

# *Saccharomyces cerevisiae* as a Model for Space Biology

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**Abstract** Manned spaceflight continues to be in the agenda of most of the countries involved in space research. Development of human settlements in planets and sustainable space ecosystems where crops can be grown and waste recycled are the exciting aims of some of the future space missions. There is considerable concern on the health of the space travelers during long term travel and stay in these unexplored terrains. Astronauts may be exposed to ionizing radiations and weightlessness due to alterations in gravitational force. Studies on astronauts during and after space travel indicate effects on the immune system, cardiovascular system, bone density etc. It is not clearly known how the space missions may influence DNA replication, transcription, and translation and cell division cycle in humans. Information on these will be vital. Experiments on humans and animals could be cumbersome in space. As such use of eukaryotic models like *Saccharomyces cerevisiae* could be rewarding. The yeast *S. cerevisiae* is considered as an excellent model for studying eukaryotic biology and has contributed significantly to our understanding of cancer biology and fundamental metabolic processes in humans. In this review, the potential of *S. cerevisiae* as a model for space biology has been discussed.

**Keywords** *Saccharomyces cerevisiae* · Space biology · Model · Yeast · Gravitation · Space medicine

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# 1 Introduction

Life forms have evolved on Earth under the influence of gravitational force. Life forms possess the ability to perceive and respond to gravity (Volkman and Baluska 2006). Gravity is a major player in the evolution of life from water to land and contributes to natural selection on the basis of suitable body sizes. To grow under normal gravity condition on Earth, living organisms need to grow defying gravity, uninterrupted fluid transport and structural support for rigidity and locomotion (Benoit and Klaus 2007). Gravity may contribute to changes in cellular behavior and cell structure (Bizzarri et al. 2015). During the 1960s, Russian scientists conducted experiments to reveal the changes under zero gravity conditions on human cells but failed to reveal any effect (Montgomery et al. 1978; Tairbekov et al. 1983). Later, several experiments carried out in space environments revealed that, biological functions are indeed affected when microgravity field is applied on it (Van Loon 2007; Hammond and Hammond 2001).

It is not easy to study the actual influence of gravitational force on physiology, metabolism and other life processes. Since it is hard to create gravitation free environment on Earth, such experiments need to be conducted on a space craft during space flight, in space stations or under simulated microgravity conditions. The advent of ground based simulation facilities have generated considerable interest in space biology. These facilities have contributed to studies on the role of gravity on growth, morphology, function of cells and developmental processes in animals and plants (Clément and Slenzka 2006).

Humans under space environment experience various unusual conditions which include absence of effective gravity and exposure to cosmic radiation. These conditions can be hazardous to the space travelers during spaceflight (Clément and Slenzka 2006). Space medicine is an area of space biology addressing studies on human health and combating diseases in the space environment. Space conditions provide unique environment to study the physiological responses in organisms ranging from unicellular organisms to the most organized species to microgravity. It is known that, humans can adapt to changes during spaceflight. Prolonged exposure to space environment can lead to nausea, disorientation, shift in body fluids, disruption of sleep pattern and reduced immune power. However, most of the changes may disappear on return to Earth. The changes may occur immediately, gradually, or may take several years to repair it or even some changes may remain permanent. It is difficult to produce real microgravity conditions and natural spectrum of space radiation effectively on the ground. Space environment is considered to be an ideal environment to study the effect of these conditions on living organisms (Clément and Slenzka 2006).

## 2 Successful Journey of Humans to Space

Humans are not the first living creatures who were sent to space. On 20 September 1951, the former Soviet Union launched a sounding rocket. A monkey and eleven mice were transported with this rocket. This became the first successful space travel for living beings. Yuri Gagarin, Soviet cosmonaut was the first human who travelled from Earth to orbit. He travelled in Vostok 1 to an orbit about 24,800 miles from Earth. On July 20, 1969, Sir Neil Armstrong became the first person to walk on moon through the Apollo 11 mission (Klaus et al. 2004).

In an orbiting space vehicle, microgravity conditions are generated and are typically in the range of  $10^{-6}$ – $10^{-4}$  g. This condition leads to abnormal physiological changes in humans (Clément 2011). For example, calcium level in the bone is reduced that can lead to 1–2% loss in bone density per month and the loss in muscle fiber results in up to 40% reduction in muscle function. In addition, other conditions such as space radiation, sensory deprivation and absence of circadian rhythms and the artificial environment can adversely affect human beings. These conditions may disappear on coming back to Earth but conditions such as bone calcium may take a long time to recover or may not be even recovered (Dayanandan 2011).

A long-term spaceflight may cause high health risks such as bone demineralization, skeletal muscle atrophy, and immune system suppression. Spaceflight condition which serves as closed environment causes another risk associated with pathogenesis because there are chances of the development of pathogens in a closed environment, where air, food, waste, and water are recycled. Pathogens can be transferred among astronauts which may lead to pathogenesis (Pierson et al. 1995, 1996).

A significant number of episodes of microbial infections, including conjunctivitis and acute respiratory and dental infections are reported among astronauts (Ball and Evans 2001). In-flight cross-contamination with opportunistic pathogens such as *Staphylococcus aureus* has been reported (Decelle and Taylor 1976; Pierson 2001). The threat of developing infectious diseases during space flight may become a serious concern in the future as the duration and frequency of space missions increase (Gueguinou et al. 2009; Horneck et al. 2010).

## 3 Gravity and Microgravity

During 1665–1666, Sir Isaac Newton proposed the law of gravity and motion which gave insights into understanding planetary revolution and space. Gravity is a well known but not a well understood physical phenomenon. It has been constant throughout the evolutionary history of Earth (Morey-Holton 2003). Any object having a mass on the surface of the Earth accelerates towards the Earth's centre approximately at  $9.8 \text{ m/s}^2$  (Dayanandan 2011). This gravity acceleration on Earth is considered as 1 g. Even though gravitational force is the weakest force among the four fundamental forces including nuclear strong force, electromagnetic force and

nuclear weak force, it has important roles in the evolution of life on Earth (Morey-Holton 2003).

The term microgravity is also referred to as ‘weightlessness’ and ‘zero gravity’. However, in microgravity condition, gravitational force is not exactly zero but less compared to gravity on the surface of Earth and is usually in the range of  $10^{-4}$ – $10^{-6}$  g. Real microgravity conditions can be achieved for a very short time by generating free fall conditions close to Earth’s surface with sounding rockets and airplanes in parabolic flight and drop facilities (Clément and Slenzka 2006). Real microgravity conditions can be achieved in drop towers or drop shaft (for 2–10 s), balloons (3–60 s), parabolic flights of aircrafts (20–25 s) or sounding rockets (up to 15 min). Since 1998, the International space station (ISS) has been established in space which offers laboratory conditions for systemic studies in microgravity (Herranz et al. 2013).

## 4 Tools Used in Space Biology

It is difficult to create spaceflight conditions on Earth and such studies are technically difficult and expensive. To achieve such conditions, different ground based facilities has been designed to simulate ‘weightlessness’ in laboratories on Earth. The environment created on Earth within ground based simulated microgravity condition is often considered as ‘simulated microgravity’ (Klaus 2001). Simulated microgravity conditions can be created on Earth by using different kind of simulators and are collectively referred as ground based microgravity simulators. Ground based facilities for simulation of microgravity includes simulators such as 2-D clinostat, 3-D clinostat, magnetic levitation and random positioning machines (Herranz et al. 2013).

### 4.1 Clinostat

Clinostat is used to create weightlessness on Earth even though 1 g force is exhibited. It cannot completely nullify the force of gravity but its influence can be changed by slowly by constantly rotating objects in a horizontal axis i.e. perpendicular to Earth’s gravitational field (Dayanandan 2011). Different models of clinostat have been established on the basis of speed, direction of rotation and number of rotation axes. 1D or 2D clinostat usually refers to clinostat with one rotation axis which rotates in horizontal axis (Klaus 2001).

Clinostat is used since 1965 to investigate effects of simulated microgravity. In 1958, Muller presented a human clinostat in which human test subject was rotated in a horizontal axis within a cylinder. On the basis of this human clinostat and many theories, Briegleb developed a rotating clinostat to investigate the influence of simulated microgravity on the behavior of microorganisms such as *Chlorella*

*pyrenidosa* and blue green algae, *Phormium autumnale*. However, there was no change observed in the metabolism of *Chlorella* or *Phormium* compared to 1 g control. Briegleb extended his research to germ differentiation of the beetle *Tribolium castaneum* and revealed that the embryonic development and the survival time of hatched larvae were not changed compared to 1 g control (Cogli 2007). Clinostats have been used to study the influence of gravity for a long period of time in the field of plant physiology (Yamada et al. 1993).

## 4.2 Random Positioning Machine

Two dimensional or three dimensional clinostat offer two rotation axes which can be operated at different speed with different directions. It is also referred as 'Random Positioning Machine' (RPM). 3 D clinostat was first developed in Japan and Netherlands (Van Loon 2007).

## 4.3 Rotating Wall Vessel (RWV)

Rotating wall vessel (RWV) is a bioreactor specially designed for the culture to create simulated microgravity condition. It was developed by the National Aeronautics and Space Administration (NASA) USA. High aspect ratio vessel (HARV) is one of the versions of rotating wall vessel (RWV). HARV have been used in gravitational biology research and typically used for the suspension culture and mammalian tissue growth (Hammond and Hammond 2001, Hammond et al. 2000; Klaus 2001; Sikavitsas et al. 2002). HARV is a rotating device which revolves around horizontal axis. Cells suspended in this vessel do not settle or are not constantly agitated but revolve around horizontal axis allowing exchange of gases, nutrients and wastes within culture medium in the vessel. This creates 'low gravity environment' by randomizing the unidirectional pull of gravity and reducing shear forces (Hammond and Hammond 2001; Klaus 2001; Unsworth and Lelkes 1998).

Low Earth orbits (LEO) is the most preferred and suitable area to carry out biology experiments which is at altitudes less than 1% of the distance from the Earth to the Moon. Space vehicles such as Sputnik I (215 km), ISS (350 km), Mir (390 km) and space shuttles (300–400 km) belongs to LEO era. At these distances, the force of gravity still remains high as 90% as on Earth but space vehicles moving in circular LEO experience actual microgravity environments (Dayanandan 2011; Hammond et al. 2000).

## 5 Studies Done on Models

Human blood, kidney, liver, tonsil cells and colon cancer cells are cultivated under microgravity conditions in bioreactors on ISS. These cell types and tissue were analyzed and compared to those cells and tissues in ground. It was found that cells function normally when cultivated in microgravity condition. Movement of motile flagellates such as *Chlamydomonas* and *Euglena* and ciliates such as *Paramecium* and *Loxodes* showed gravitactic response. Experiments done in the International Microgravity Laboratory on Columbia and parabolic rocket flight have revealed that gravitaxis in these organisms require a threshold of 0.16 g (Hader et al. 2003).

Various experiments were done during the past twenty years on the cultivation of plants in microgravity successfully. Plants were grown for more than one generation in microgravity condition. *Arabidopsis thaliana* was successfully grown in the 'Astroculture greenhouse' to obtain seeds of the third space generation (Souza et al. 2009; Wolverton and Kiss 2009). *Brassica repa* seeds germinated and produced normal plants and viable seeds in microgravity. Successful growth of cereal plants in space is reported (Levinskikh et al. 2000).

In plants, roots respond to gravitropic responses to get water and nutrients from soil. Land living organisms experience mechanical load on them due to gravity that is thousand times greater than the load that is experienced by living organisms in water. The production of anti gravitational substances such as lignin, cellulose and pectin support the increased load on plants while animals strengthen their bones with hydroxyapatite a mineral form of calcium associated with collagen in response to mechanical load exerted on them (Volkman and Baluska 2006).

In the animal kingdom, gravitropic responses are studied in all major groups. Various organs in animal the kingdom have evolved sensory motors to recognize the gravity factor and to orient and move the organism, musculoskeletal system to support body mass and provide structural and postural stability to land animals and vestibular system for efficient swimming in fish (Highstein et al. 2004).

The nematode, *Caenorhabditis elegans* was found to reproduce and develop through several generations in microgravity without resulting in major structural differences. Studies have been carried out on *C. elegans* on ISS for eleven days. RNA interference treatment was used to regulate gene expression. Treated tissues functioned normally in microgravity, thus providing scope for treatment and control of muscle degradation (Etheridge et al. 2011).

Fish, birds, amphibians and small mammals are used model organisms for developmental studies in microgravity. Normally, upon sperm penetration, fertilized in frog egg rotates which may be essential for normal development. Upon fertilization, eggs divide and develop embryo that emerges from egg as a tadpole. When artificially inseminated female frogs were sent into space, it was found that eggs fail to rotate but normal tadpoles emerged. On coming back to Earth within 2–3 days, the tadpoles metamorphosed and matured into normal frogs. Although development appeared normal during Spaceflight, some morphological changes were observed in the embryo and the tadpoles such as thicker blastocoel roof of

embryo. Flight tadpoles did not inflate their lungs until they returned to Earth (Souza et al. 1995; Morey-Holton 2003).

Microbes respond less to gravity than larger animals so should have less difficulties in adapting to different gravity than humans (Morey-Holton 2003). Studies are done on the bacterium *Escherichia coli* in culture. During space flight, *E. coli* shortens the lag phase and increases the time period of exponential phase and approximately doubles the final population density compared to ground control. Microorganisms such as *Salmonella enterica*, and *Bacillus subtilis* also changes their cell growth characteristics (Klaus et al. 1997; Horneck et al. 2010). It is reported that, microgravity has a key role in microbial physiology, regulation of gene expression and pathogenesis (Hammond et al. 2000; Collister et al. 2002). Bacteria are able to proliferate more readily in space, supporting that space environment is more suitable to initiate growth that could lead to contamination, colonization and infection. Under these conditions, microbes may become opportunistic pathogens and cause infectious diseases (Gueguinou et al. 2009).

## 6 The Yeast, *Saccharomyces cerevisiae* as a Model

The yeast, *S. cerevisiae* is a single celled eukaryotic organism. It is commonly known as baker's yeast (Sicard and Legras 2011). In the 1860s, Louis Pasteur discovered the involvement of *S. cerevisiae* in fermentation (Pasteur et al. 1860). Since then *S. cerevisiae* has been widely used as a model organism in biological sciences. *S. cerevisiae* has extensively contributed to our understanding of fundamental biological processes (Hartwell et al. 1970). Yeast has a ~12 Mb sized genome which comprises 6,607 open reading frames on 16 chromosomes (Forsburg 2007). The term yeast refers to any unicellular fungus. There are hundreds of different kinds of yeasts which may differ in their taxonomic status. For convenience in this review we will be using the term yeast to refer to the *S. cerevisiae*.

Yeast exhibits high level of conservation between its cellular processes and those of mammalian cells. Additionally, yeast is advantageous because of its simple growth requirements, rapid cell division, and ease of genetic manipulation and availability of experimental tools for genome-wide analysis (Botstein et al. 1997; Simon and Bedalov 2004). Yeast mutants have helped in the study of the biochemical function of gene products and the reasons for the failure of gene to function. Yeast strains were mutated in many studies by researchers as needed. Information on mutations in yeast strains concerning to diverse biological assays provides understanding of the biological functioning of genes (Ghaemmhami et al. 2003; Huh et al. 2003). The *Saccharomyces* Genome Database (SGD) provides extensive information about systemic study of every *Saccharomyces* gene. Nearly, 5800 genes of *S. cerevisiae* are known for their biological function. About ~17% of yeast genes are orthologous with the genes associated with human disease (Heinicke et al. 2007). Among these orthologous genes, majority of genes are functional in yeast. These homologous genes can

complement respective yeast deletion mutants (Dolinski and Botstein 2005, 2007). Yeast studies cannot give complete insights to all the biological processes in humans because of the complexities of human tissues, but it shows high level of homology with a number of human genes. About 70% of all essential yeast genes show homology with human genes (Botstein and Fink 2011). *S. cerevisiae* is the first eukaryotic organism whose complete genome database was studied and is available at SGD. Additionally, yeast whole genome wide knockdown collection is available. It carries a collection of strains of single gene deletion. These libraries have been widely used to study survival of *S. cerevisiae* at different environmental stress conditions and to reveal the genes required for survival (Giaever and Nislow 2014). Yeasts and their heterologous expression contribute to analyse the function of human proteins related with a specific disease state. Use of yeasts as models for study of human genes may be beneficial because of two reasons. First, a study on human defective proteins with the aid of yeast experiments reveal the diverged enzyme function that may not be obvious from assay in humans or from assessment of protein sequences. Second, studies of human gene function in yeast may be useful for the treatment of disease caused due to deficiencies (Botstein and Fink 2011). Physical and biochemical parameters of yeast can be controlled by using defined medium. Databases, plasmid and genomic libraries give information available on metabolic pathways of yeast (Giaever et al. 2002).

Studies on yeast have contributed significantly to cell cycle control, damage responses, chromosome segregation to protein secretion in humans and other eukaryotes (Forsburg 2007).

*S. cerevisiae* can be ideal model for Eukaryotes for Space Biology because:

- (1) Although spaceflight can interfere with its physiological response in space, it grows well in space. Spaceflight studies done during last decade on yeast revealed changes related to morphology, survival rate, genomic and metabolic pathways. Additionally, ground based facilities helped researchers to reveal the changes specific to microgravity and spaceflight. However, comparative studies are essential to support these findings.
- (2) It is a well studied eukaryotic model used for human diseases and human genes. Studies related to yeast spaceflight may be beneficial for newly emerged fields like space medicine that can contribute to astronaut's health in space.
- (3) Genes and the regulatory mechanism in yeast are well conserved throughout evolution.

## **6.1 *Spaceflight Induced Effects on S. cerevisiae***

### **6.1.1 Effect of Spaceflight on Chronological Lifespan of Yeast**

Yi et al. (2011) studied the viability of wild type *S. cerevisiae* during spaceflight. It was grown on onboard 'Practice 8' a recoverable satellite in orbit for 15 days. The



satellite was launched by China at Jiu-Quan Satellite Launch Center in September 2006 and recovered after 15 days from the central Szechwan province. Viability of yeast cells grown during spaceflight was enhanced to three folds compared to ground control. Viability of yeast cells were determined by counting the number of colony-forming units per ml (CFU/ml) on YPD agar plates. Spaceflight grown cells were cultured in water and YPD liquid media and viability was assessed. Spaceflight grown culture showed higher viability compared to control cells grown on ground. Spaceflight grown culture showed 17.6% of viability in water and 78.8% of viability in YPD whereas ground control culture exhibited only 5.7% in water and 25.7% in YPD.

Spaceflight environment affects the cell cycle of wild type *S. cerevisiae* grown during spaceflight. Post-mitotic *S. cerevisiae* cells grown during spaceflight exhibited increased G2/M cell population and decreased sub-G1 cell population. The postmitotic cells from saturated culture were 11% in sub-G1 cell population at day 0. After fifteen days, control yeast culture were 62% (in water) and 57.9% (in YPD) sub-G1 cell population indicating that apoptosis occurred in control cells. Yeast cells grown during spaceflight showed 32.5% sub-G1 cell population in water and 1.6% sub-G1 cell population in YPD. Spaceflight environment decreased apoptosis and increased viability in yeast cells (Yi et al. 2011).

Lipid peroxidation level decreased in the postmitotic *S. cerevisiae* grown during spaceflight supporting that decreased ROS level favors survival of yeast in space. Glycogen content was reduced in *S. cerevisiae* grown during spaceflight. The activity of enzymes involved in carbohydrate metabolism such as hexokinase (HK), malate dehydrogenase (MDH) and succinate dehydrogenase (SDH) in *S. cerevisiae* grown during spaceflight is reported (Yi et al. 2011). Yeast cells grown in spaceflight were having significantly reduced level of hexokinase and succinate dehydrogenase activities than control cells (Yi et al. 2011).

The activity of malate dehydrogenase (MDH) was enhanced by 1.24-folds in water and 1.34-folds in YPD as compared to control cells, respectively. Hexokinase act as a catalyst in the reaction of the ATP dependent phosphorylation which converts glucose to glucose-6-phosphate. This reaction is known to be the first major reaction of glucose utilization such as glycolysis and glucogenesis. MDH is an enzyme involved in the tricarboxylic Acid (Krebs) cycle that yields oxaloacetate from malate and vice versa. It is involved in gluconeogenesis for the synthesis of glucose from smaller molecules. SDH is involved in the conversion of succinate into fumarate in the Krebs cycle (Lemire and Oyedotun 2002). Metabolism of carbohydrates was restored during spaceflight that led to reduced glycogen accumulation (Yi et al. 2011).

Depending upon these findings, it is hypothesized that, spaceflight or micro-gravity environment may be responsible for enhanced chronological life span of yeast by maintaining carbohydrate metabolism, ROS (reactive oxygen species) level and cell cycle progression. Nevertheless, this hypothesis is limited to only single spaceflight studies so further investigation is called for confirming this hypothesis such as yeast chronological studies in ground based simulated micro-gravity devices (Yi et al. 2011).

### 6.1.2 Budding Pattern of *S. cerevisiae* on Solid Medium

Generally, budding pattern are widely typed into three: random, unipolar and axial. The influence of microgravity on the budding pattern of the *S. cerevisiae* was investigated by Van Mulders et al. (2011). In this study,  $\Sigma$ 1278b strain and the industrial brewer's CMBSESA1 strain has been used and experiment was conducted in real microgravity condition on ISS. For  $\Sigma$ 1278b, a haploid laboratory strain when grown on 2% YPD agar, most of cells appeared in an axial budding pattern in normal gravity whereas in microgravity condition the percentage of random budding pattern was enhanced by 5.2%. In case of an industrial brewer's CMBSESA1 strain, the percentage of random budding pattern increased by 6.0% (Van Mulders et al. 2011). However, the rate of random budding pattern is higher than that observed in liquid medium and which is about 12% in real microgravity condition (Walther et al. 1996) and in LSMMG conditions in diploid strain (Purevdorj-Gage et al. 2006). This difference may be because of different growth systems and ploidy conditions of strains i.e. growth on semisolid condition rather than of liquid condition and haploid strain (Van Mulders et al. 2011). Walther et al. (1996) has opined that, microgravity influences the cytoskeleton which may lead to change in bud scar position.

### 6.1.3 Reduced Relative Colony Growth Rate and Invasive Growth

Growth of *S. cerevisiae*  $\Sigma$ 1278b and CMBSESA1 strain in real microgravity and normal gravity was studied on a semi-solid medium. These strains were grown on a semi-solid medium for twelve days in microgravity condition at the International Space Station (ISS) and normal microgravity condition on ground laboratory at Baikonur, Kazakhstan. *S. cerevisiae*  $\Sigma$ 1278b is a haploid laboratory yeast strain. It has the ability to grow invasively on semisolid medium. *S. cerevisiae* CMBSESA1 is an industrial brewer's yeast. As this strain originates from brewery environment, it has adapted to survive in stress conditions such as high osmotic and ethanol concentration but does not show invasive growth. *S. cerevisiae*  $\Sigma$ 1278b which is grown in microgravity condition at the ISS showed reduced colony surface and relative colony growth. Oppositely, *S. cerevisiae*  $\Sigma$ 1278b grown under normal condition exhibited larger colony surface and increased relative colony growth. However, these observed effect was only limited for  $\Sigma$ 1278b and not for non-invasive industrial brewer's CMBSESA1 strain.

In response to specific environmental condition such as nitrogen starvation, *S. cerevisiae* switches from a single cell morphology to elongated cell which forms short filaments. Haploid cells invade into the rich semi-solid medium after glucose depletion. Diploid *S. cerevisiae* cells responds to nitrogen starvation and undergo dimorphic switching to pseudohyphal growth. The pseudohyphal growth allows cells to forage for nutrients. Pseudohyphae grow away from the colony and invade agar medium. This process is referred as invasive growth (Cullen and Sprague 2000; Prusty et al. 2004; Gimeno et al. 1992). Van Mulders et al. (2011) found that,

Σ1278b, a Flo11p-dependent strain, showed reduced invasive growth in the middle of the colony on 2% agar under microgravity condition.

This strain dependant variation revealed that microgravity induced strain specific change. Strain specific change in colony spreading under microgravity may be due to change in colony growth morphology (Van Mulders et al. 2011). Similarly, *E. coli* exhibited thicker biofilm formation under low shear microgravity condition than normal microgravity conditions (Lynch et al. 2006). The Flo11 adhesin is involved in maintaining colony morphology, mat patterns and colony size in *S. cerevisiae* (Reynolds et al. 2008). FLO gene expression in Σ1278b *S. cerevisiae* was down regulated under microgravity condition. This down regulation may cause the smaller colony size. In *S. cerevisiae* CMBESA1 strain, expression of FLO11 were down regulated but polyploidy condition in *S. cerevisiae* CMBESA1 led to lower expression of FLO11. This remains unaffected to under microgravity conditions. A second hypothesis states that, yeast cells experience microgravity as a stress condition and alter its cellular metabolism and growth resulting in smaller colony size on semisolid medium (Van Mulders et al. 2011). *S. cerevisiae* Σ1278b is known for hyper activation of cAMP/PKA pathway. This leads *S. cerevisiae* Σ1278b for strong agar invasion but defective in stress-responsive gene induction (Stanhill et al. 1999). Microgravity is a stress condition but *S. cerevisiae* Σ1278b is not adapted for stress condition. So, it may be the reason for reduced growth in Σ1278b strain (Van Mulders et al. 2011).

#### 6.1.4 Effect of Spaceflight Environment on the Cell Wall of *S. cerevisiae*

Fungal cell wall is composed of interconnected β-glucan, mannoprotein, and chitin. It creates protection layer against osmotic pressure and helps to maintain their different shape as per cell cycle progress (Inoue et al. 1995). The yeast cell wall comprises about 20% of the cell's weight. Mannan and β-glucan comprises 40–45 and 35–45% of the yeast cell wall, respectively (Aguilar and Francois 2003). β-Glucan is an abundantly found polysaccharide in the cell wall. It is a homopolymer of glucose bound by 1, 3-β- or 1, 6-β-D-glucosidic linkage. Two types of glucose linkages are found in *S. cerevisiae*. β-glucan is a long chain of approximately 1,500 β-1,3-D-glucose units which comprises about 85% of the total cell wall β-D-glucan and short chain of approximately 150 β-1,6-D-glucose units comprise about 15% of the cell wall β-D-glucan (Klis et al. 2002). β-glucans produced from yeast are having wide application in pharmaceutical, food, and feed industries (Thompson et al. 1987; Hofer and Pospisil 1997; Liu et al. 2007a) because of its immunostimulating, anticarcinogenic, hypocholesterolemic, and hypolipidemic properties (Gordon and Siamon 2003; Jamas et al. 1996; Peter et al. 2004).

Effect of spaceflight environment on polysaccharides of *S. cerevisiae* was studied by Liu et al. (2008). This study was done by using four *S. cerevisiae* strains, namely, FL01, FL03, 2.0016, and 2.1424. These were subjected to spaceflight by loading into a recoverable satellite. The satellite was launched at Jiu-Quan Satellite

Launch Center on 9 September 2006. After fifteen days in orbit, the satellite was successfully recovered from the central Szechwan province. After spaceflight, *S. cerevisiae* 2.0016 showed drastic increase in the biomass and cell wall mass from  $591 \pm 12.7$  to  $867 \pm 7.1$  mg/100 mL and  $116 \pm 6.69$  to  $204 \pm 4.90$  mg/100 mL, respectively. However, there was no significant change observed in biomass and cell wall mass between the spaceflight and ground samples of *S. cerevisiae* FL01, FL03, and 2.1424. All spaceflight *S. cerevisiae* strains did not exhibit significant difference in cell wall thickness. However, cell wall thickness of strain *S. cerevisiae* FL01, 2.0016, and 2.1424 increased whereas *S. cerevisiae* FL03 showed decreased cell wall thickness after spaceflight. Highest increment in cell wall thickness was observed *S. cerevisiae* 2.0016 i.e. 63% increase than control.

$\beta$ -Glucan content was enhanced in cell wall of *S. cerevisiae* strain 2.0016 and this increase was three times more in spaceflight sample. Such increment was not seen in *S. cerevisiae* FL01, FL03, and 2.1424 strains. Mannan content was elevated in the cell wall of spaceflight *S. cerevisiae* FL01 and 2.0016 whereas spaceflight *S. cerevisiae* FL03 and 2.1424 strains showed decrease in glucan content. However, these changes were not statically significant (Liu et al. 2008).

$\beta$ -Glucan content was evaluated on the basis of activity of enzymes involved in synthesis and degradation of  $\beta$ -glucan (Kim and Yun 2006).  $\beta$ -Glucanases, are enzymes involved in the cleavage of the  $\beta$ -O-glycosidic linkages of  $\beta$ -glucan chains which gives glucose and oligosaccharides. It is also involved in other cellular processes such as cell budding, wall growth, conjugation, ascus formation, and other morphogenetic events (Fleet 1991; Martín-Cuadrado et al. 2008). Production of glucanases was reduced in yeasts during spaceflight due to the low-shear and microgravity conditions. Reduced glucanases could lead to change in cell wall thickness and  $\beta$ -glucan content (Liu et al. 2008).

### 6.1.5 Increased Phosphate Uptake in Yeast During Spaceflight

Wild type *S. cerevisiae* was launched into space during the Trans Earth extra vehicular Activity of Appollo 16. Appollo Microbiology Ecology Evaluation Device (MEED) was used to maintain the culture during spaceflight. Additionally, ground control was maintained at room temperature and flight control with no light exposure. Viability and survival rate of spaceflight sample did not change compared to control. Phosphate uptake rate was enhanced for spaceflight sample than ground control sample. Berry and Volz (1979) speculated that, phospholipid content of cell membrane may be altered which leads to change in ion transport including uptake of phosphate by cell membrane.

### 6.1.6 Stress Response of *S. cerevisiae* to Microgravity Condition in Spaceflight

Proteomic analysis of *S. cerevisiae*  $\Sigma$ 1278b grown at ground and space (in ISS) was carried out. The proteome map of the microgravity- grown yeast samples showed less visible proteins in the high-mass range in comparison to the proteome map of normal gravity-grown colonies. This could be because of increased protein degradation in microgravity (Van Mulders et al. 2011).

Key glycolytic enzymes, glyceraldehyde-3-phosphate dehydrogenase, pyruvate kinase, and a subunit from pyruvate decarboxylase are upregulated and tricarboxylic acid cycle and some oxidative phosphorylation enzymes are less abundantly found in microgravity conditions. Reduced production of gluconeogenic enzymes such as fructose 1, 6 biphosphate aldolase, and of acetyl-CoA hydrolase in yeast samples was also found in microgravity condition. These enzymes are known to be involved in oxidative metabolism during propagation (Kobi et al. 2004). There is also reduced abundance in several heat shock proteins involved in protein folding or protein translocation into mitochondria (Van Mulders et al. 2011). Some of the components of ribosome biogenesis were found to be down regulated in microgravity condition. Ribosome biogenesis is controlled by Sfp1, which was known for unique microgravity response (Coleman et al. 2008). Additionally, ribosomal genes show reduced expression in modeled microgravity condition (Sheehan et al. 2007). There is also down regulation observed in several components of the proteasome. Outcomes of this study found that protein abundance changes such as enzyme involved in glycosylation of cell wall proteins and microgravity is responsible to change the integrity of the cell wall. Proteins that deal with glycerol stress were over expressed which supports the induction of the HOG pathway. The induction of High Osmolarity Glycerol (HOG pathway) and CWI (Cell Wall Integrity) signaling pathway in response to microgravity underlies that microgravity is one of the stress conditions experienced by yeast cells which resembles with the osmotic stress. This stressful condition is investing cellular energy more into the protective measure such as cell wall biosynthesis (CWI pathway activation), and the production of compounds (such as glycerol and trehalose) to increase the osmotolerancy of the yeast (HOG pathway activation) (Nickerson et al. 2004; Sheehan et al. 2007; Johanson et al. 2002, 2007; Willaert 2013).

### 6.1.7 Oxidative Stress Response in Spaceflight

High energy free radicals are originated from various radiations in space environment. These high energy free radicals may be responsible for increased oxidative damage. In general, cells respond to oxidative damage by exhibiting different oxidative stress responses. Oxidative stress response includes detoxifying enzymes such as catalase, superoxidase dismutase and peroxidase and thiol systems i.e. glutathione (GSH) and thioredoxin (Jones 2008). Glutathione is involved in many of the critical cellular processes such as differentiation, proliferation and apoptosis.

GSH homeostasis is associated with a number of human diseases such as cancer, cystic fibrosis, neurodegenerative disorders and aging related diseases (Ballatori et al. 2009).

*S. cerevisiae* releases endogenous GSH in culture medium upon exposure to oxidative stress in simulated microgravity condition (Bradamante et al. 2010a, b; Versari et al. 2005). The oxidative stress response of *S. cerevisiae* LS267 under real microgravity is investigated. The SCORE (*Saccharomyces cerevisiae* Oxidative Stress Response Evaluation) experiment was conducted during a 24 day FOTON-M3 space mission of Italy. Gravitational forces on space station or on a space craft down to  $10^{-4}$ – $10^{-6}$  g which creates microgravity condition. SCORE experiment was aimed to investigate oxidative stress response specifically any changes in GSH homeostasis under real microgravity. Findings recorded in SCORE experiment were compared with ground experiment carried out in simulated microgravity condition (Bradamante et al. 2010a, b). To create simulated microgravity condition on ground, Rotating Wall Vessel bioreactor (RWV) was used. In this bioreactor, a rotating high aspect ratio vessel (HARV) revolves at 28 rpm around a horizontal vessel but permits gas diffusion across a semi permeable membrane. In RWV, an average gravitational force is found reduced to  $10^{-2}$  g (Rucci et al. 2007; Unsworth and Lelkes 1998; Meaney et al. 1998).

SCORE experiment showed that, 24 h of hyperoxia enhances extracellular release of GSH (40%) in spaceflight samples. This result supports the hypothesis that microgravity is having a role in induction of glutathione production. Further, yeast cells showed stress response by activating high-osmolarity glycerol (HOG) MAP kinase and cell integrity/protein kinase C (PKC) pathways (Bradamante et al. 2010a, b). High-osmolarity glycerol regulates cell morphology by swelling or shrinking and responds to oxidative stress. Protein kinase C senses variation in cell morphology and regulates genes involved in cell wall biogenesis and the maintenance of the actin cytoskeleton in response to this variation (Hohmann 2002). In SCORE experiment, Hog1 and slt2 varied in their activity on space station which may be a combined effect of microgravity and oxidative stress. Hog1 was highly activated under hyperoxic conditions whereas Slt2 was activated in both hyperoxic and normoxic conditions on a space station (Bradamante et al. 2010a, b).

Bradamante et al. in 2010 carried out ground experiments to investigate the oxidative stress effect on *S. cerevisiae* under hyperoxic and normoxic conditions simulated microgravity condition. Ground experiment showed that the rate of extracellular release of GSH was increased (10%) with protein carbonylation in both hyperoxic and normoxic simulated microgravity condition. Increased extracellular release may be due to alteration of cytoskeleton induced by microgravity. To check this possibility, researchers treated cells with an inhibitor of actin polymerization i.e. Dihydrocytochalasin B (DHCB) and (R)-(+)-trans-4-(1-aminoethyl)-N-(4-pyridyl) cyclohexanecarboxamide dihydrochloride monohydrate (Y-27632), a potent and selective rho associated kinase inhibitor. DHCB and Y-27632 treated cells also exhibited high rate of extracellular release of glutathione (12%) with protein carbonylation under hyperoxic conditions and Hog1 and Slt2 activation (Bradamante et al. 2010a, b).

Gene expression of *S. cerevisiae* under oxidative and simulated microgravity condition was studied. A gamma glutamylcysteine synthetase (GSH1) and glutathione synthetase (GSH2) catalyses main step in glutathione synthesis. GTT1, encodes GSH transferase 1; BPT1 and YCF1, encodes two vacuolar GSH S-conjugate transporters of the ATP binding cassette family. Heat shock protein 26 (HSP26) and CTT1 (cytosolic catalase T) are two genes involved in stress response pathway. HSP26 and CTT1 transcripts are highly upregulated between 4 and 9 h in samples grown under simulated microgravity condition. This remains noticeable after 11–12 h. Same effect was observed in DHCB treated cells. These findings support that cells may have activated MAP kinases pathway. Additionally, GSH1 and GSH2 transcripts were activated in simulated microgravity and DHCB cells after 4 h and were same until extracellular release stops. However, genes involved in vacuolar transport were not affected. GTT1, BPT1 and YCF1 mRNAs were present from initial point supporting that these genes remain same with high upregulated Bpt1 in the early stationary phase. It reveals that, simulated microgravity condition under hyperoxic conditions influence expression of genes involved in GSH biosynthesis and stress response but does not affect genes involved in vacuolar transport (Bradamante et al. 2010a, b).

### 6.1.8 Metabolic and Genomic Pathways Affected During Spaceflight

Nislow et al. (2015) identified genomic and metabolic pathways required during spaceflight by yeast. Strain fitness test was performed for two yeast deletion collection i.e. ~4800 homozygous strain and ~5900 heterozygous strains grown during spaceflight. These strains contain unique DNA barcode as strain identifiers. For spaceflight experiment, yeast samples were delivered on space shuttle mission via opticell processing module (OPM). OPM is a specially designed instrument used for spaceflight experiment in space. It can maintain yeast deletion pool for about 20 generation in microgravity. Simultaneously, same experiment was carried out as ground control at normal gravity condition in the orbital environmental simulator at Kennedy space centre (USA). Additionally, osmotic stress effect was also tested on survival of yeast in space by addition of 0.5 M NaCl (Nislow et al. 2015).

Growth rate of yeast grown in the opticell during spaceflight was quantified. Population doubling time of sample grown for ~21 generations in microgravity was ~100 min whereas population doubling time for ground based control was recorded as ~90 min. Morphological characters such as budding pattern, overall shape and size was not found different than ground control when observed by light microscopy. Differences were observed in budding pattern and polarity on scanning electron microscopy. However, these changes were not consistent (Nislow et al. 2015).

Gene ontology enrichment analysis is carried out for homozygous and heterozygous deletion strains. Some strains showed reduced abundance from pool in spaceflight. These strains showed significant requirement of genes involved in RNA metabolism and DNA integrity pathway. Different biological processes linked



to RNA metabolism and DNA integrity pathway were required for survival of yeast in spaceflight. These processes include ribosome biogenesis, regulation of ribosomal protein, transcription, cytoplasmic RNA translation, rRNA processing, tRNA modification and mRNA decay from RNA metabolism, DNA repair, recombination, replication, chromatin modeling, mitochondrial maintenance and proper protein localization to the mitochondria (Nislow et al. 2015). Such kind of effects was also found when DNA damage was induced. Additionally, such enrichment was found with therapeutics that acts as nucleotide analogs such as 5-fluorouridine and flurocytosine. Enrichment of genes needed for processing of DNA and RNA has been increased on combining spaceflight condition with hyperosmotic stress exerted by addition of 0.5 M NaCl in medium. Nislow et al. (2015) speculated that, higher osmotic stress enhances the DNA damage effect of space flight. Gene ontology profile showed strong concordance between pathway responded by yeast in spaceflight and effect of 5-fluorouridine, a FDA approved anticancer drug on yeast. Similarly, marked concordance was found with carmoflur, 5-fluorocytosine and 8 methoxysoralen. Strong concordance was also observed with diallyl disulfide which is involved in detoxification of cells with increase in glutathione—S-transferase (GST).

Survival of haploid deletion strains during spaceflight was studied. Yeast strains stored in distilled water were delivered on space through space shuttle Atlan mission STS 112 in October 2001. Yeast deletion strain without PEX 19 showed 133 fold survival advantages in space. PEX 19 is a chaperon and import receptor for class I peroxisomal membrane protein. Consequently, yeast deletion strain lacking components of aerobic respiration, isocitrate metabolism mitochondrial electron transport exhibited 77–40 fold advantage for survival in space. Strains deleted with hydrolases, oxidoreductase and transferase exhibited significant advantage for survival during spaceflight in rich medium (Johanson et al. 2007) (Table 1).

## **6.2 Low Shear Modeled Microgravity (LSMMG) Effect on *S. cerevisiae*: Studies Done by Using Simulated Microgravity Device (Rotating Wall Vessel)**

### **6.2.1 Growth Rate, Viability and Growth Kinetics**

Purevdorj-Gage et al. (2006) studied low shear microgravity induced effect on *S. cerevisiae* BY4743 in HARV (high-aspect-ratio vessel). Growth rate, overall metabolic activity and rate of viability did not differ significantly between *S. cerevisiae* grown under LSMMG (low shear modeled microgravity) condition in HARV (high-aspect-ratio vessel) and control. Samples from both conditions showed similar doubling time. However, growth kinetics was noticed different for LSMMG sample and control. In LSMMG sample, lag phase was shorter with slightly higher yield than control (Purevdorj-Gage et al. 2006). McPherson (1997)



**Table 1** Spaceflight induced effects on *S. cerevisiae*

Sr. no.	Spaceflight induced effect on <i>S. cerevisiae</i>	Strain used	Period	References
1.	Higher viability of yeast cells	Wild type	15 days	Yi et al. (2011)
2.	Increased G <sub>2</sub> /M population, Decreased sub G <sub>1</sub> population	Wild type	15 days	Yi et al. (2011)
3.	Decreased glycogen content	Wild type	15 days	Yi et al. (2011)
4.	Increased malate dehydrogenase level	Wild type	15 days	Yi et al. (2011)
5.	Decreased succinate dehydrogenase and hexokinase level	Wild type	15 days	Yi et al. (2011)
6.	Random budding pattern	Σ1278b	12 days	Van Mulders et al. (2011)
7.	Reduced colony growth rate	Σ1278b	12 days	Van Mulders et al. (2011)
8.	Reduced invasive growth	Σ1278b	12 days	Van Mulders et al. (2011)
9.	Increased in biomass and cell wall mass	2.0016	15 days	Liu et al. (2008)
10.	Increased β-glucan and mannan content in cell wall	2.0016	15 days	Liu et al. (2008)
11.	Increased phosphate uptake	Wild type	7 Min., 7 s	Berry and Volz (1979)
12.	Induction of CWI and HOG pathway	Σ1278b	12 days	Van Mulders et al. (2011)
13.	Increased glutathioine content	LS267	24 days	Bradamante et al. (2010)

hypothesized that, low fluid shear environment of weightlessness may lead to unusual mass transfer of cellular byproducts in microenvironment surrounding cell and trigger rapid growth. It resulted in shorter lag phase (McPherson 1997; Mennigmann 1986). However, metabolic activity remained same in LSMMG condition and control during the experiment.

### 6.2.2 Random Budding Pattern in Liquid Medium

Under LSMMG condition *S. cerevisiae* BY4743 showed random budding pattern during early stationary phase and late stationary phase. Purevdorj-Gage et al. (2006) states that the disturbances in budding pattern in LSMMG condition may not be due to change in the cytoskeleton organization but rather due to changed expression of genes having role in budding processes and polarity establishment. They found that there is a significant difference in the expression level of genes involved in polarity establishment (*BUD5*) and bipolar budding phenotype (*RAX1*, *RAX2*, and *BUD25*). *BUD25* is involved in budding process, and its deletion resulted in random budding

phenotype whereas *BUD5* gene deletion or over expression lead to random budding phenotype (Ni 2001). *BUD25* is over expressed in LSMMG compared to the control. Reduced expression in both *RAX1* and *RAX2* in LSMMG sample resulted in aberrant budding phenotype (Purevdorj-Gage et al. 2006). *BUD5* is involved in maintaining cell polarity during budding process (Kang et al. 2001). Overexpression of *BUD5* in LSMMG sample lead into random budding pattern (Purevdorj-Gage et al. 2006). There was no significant difference observed on cell size of *S. cerevisiae* BY4743 between control and LSMMG sample in HARV when examined at logarithmic, late-logarithmic, early-stationary and late-stationary growth phases (Purevdorj-Gage et al. 2006).

### 6.2.3 Cell Clumping

Five percentage of the LSMMG cell population was clumped (of five or more than five yeast cells) in the late-logarithmic growth phase and ten times more clumped during early-stationary growth than control (Purevdorj-Gage et al. 2006). Clumped phenotypes in yeast are found due to the improper functioning of chitinases and glucanases that result into the defective mother-daughter cell separation process (Baladron et al. 2002; Doolin et al. 2001; Ufano et al. 2004). Chitinase activity was normal in LSMMG sample. *EGT2*, *DSE1*, and *DSE2* involved in mother-daughter cell separation were down regulated in LSMMG grown sample (Colman-Lerner et al. 2001). This process is dependent on the cell polarization process (Amon 1996; Chant 1999). Bud scarring and formation of aggregate in LSMMG may be because of cell polarity defects (Purevdorj-Gage et al. 2006).

### 6.2.4 Change in Gene Expression in Response to Microgravity

Johanson et al. (2002) provides first data on change in gene expression of *S. cerevisiae* (strain FE18984) in response LSMMG condition in RWV culture. Microarray analysis was carried out to reveal the changes in gene expression in response to LSMMG condition compared to ground control incubated in Rotating Wall Vessel (RWV) at 1 g condition at different time points (at 20, 60 and 180 min). Genes expressed differentially in response to LSMMG at different time periods were grouped on the basis of cluster analysis. Promoter analysis was performed for DNA sequences of identified genes. This analysis identified motifs that have similar core sequence with stress responsive element (STRE) and *Rap1* transcription factor (Johanson et al. 2002).

Genes identified with STRE sites were grouped into two categories. Stress responsive group of genes contains *HLJ1* and *SSA4* which are involved in stress response. Another group of genes are related to metabolic cycle and is comprised of *PGM2*, *GPM2*, and *COX5B*. Gene product of *PGM2*, phosphoglucomutase is involved in one of the steps in conversion of glucogen to glucose i.e. glucose-1-phosphate to glucose-6-phosphate. *GPM2* encodes for phosphoglycerate mutase

which catalyses last step of glycolysis. *COX5B* gives product cytochrome-*c* oxidase chain Vb, and cytochrome oxidase and are involved in ATP synthesis. In addition to this, *Rap1* binding motifs were found in 13 upregulated genes after 20 min. *Rap1* regulates expression of genes which are differentially expressed in response to change in growth rate. Consequently, increased glucose utilization was observed in LSMMG yeast sample compared to control yeast sample. Authors speculated that, enhanced glucose utilization in RWP serve as a model for increased *Rap1*-mediated transcription and indicating that *Rap1* may be involved in RWP induced pathway of yeast (Johanson et al. 2002).

Sheehan et al. (2007) carried out microarray analysis of *S. cerevisiae* diploid strain BY4743 grown in High Aspect Ratio Vessel (HARV), which provides in Low shear modeled microgravity condition (LSMMG) in comparison with HARVs under normal gravity conditions. Cells were exposed to various stress factors in the environment. These conditions may lead cells to evolve stress specific pathways to survive in these conditions. There are various stress inducible genes studied in *S. cerevisiae*. Gasch et al. (2000) studied stress responsive genes from *S. cerevisiae* and these set of ~900 genes are referred as the 'environmental stress response' (ESR) genes. Microgravity may be as of the stress conditions experienced by an organism. Authors compared genes having altered expression in HARVs with Gasch et al. (2000) datasets and categorized microgravity responsive genes of which 26% of the genes were defined as ESR genes whereas remaining 74% genes may be specific in response to microgravity. Additionally, such microgravity related stress response was also observed in *E. coli* (Sheehan et al. 2007). It is revealed that, simulated microgravity conditions lead cells to be more resistant to other stress conditions such as hyperosmosis and low pH (Collister et al. 2002). Stress responsive genes were also identified in *Salmonella enteric* in a study conducted in HARVs (Wilson et al. 2002).

Sheehan and group identified the set of genes whose expression is altered significantly at 5 and 25 generations of growth. At 5 generations, 278 genes were significantly changed. Of these, 161 genes were up regulated and 117 were down-regulated whereas at 25 generation 197 genes were significantly changed in expression. Of these, 106 were up-regulated and 91 were down regulated in their expression. 897 genes did not alter their expression between 5 and 25 generations (Sheehan et al. 2007). Some of the genes involved in budding and cell wall integrity pathway are discussed below.

Normal yeast cells do not have the tendency for random budding pattern. NSR1 gene which is having role in budding pattern was found to be diminished in its expression in LSMMG grown cells in HARVs. On deletion of BUD21 in yeast cell, cells exhibit random budding phenotype (Hahn and Thiele 2002). BUD21 was found to be down regulated in its expression in LSMMG grown yeast cells in HARVs. Additionally, 46% of bud localized transcripts were significantly altered in the same study. This include down regulation of genes EGT2, ASH1 which are involved in cell separation (Nickerson et al. 2004), daughter cell-specific transcription WSC2 (Martin et al. 2005). WSC2 encodes for heat shock sensor in MAP1 pathway (Saito and Tatebayashi 2004; Sheehan et al. 2007).

**Table 2** Differential regulation of genes in *S. cerevisiae* under simulated microgravity condition

Function	Differentially regulated genes			
	Down regulated genes	Fold change	Up regulated genes	Fold change
Bud site selection	BUD 2	1.1	BUD 5	1.6
	BUD 3	1.4	BUD 7	1.1
	BUD 4	2.0	BUD 13	1.4
	BUD 6	1.4	BUD 20	1.1
	BUD 8	1.3	BUD 25	1.7
	BUD 9	1.9	BUD 27	1.2
	BUD 16	1.0	AXL 1	1.1
	BUD 17	1.0	THP 1	1.2
	BUD 22	2.0		
	BUD 23	2.4		
	BUD 31	1.3		
	BUD 32	1.3		
	STE 20	1.0		
	RSR 1	2.0		
	RAX 1	5.1		
	RAX 2	3.2		
Axial bud site selection	–		ERV15	1.8
Cell bud growth	TOS2	1.7	LAS1	1.2
Cell wall integrity, stress response	–		SLG1	1.6
Regulation of cell size	PRS3	2.0	MUB1	1.2
Bud site selection, cell polarity	RSR1	2.0	–	–
Cell polarity	RRP14	1.4	–	–
Ribosomal protein	RSP18B	3.1	–	–
	RPL22A	4.4		
Lipid metabolism	FEN1	3.2	–	
	SUR4	2.5		
Nuclear protein	NSR1	7.5	–	
Cell wall	GAS1	2.0	–	
Hypothetical ORF	Unspecified	2.4	–	
Bipolar bud selection	–		TWF1	1.6

Reference Sheehan et al. (2007)

For the survival in stress condition such as exposure to heat, osmotic stress, and/or oxidative stress, yeast cells should retain its cell wall integrity (Saito and Tatebayashi 2004). The alterations in cell wall due to stress on cell wall is basically regulated by cell wall integrity (CWI) MAPK signaling Pathway. Sheehan et al. (2007) found that, SDP1 was up regulated at fold change of 6.71 at 5 generations and 8.12 at 25 generations. Sdp1 is a stress inducible negative regulator of MAP kinase cascade and have dual specificity as MAP kinase phosphatase. Another down regulator of MAP kinase pathway, PTP2 i.e. protein tyrosine phosphatase is

highly expressed in LSMMG grown cells than control in HARVs (Sheehan et al. 2007). Sheehan et al. (2007) suggested that, simulated microgravity affects multiple signaling pathways and cells sense low shear microgravity environment through MAP kinase pathway (Sheehan et al. 2007) (Table 2).

## 7 Conclusions and Future Perspectives

Health of the astronauts during and after space flight, long term stay aboard in spaceships and future colonies is a major concern. Data available from experiments on humans and model organisms support this concern. Experiments on human and animals aboard a space ship are difficult to perform. Data available so far indicate major changes in human physiology in the space environment with impact on the astronaut's health. The way we treat Earth bound humans may not be valid for the astronauts considering the major changes they experience in space. A thorough understanding of the human biology in the space may be required for effectively treating astronauts or for maintaining their health. Information available on the impact of microgravity and space flight on basic eukaryotic biology is far from complete. Research using the yeast model is expected to give significant insights to space biology especially space medicine.

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