studied. The modeled microgravity was found to affect gene expression of *Escherichia coli* (Vukanti et al., 2008; Tucker et al., 2007), *Salmonella enterica* (Wilson et al., 2002), *Pseudomonas aeruginosa* (Crabbe et al., 2010) and *Streptococcus pneumoniae* (Allen et al., 2006). So we attempted to study global transcriptomic change and change in the virulence factors of *S. pyogenes*, under low shear modeled microgravity by microarray analysis.

2. Materials and methods

2.1. High aspect ratio vessel

High aspect ratio vessel (HARV) also called as rotating wall vessel (RWV) is commonly used to maintain the low shear modeled microgravity. It is a cylindrical bioreactor having a capacity of 50 ml with one filling port and two sampling ports. In the HARV, modeled microgravity is obtained by rotating the reactor perpendicular to the gravity vector, thus nullifying the downward gravity vector (Nickerson et al., 2000). Oxygenation of the bioreactor was achieved through the gas permeable membrane present on back of the HARV reactor.

2.2. Bacterial strains and culture conditions

Experiments were performed with *S. pyogenes* obtained from Korean Agricultural Culture Collection (KACC 11858). Brain heart infusion agar was used to maintain the culture and stored at 4 °C until use. The overnight liquid cultures were cultured in brain heart infusion at 37 °C. Overnight cultures were used as starter cultures to perform further experiments. 100 µl of culture was inoculated into the HARV vessel for culturing *S. pyogenes* under low shear modeled microgravity and into the conical flask for culturing bacteria under normal gravity which served as control. HARV bioreactor was packed with brain heart infusion broth and removed the air bubbles formed to maintain the low shear modeled microgravity conditions. The normal gravity cultures were grown at 37 °C in the incubator as shake flask cultures shaking at 25 rpm. HARV reactor was also maintained at same 37 °C, rotating at an rpm of 25.