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ORIGINAL ARTICLE

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

Duraisamy Kalpana [a,b], **Chanki Im** [a], **Yang Soo Lee** [a,*]

[a] *Department of Forest Science and Technology, Institute of Agricultural Science and Technology, Chonbuk National University, Jeonju, South Korea*
[b] *Department of Bioresources and Food Science, College of life and Environmental Sciences, Konkuk University, Seoul, South Korea*

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KEYWORDS

Low shear modeled microgravity;
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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.

* Corresponding author. Tel.: +821097402622
E-mail address: ysoolee@chonbuk.ac.kr (Y.S. Lee).
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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.

2.3. Growth analysis

Sampling was done by injecting 1 ml of sterile brain heart infusion medium into one of the sampling ports which displaced 1 ml of culture into the other syringe inserted at other sampling ports. The reactor was rocked well before collecting sample, to obtain representative sample for the growth analysis. Similarly for normal gravity grown culture, the flasks were shaken well and 1 ml of the culture was collected and 1 ml of fresh brain heart infusion medium was introduced into culture flask to maintain a uniform volume of 50 ml and to maintain same culture volume as of the low shear modeled microgravity culture throughout the study. The cultures were collected at every 4 h of intervals until 24 h and absorbance was measured at 600 nm in UV–Vis spectrophotometer.

2.4. Bio-SEM and Bio-TEM analysis

Bio-SEM and Bio-TEM analysis were carried out to check if any alteration in the morphology of *S. pyogenes* under low shear modeled microgravity has occurred. The control cells cultured under normal gravity and low shear modeled microgravity cultured cells were collected by centrifugation at 10,000 rpm for 2 min at room temperature. The pelleted cells were washed twice with phosphate buffered saline and then fixed with 1.5% glutaraldehyde solution for around two hours in room temperature. The cells were centrifuged after fixation, washed once with 70% ethanol and twice with 100% ethanol. The washed cells were resuspended in sterile Millipore water for Bio-TEM analysis and cells were air dried for Bio-SEM analysis. Bio-TEM was performed at an accelerating voltage of 100 kV with HITACHI-JP/H7600 instrument. The air-dried bacterial cells were mounted on metallic stub and sputter coated with Osmium for Bio-SEM analysis.

2.5. Antibiotic sensitivity assay

The antibiotic sensitivity assay was performed by disc diffusion method. The *S. pyogenes* cultures were cultured both under normal gravity and low shear modeled microgravity for 16 h to reach the mid log phase of growth. 100 µl of mid log phase cultures was spread uniformly on the brain heart infusion agar plates. Antibiotics ampicillin, penicillin, rifampicin, kanamycin, streptomycin, and hygromycin were selected for the present study. The antibiotics were loaded on Whatman filter paper antibiotic assay discs at different concentrations of 50, 100, 150, 200 µg/disc for the antibiotics penicillin, rifampicin, kanamycin, streptomycin and 25, 50, 75 and 100 µg/disc for ampicillin. The antibiotic loaded discs were dried under sterile conditions inside the laminar air flow chamber. The sterile antibiotic discs were then placed on the *S. pyogenes* culture spread plates. The plates were incubated at 37 °C for 12 h and the zone of inhibition of growth of bacteria produced by different antibiotics was measured in mm. The experiments were carried out as triplicates of three independent cultures.

2.6. Thermal stress studies

The ability of *S. pyogenes* to with stand thermal stress was analyzed at three different temperatures of 45, 50 and 55 °C. The *S. pyogenes* were grown for 16 h under low shear modeled microgravity at 37 °C. 5 ml of this culture was separately incubated at 45, 50 and 55 °C. The samples were collected before and after 30 min of incubation; cultures were then serially diluted and spread on the brain heart infusion agar plates. The colonies were counted after 12 h of incubation at 37 °C. A similar treatment was carried out for normal gravity cells.

2.7. Oxidative stress analysis

The oxidative stress was induced in normal gravity grown and low shear modeled microgravity cultured *S. pyogenes* by adding 30% of hydrogen peroxide to make a final concentration of 30 mM in 5 ml of culture. Sample at 0 min was collected before the addition of hydrogen peroxide. The hydrogen peroxide added cultures were then incubated at room temperature for 30 min. After 30 min the cultures were again sampled and 100 µl of the serially diluted samples were plated on brain heart infusion agar and incubated overnight at 37 °C and the colony forming units were calculated.

2.8. Acid survival test

The normal gravity grown control cultures and low shear modeled microgravity cultures of *S. pyogenes* were tested for their survival at acidic pH by using citrate buffer of pH 3.5. Citrate buffer was prepared using sterile distilled water and the pH was adjusted to 3.5. The mid log phase grown cultures at two different conditions were introduced into the citrate buffer. The samples were mixed thoroughly and collected immediately which is indicated as t0 min culture and samples were collected at every 10 min up to 40 min. 100 µl of the samples collected at different time intervals were cultured on the brain heart infusion agar plates and colonies were counted.

2.9. Transcriptomic studies

2.9.1. RNA extraction and quality analysis

S. pyogenes were cultured both under normal and low shear modeled microgravity for 16 h and used for RNA extraction. RNA isolation and purification was further carried out using the RNA isolation kit (GeneAll Hybrid-R, total RNA purification kit). The quality of isolated RNA was estimated by evaluating the OD at 260 and 280 nm. The integrity of the RNA was analyzed using Agilent 2100 Bioanalyzer (Agilent Technologies, USA).

2.9.2. Synthesis and labeling of cDNA

cDNA was synthesized using double stranded cDNA synthesis kit (Invitrogen Life Technologies, Carlsbad, USA). Briefly reverse transcription of 10 µg of total RNA was performed using oligo dT primer and then second strand of cDNA was synthesized. The cDNA was purified, quantified spectrophotometrically (Nanodrop, Wilmington, USA) and the purified cDNA was labeled with Cy3-nonamer using one-color labeling kit (Nimblegen, Nadison, USA) following Nimblegen expression protocol.

2.9.3. Hybridization and data analysis

Hybridization of purified labeled cDNA to Nimblegen Expression array was performed at 42 °C for 16–20 h. The arrays

were scanned on Nimblegen MS2000 microarray scanner at 532 nm at a resolution of 2 μm. The images were exported and analyses were done using Nimblescan v2.5.

3. Results

The growth of *S. pyogenes* was monitored at every 4 h interval up to 24 h. The absorbance was measured at 600 nm, the experiments were repeated three times, the mean values of the triplicates were calculated and growth curve was plotted (Fig. 1). The growth of the bacteria was found to be reduced under low shear modeled microgravity compared to the normal gravity cultured bacteria. The morphological images of *S. pyogenes* were obtained with TEM and SEM after culturing the bacteria under normal and low shear modeled microgravity (Figs. 2 and 3). Low shear modeled microgravity did not alter the morphology of *S. pyogenes* as no significant differences in size and shape could be observed from the TEM and SEM images and extracellular matrix formation was also not noticeable. The sensitivity of bacteria grown under normal gravity to antibiotics was analyzed and the alteration in the antibiotic sensitivity of low shear modeled microgravity grown bacteria was compared (Table 1). The results obtained indicated that *S. pyogenes* was resistant to hygromycin producing no zone of inhibition of growth. Ampicillin produced highest zone of inhibition, overlapping zones were produced when higher concentrations of 50, 100, 150, 200 μg/disc were used, so the concentration of ampicillin was reduced to half and discs of final concentrations of 25, 50, 75, 100 μg/disc were prepared and the antibiotic sensitivity test was carried out.

The *S. pyogenes* grown under normal gravity and low shear modeled microgravity at 37 °C were tested for thermal stress tolerance 45 °C, 50 °C and 55 °C in incubators for 30 min. The percentage of cells survived after 30 min of thermal stress were estimated and presented in the Fig. 4. The bacteria cultured under low shear modeled microgravity showed higher resistance to thermal stress of 45 °C and 50 °C as compared with the culture grown under normal gravity. Both the cultures grown under low shear modeled microgravity and normal gravity could not resist thermal stress when exposed to 55 °C for 30 min. The alteration of low shear modeled microgravity grown *S. pyogenes* to oxidative stress using 30 mM hydrogen peroxide was analyzed and given in Fig. 5. Low shear modeled microgravity cultures resisted the thermal stress more than normal gravity grown cultures, but the oxidative stress resistance was decreased when the bacteria was cultured under low shear modeled microgravity. The acid stress analysis performed using citrate buffer of pH 3.5 revealed *S. pyogenes* were not able to withstand the acid stress for long time interval, this was same with the case of both normal gravity and low shear modeled microgravity grown cultures (Fig. 6). But low shear modeled microgravity cultures were able to withstand the acid stress slightly more than normal gravity grown cultures for about a short period of 10 min. The whole genome expression study carried by microarray analysis revealed down regulation of 22 genes and upregulation of 26 genes in low shear modeled microgravity to normal gravity (Fig. 7) among the 1886 genes analyzed. Among the 22 down regulated genes majority of genes were involved in the production of proteins which are building components of 50 S and 30 S ribosomal units (Table 2). The upregulated genes included mainly unknown phage protein and hypothetical proteins of five and seven respectively (Table 3).

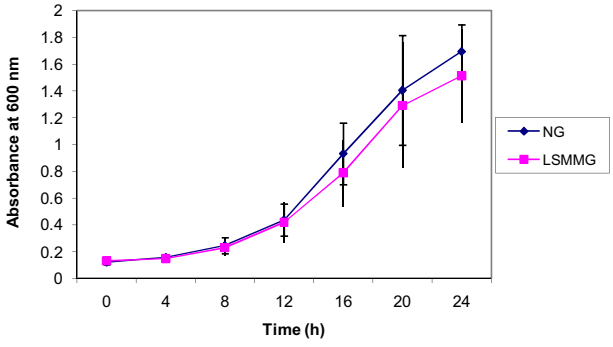

Figure 1 Growth curve of *S. pyogenes* cultured under normal gravity (NG) and low shear modeled microgravity (LSMMG) in BHI broth.

4. Discussion

4.1. Growth kinetics

Growth curve for both the normal gravity cultured and low shear modeled microgravity grown cultures revealed a similar growth rate up to 14 h and later low growth rate was observed with low shear modeled microgravity cultured *S. pyogenes*. A similar result was obtained with Gram positive bacterium *Staphylococcus aureus*, the growth of bacteria grown under low- shear modeled microgravity was lower than normal gravity cultured *S. aureus* (Rosado et al., 2006). The growth of *S. enterica* and *E. coli*, Gram negative bacteria was found to be faster when cultured under modeled microgravity compared with the normal gravity grown cultures (Wilson et al., 2002; Vukanti et al., 2008; Arunasri et al., 2013). The Gram negative bacterium *P. aeruginosa* was found to have no significant differences in growth under modeled microgravity (England et al., 2003). The growth curve of *E. coli* in space flight indicated shortened lag phase, increase in the duration of exponential growth phase and approximately doubled final cell density population (Klaus et al., 1997). The final cell population of *E. coli* and *Bacillus subtilis* grown abroad space shuttle was found to be higher than the static 1 g controls. These studies suggested that access of bacteria to nutrients and removal of waste products is more favorable under microgravity due to lack of sedimentation of the bacterial cells at the bottom of the culture flasks. The low gravity also makes the metabolism of nutrients more efficient and cells multiply repeatedly before the nutrients are depleted (Kacena et al., 1999). But increase in growth pattern was not observed with *S. pyogenes* under low shear modeled microgravity. The kind of nutrient medium either the complex media or the minimal essential media and the motility of the bacterium was also found to play a role in varying the growth pattern of bacteria under microgravity conditions (Wilson et al., 2002; Benoit and Klaus, 2007). *S. pyogenes* cannot be cultured on minimal essential medium so the influence of medium composition on growth of the bacteria under low shear modeled microgravity was not studied in detail.

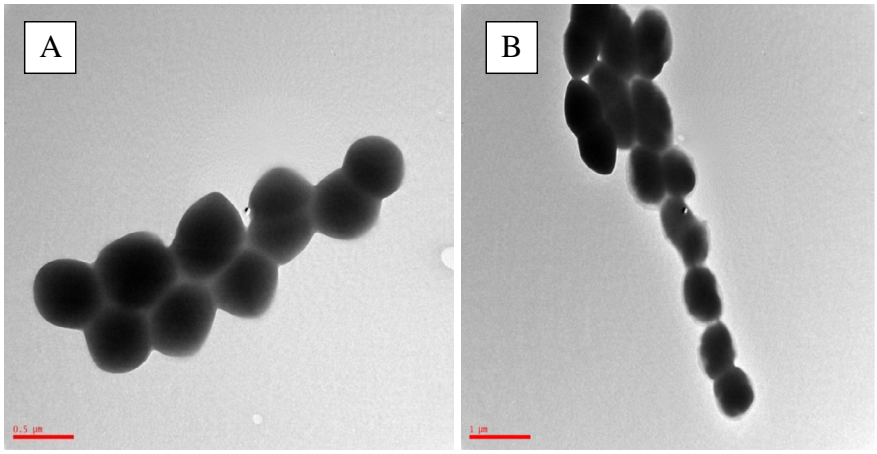

Figure 2 Morphology of *S. pyogenes* under transmission electron microscope grown under (A) normal gravity and (B) low shear modeled microgravity. (Magnification: ×62,600).

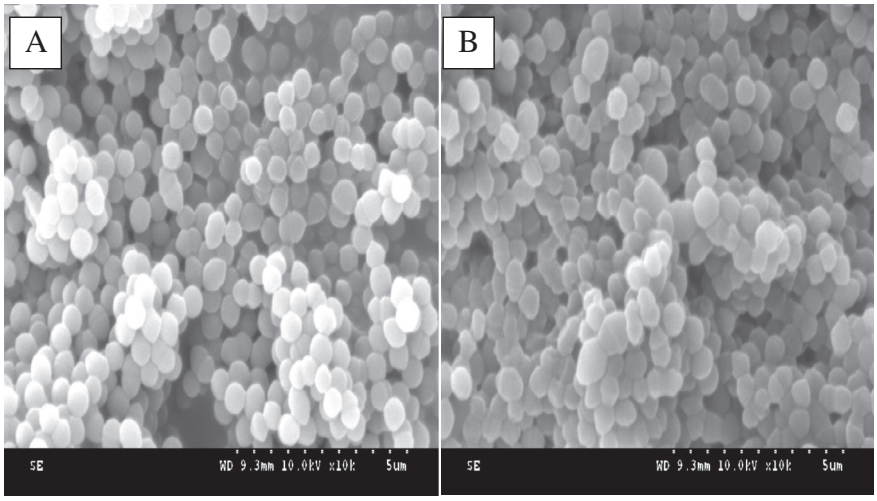

Figure 3 Scanning electron microscopic images of *S. pyogenes* grown under (A) normal gravity and (B) low shear modeled microgravity. (Magnification: ×18,000).

Table 1 Effect of low shear modeled microgravity on antibiotic resistance of *S. pyogenes* to various antibiotics (NG: normal gravity, MMG: low shear modeled microgravity).

Antibiotic	Concentration of the antibiotic (μg/disc)							
	50		100		150		200	
	NG	LSMMG	NG	LSMMG	NG	LSMMG	NG	LSMMG
Streptomycin	16.0 ± 1.0	18.0 ± 1	19.3 ± 1.1	18.6 ± 1.1	19.6 ± 1.1	19.6 ± 1.1	21.3 ± 1.5	20.3 ± 1.5
Penicillin	23.6 ± 2.3	24.3 ± 2.0	25.6 ± 1.5	26 ± 1.7	26.6 ± 1.5	27.3 ± 2.3	28.3 ± 0.5	29.3 ± 2.5
Kanamycin	18.0 ± 0.0	19 ± 1.0	19.6 ± 2.0	19.6 ± 0.5	20.6 ± 2.0	21 ± 1.0	22.6 ± 3.0	22.3 ± 1.5
Rifampicin	25.6 ± 1.5	28.6 ± 0.5	27.6 ± 1.1	31.6 ± 2.5	28.3 ± 2.8	33.3 ± 1.1	30.3 ± 2.0	34.3 ± 2.0
	25		50		75		100	
Ampicillin	22.6 ± 1.1	28.3 ± 1.5	25 ± 1.0	31.3 ± 1.5	29.3 ± 0.5	33.6 ± 1.5	30.6 ± 1.1	35.3 ± 0.5

Zone of inhibition in mm and values represent mean ± SD of triplicates.

4.2. Structural analysis of S. pyogenes under normal and low shear modeled microgravity

From the images obtained from TEM and SEM analysis, alteration in the morphology of *S. pyogenes* was not noticed. This could be attributed by the presence of thick peptidoglycan layer in *S. pyogenes*, which protects from low shear modeled microgravity. No variation in the size and shape was observed with *Salmonella typhimurium* during the space flight, but the aggregation of the cells and clumping of bacteria associated

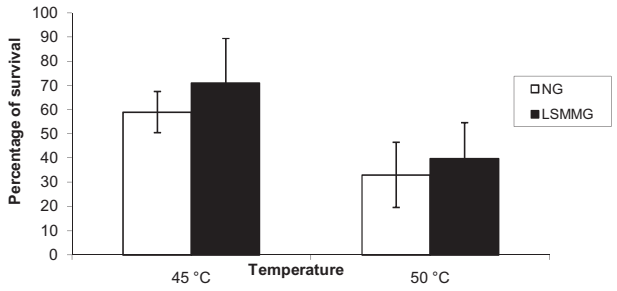

Figure 4 Resistance of *S. pyogenes* to 45 °C, 50 °C thermal stress, after culturing under normal gravity (NG) and low shear modeled microgravity (LSMMG).

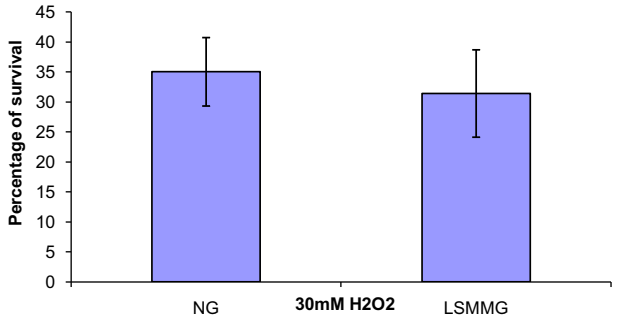

Figure 5 Effect of hydrogen peroxide on the survival rate of *S. pyogenes* grown under normal gravity (NG) and low shear modeled microgravity (LSSMG).

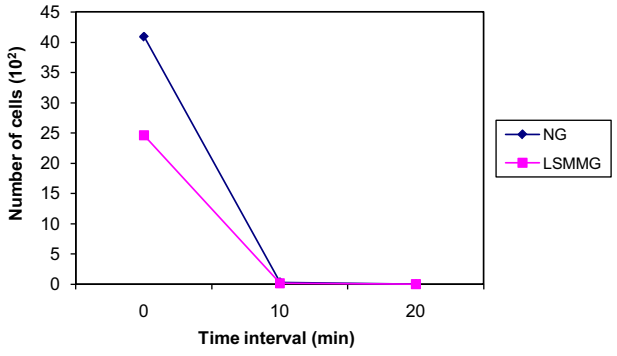

Figure 6 Susceptibility of *S. pyogenes* to acid stress (pH 3.5) induced on low shear modeled microgravity (LSMMG) and normal gravity (NG) produced cultures.

with the extracellular matrix formation was observed in SEM studies (Wilson et al., 2007). In the present study no aggregation of *S. pyogenes* cells was observed under low shear modeled microgravity compared with normal cultured *S. pyogenes*. In case of *S. aureus* also distinguishable differences in the morphology of bacterial cells cultured under low shear modeled microgravity were not found (Rosado et al., 2006) but *S. aureus* strain which was isolated and cultured in the complex nutritional media from the auto microflora of astronauts revealed thickened cell wall in comparison with the earth grown controls (Tixader et al., 1985; Lapchine et al., 1985).

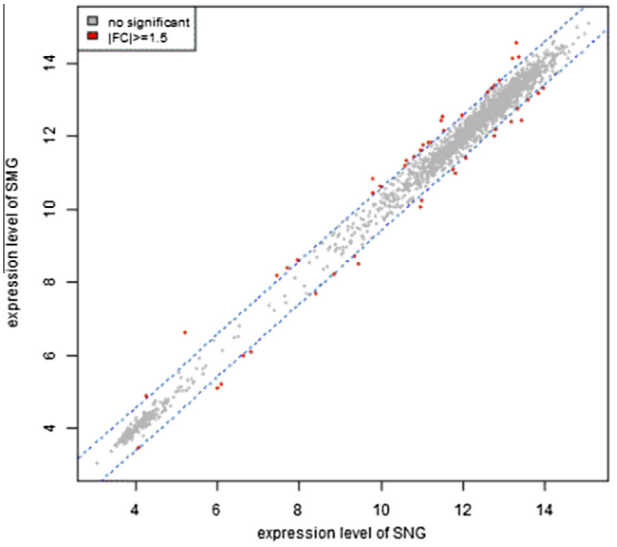

Figure 7 Plot of gene expression level between low shear modeled microgravity (SMG) vs Normal gravity (NG) grown *S. pyogenes*.

4.3. Antibiotic sensitivity assay

The low shear modeled microgravity grown cultures were found to be more resistant to ampicillin and rifampicin compared with the normal gravity grown cultures. The other antibiotics streptomycin, kanamycin and penicillin produced a similar zone of inhibition of growth pattern with both the normal and low shear modeled microgravity cultures (Table 1). The antibiotics ampicillin, penicillin belong to β-lactam antibiotics, and streptomycin, hygromycin and kanamycin belong to aminoglycoside class of antibiotics. The action of β-lactam antibiotics against bacterial cells is by inhibiting the bacterial cell wall biosynthesis and action of aminoglycosides is either by one of the modes by inhibiting protein synthesis, inhibition of ribosomal translocation or disruption of bacterial cell membranes. The expression of gene coding for enzyme β-lactamase responsible for resistance to antibiotic penicillin, was found to be unaffected under low shear modeled microgravity. Microarray analysis data indicated the expression of β-lactamase enzyme coding gene was underexpressed only by 1.2 times. A similar result was obtained with *S. aureus* cultured under microgravity for the β-lactam antibiotic flucloxacillin, macrolide antibiotic erythromycin and glycopeptide vancomycin with no difference in antibiotic resistance (Rosado et al., 2010).

4.4. Thermal stress resistance

The low shear modeled microgravity grown cultures showed a 1.2 fold higher resistance to thermal stress than the normal gravity grown cultures to a heat shock of 45 °C for 30 min (Fig. 4). A 0.8 fold of higher resistance to 50 °C for 30 min was observed in *S. pyogenes* cultured under low shear modeled microgravity. The *P. aeruginosa* grown under the modeled gravity showed 2.8 fold higher resistance compared with normal gravity grown cultures to heat shock of 50 °C for 15 min and significant difference in resistance was not observed when

Table 2 Down regulated genes of *S. pyogenes* under low shear modeled microgravity compared with the normal gravity.

Gene	Fold change	Function
M6_Spy0007	1.52	Heat shock protein 15
M6_Spy0079	1.57	Phosphoribosylaminoimidazole carboxylase catalytic subunit
M6_Spy0101	1.52	50S ribosomal protein L29
M6_Spy0266	1.60	Hypothetical protein
M6_Spy0375	1.72	Hypothetical protein
M6_Spy0669	1.50	Hypothetical protein
M6_Spy0873	1.83	Hypothetical protein
M6_Spy0974	1.85	Signal peptidase I
M6_Spy1122	1.56	ComE operon protein 3
M6_Spy1556	1.62	Major tail protein
M6_Spy1696	1.58	Hypothetical protein
M6_Spy1699	1.56	Hypothetical cytosolic protein
M6_Spy1775	1.96	Histidine ammonia-lyase
M6_Spy1779	1.78	30S ribosomal protein S2
M6_Spy1780	1.65	Elongation factor Ts
M6_Spy1826	1.50	ArgR
M6_Spy1829	1.50	Hypothetical membrane spanning protein
M6_Spy1830	1.50	Hypothetical membrane spanning protein
M6_Spy1834	1.86	50S ribosomal protein L32
M6_Spy1835	1.67	50S ribosomal protein L33
M6_Spy1842	1.90	Hypothetical protein
M6_Spy1846	1.58	Transcriptional regulator, PadR family

Table 3 Upregulated genes of *S. pyogenes* under low shear modeled microgravity with respect to normal gravity.

Gene name	Fold change	Function
M6_Spy0025	1.56	Unknown phage protein
M6_Spy0068	1.60	Unknown phage protein
M6_Spy0419	1.58	Hypothetical protein
M6_Spy0457	1.53	ABC transporter permease protein
M6_Spy0724	1.67	Cystine transport system permease protein
M6_Spy0871	1.53	Sortase
M6_Spy0872	1.92	Lactoylglutathione lyase
M6_Spy0900	1.52	Hypothetical protein
M6_Spy0903	1.52	Phage transcriptional repressor
M6_Spy0935	1.58	30S ribosomal protein S20
M6_Spy0965	2.41	Integral membrane protein
M6_Spy0984	1.56	Sla
M6_Spy0994	1.57	Phage endopeptidase
M6_Spy1007	1.53	ATP-dependent Clp protease proteolytic subunit
M6_Spy1106	1.56	Transcriptional regulator, Cro/CI family
M6_Spy1117	1.50	Hypothetical protein
M6_Spy1194	1.52	Unknown phage protein
M6_Spy1203	1.66	Hyaluronoglucosaminidase
M6_Spy1240	2.66	Unknown phage protein
M6_Spy1345	2.07	Unknown phage protein
M6_Spy1399	2.01	Hypothetical cytosolic protein
M6_Spy1438	1.71	Hypothetical protein
M6_Spy1467	1.85	Hypothetical protein
M6_Spy1541	1.57	Streptodornase
M6_Spy1561	1.65	Terminase large subunit
M6_Spy1706	1.53	Hypothetical protein

exposed to 60 min (Crabbe et al., 2010). But in *S. pneumoniae*, a decrease in the resistance of modeled microgravity grown cultures to the thermal stress was observed and the bacteria also thrived at 55 °C for duration of 30 min (Allen et al., 2006). In case of *S. pyogenes*, they were able to resist 45 °C and 50 °C for 30 min, but cannot resist the 55 °C thermal stress and higher resistance was observed with low shear modeled microgravity than the normal gravity grown cultures. Similarly wild type *Salmonella* and *rpoS* mutant were able to resist the higher thermal stress of 55 °C and the modeled microgravity grown cultures can withstand the thermal stress more than 1 × g grown cultures (Wilson et al., 2002).

4.5. Oxidative stress resistance

A 0.8 fold decrease in oxidative stress was noticed with low shear modeled microgravity compared to normal gravity cultured *S. pyogenes*. Similar results were observed with *Salmonella*, the sensitivity of the bacterium to oxidative stress increased when they are cultured under modeled microgravity. The wild strain Salmonella X^{3339} showed more resistance to hyperoxidative stress than the *rpoS* mutant X^{4973} and both wild strain and mutant strain had decreased resistance to oxidative stress under modeled microgravity (Wilson et al., 2002). The oxidative stress resistance of *P. aeruginosa* was found to increase by 4.6 fold when the treatment time is for 15 min and 6.6 fold when the treatment was prolonged for 60 min. The alginate production was found to increase during the growth of *P. aeruginosa* under the modeled microgravity and the alginate was thought to scavenge oxygen radicals. Increased alginate production acts as a long-lasting barrier and protects the cells from hydrogen peroxide. The induced transcription of *katA* gene encoding for catalase enzyme could have converted the H_2O_2 to H_2O and O_2 (Crabbe et al., 2010).

The results from microarray analysis revealed down regulation of Hsp-33 like chaperonin coding gene by 1.06 fold, which becomes active during oxidative stress. Also methionine sulfoxide reductase B and bifunctional methionine sulfoxide reductase B genes playing a protection role against oxidative stress and removal of damaged proteins formed during oxidative stress were found to be expressed at altered levels of fold change of only 1.03 and 1.17, respectively. The lack of difference in response to oxidative stress by *S. pyogenes* when cultured under low shear modeled microgravity could be because of change in the production of oxidative stress protection proteins under low shear modeled microgravity. In *P. aeruginosa* the *algU* expression was upregulated under low shear modeled microgravity, which involves in the resistance of oxidative stress, so the bacteria could withstand oxidative stress higher than the normally grown bacteria (Crabbe et al., 2010).

4.6. Acid stress analysis

The survival of normal gravity and low shear modeled microgravity cultures from triplicates was calculated to be 0.6% and 1.11%, respectively after 10 min of exposure to acid stress. The *S. pyogenes* under low shear modeled microgravity has gained resistance to acid stress compared to bacteria grown under normal gravity, although it could not resist the acid stress more than ten minutes. A fold change of 1.92 was observed in the gene expression of lactoylglutathione lyase which is responsible for increased acid stress resistance of *S. pyogenes* cultured under low shear modeled microgravity. Lactoylglutathione lyase is the enzyme involved in catalyses of formation of S-D-lactoylglutathione from methylglyoxal. Methyl glyoxal is formed by glycolysis pathway which is toxic to cells and controls the growth of cells. When *Streptococcus mutans* was subjected to acid stress lactoyl glutathione lyase (LSL) was found to be upregulated (Korithoski et al., 2007). *S. pyogenes* was found to contain to a major and minor σ factors, which plays a similar role of σ^E present in *E. coli*. This homolog in *S. pyogenes* is expected to involve in transcription of heat induced proteins when the organism is exposed to higher temperatures in the host (Ferretti et al., 2001).

Acid stress study carried for *S. enterica*, indicated the ability of bacterium to withstand the acid stress up to 60 min of exposure to acid stress and higher acid resistance was noted with microgravity grown cultures (Nickerson et al., 2000). *S. pneumoniae* cultured under modeled gravity were less resistant to acid stress when compared with $1 \times g$ cultured bacteria, and the bacteria was able to withstand the acid stress upto 30 min (Allen et al., 2006). *E. coli* MG1655 strain showed no significant differences in stress survivals between modeled microgravity and control grown cells (Tucker et al., 2007). The *S. pyogenes* is a commonly occurring bacterium on the mouth and pharynx, rarely on the skin and gastrointestinal tract, so the bacteria is generally not adopted to acidic pH of stomach or intestines, which makes it sensitive to acidic pH of 3.5 and this sensitivity could make *S. pyogenes* not able to thrive more than 10 min of acid stress compared to *S. enterica* and *S. pneumoniae*.

4.7. Changes in gene expression under low shear modeled microgravity

Earlier studies indicated that bacteria responds to microgravity, gain resistance to multiple stresses and changes their expression of genes involved in a variety of functions. *P. aeruginosa* under low shear modeled microgravity over expressed alginate production genes and genes coding for stress related proteins. Down regulation of genes was noticed with *S. pneumoniae* and differential expression of genes involved in lipopolysaccharide biosynthesis, iron utilizing enzymes, transcriptional regulation and virulence factors was observed with *S. enterica*. Upregulation of genes exposed to starvation, multiple stresses, lipid biosynthesis, genes involved in formation of biofilm and curli was found in *E. coli* under modeled microgravity conditions (Wilson et al., 2002; Vukanti et al., 2008; Allen et al., 2006).

The present study with *S. pyogenes*, Gram positive respiratory pathogen revealed both up and down regulation of different genes. Among differentially expressed genes high number of genes was upregulated. Previous study by Tucker et al. (2007) suggested that the response of different organisms to low shear and space environment differs. Microarray analysis carried out with *S. typhimurium* cultured abroad in space shuttle mission STS-115 and ground grown cultures indicated differential expression of 167 genes, of which 69 are upregulated and 98 are down regulated (Wilson et al., 2007). Gene expression studies of *E. coli* under modeled reduced gravity identified a change in expression of 430 genes, of those 221 were upregulated and 209 were downregulated (Vukanti et al., 2008). The transcriptional analysis of Gram positive bacterium *S. pneumoniae* showed a different expression pattern of consistent down regulation of genes in response to low shear modeled microgravity (Allen et al., 2006). The change in number of genes expressed differentially was found to be higher with Gram negative bacteria, but with the case of Gram positive bacteria relatively few genes were differentially expressed. Three isolates of *S. aureus* RF1, RF6, RF11 were cultured under low shear modeled microgravity and significant change in gene expression of only 25, 12 and 3 genes were noticed respectively by microarray analysis (Rosado et al., 2010).

Down regulation of heat shock protein 15 coding gene of about 1.5 times was observed when *S. pyogenes* was cultured under low shear modeled microgravity. Hsp15 is associated with ribosome binding and found to be produced more during the increase in temperature. Hsp15 is involved in the recycling of free 50 S subunits (Korber et al., 2000). ComE operon protein 3 expressing gene involved in the uptake of DNA was found to be underexpressed in low shear modeled microgravity, thereby the competency of the *S. pyogenes* was found to be affected under low shear modeled microgravity. The gene *argR* was observed to be downregulated, the gene code for enzymes involved in arginine biosynthesis. Another important gene coding for the signal peptidase I enzyme was found to be underexpressed. This enzyme is important in removing the signal peptides from nascent proteins to produce mature proteins. The underexpression of gene about 1.85 folds suggests that low shear modeled microgravity affects the post translation process in *S. pyogenes*. Five genes coding for hypothetical proteins were also found to be downregulated whose function is unknown.

The gene coding for enzyme sortase was found to be overexpressed by 1.53 fold under low shear modeled microgravity. The role of this enzyme is to assemble pilins into pili and attach the pili to the cell wall and it is also involved in the attachment of proteins to cell surface, which is important for virulence and pathogenicity of bacteria. But the genes coding for streptolysin O protein were not found to be highly upregulated, upregulation by only 1.17 fold was noticed with *S. pyogenes* cultured under low shear modeled microgravity. Streptolysin S biosynthetic proteins (SagB, SagC, SagD, SagF), streptolysin putative self-immunity protein (SagE), streptolysin S export ATP-binding (SagG) and streptolysin S export transmembrane protein (SagH, SagI) coding genes were not found to be affected by low shear modeled microgravity. Similarly the gene coding for surface antigen, enterotoxin, and virulence factor (Mvi) were found to be under expressed with a fold change of 1.20, 1.02 and 1.09, respectively. But 1.17 fold change of upregulation was noticed with streptolysin O protein coding gene. Peptidase T, enzyme which is found to be involved in biofilm formation had no change in their gene expression under low shear modeled microgravity. The microarray analysis revealed that many of the genes responsible for virulence of *S. pyogenes* had no effect under low shear modeled microgravity. Gene coding for streptodornase, group of DNases which prevents the bacteria from the neutrophil extracellular traps was found to be overexpressed by 1.57 times when *S. pyogenes* were grown under low shear modeled microgravity. The secreted neutrophil extracellular traps are found to be composed of DNA and histones, these DNases produced by *Streptococcus* digests the DNA and thereby protect the bacteria from the neutrophils (Buchanan et al., 2006). The expression of gene *hla* encoding for virulence factor α-toxin was found to be under expressed about fivefold in *S. aureus* under low shear modeled microgravity (Rosado et al., 2010). Interestingly the genes coding of virulence factors, virulence proteins and biofilm formation were under expressed but genes coding for pili formation and DNases to protect the bacteria from host response were over expressed in the current study.

Though low shear modeled microgravity cannot mimic the exact conditions of space such as radiations, results from the present study provides an understanding of response of *S. pyogenes* to low shear modeled microgravity. Study concludes that the growth of bacteria was reduced under low shear modeled microgravity, bacteria gains resistance to thermal stress and acid stress. The study also suggests that bacteria develops defence to host immune system by the expression of streptodornase, whereas virulence proteins were down regulated indicating alteration of virulence of *S. pyogenes* under low shear modeled microgravity.

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