



General Overview of Yeast and Yeast Cell as a Novel Model for Investigations

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Abstract

Biological scientists have been looking for a suitable experimental organism which can provide better understanding of biomolecular mechanisms involved in the physiological processes and observations can be readily interpolated. It can be easily procured, cost effective, and better maintained in laboratory conditions. Yeast is practically omnipresent in most of the biomes and exhibits higher genetic diversity in comparison to most of the angiosperms or chordates. These eukaryotes have very simple and short life cycle exhibiting budding, and sexual reproduction. Mostly, there are no ethical issues related to this organism being used as experimental model. Its small genome is the prime factor that makes easy manipulations in the field of molecular genetics, genomics, evolutionary genomics, senescence, cell cycle, biomedical genetics, and biotechnology. This experimental model opens new horizons in the direction of functional genomics that may be helpful in encoding metabolic mechanisms and ecological diversities.

Keywords: Intracellular-Transportation; Non-Ionization-Radiations; Viewing of Yeast-Cells; Wi-Fi Radiations; Yeast

Abbreviations: GPI: glycosylphosphatidylinositol; pmaER: Plasma Membrane Associated Endoplasmic Reticulum; cecER: Central Region Central-Cisternal Endoplasmic Reticulum; TOR: Target of Rapamycin; PKA: Protein Kinase-A; CDK: Cyclin Dependent Kinase; GSM: Global System for Mobile Communication; SHF: Super High Frequency.

Overview

Yeast is a bio system that has undergone tremendous investigations in the recent past. This organism is one of the most suitable models for studying gene sequence and comparative genomic investigations. The molecular investigations along with gene sequencing related to phylogeny helps to evaluate the biotechnological applications,

and metabolic pathways [1].

General Biology and Structure

Yeast as a species comes under unicellular fungi, and the cells of yeast are different from bacteria. These organisms have well defined cell organelles such as nucleus, Golgi apparatus, mitochondria, endoplasmic reticulum, vacuole and cytoskeleton, like any matured cells of eukaryotes. These cells typically measures $5 \times 10 \mu\text{m}$. Yeast reproduces via budding, sometimes fission, and sexual reproduction (preferably during unfavorable conditions). Their identification is based on cell morphology, physiology, immunological and molecular biological techniques. These are present in soil, water, plants, animals, insects which have special habitat of plant tissues [2].

Viewing A Yeast Cell

Under normal light microscope, yeast cell cannot be seen distinctly, even at 1000X magnification, mostly, vacuole and cytosolic inclusions are visible. To view yeast cell distinctly, phase contrast microscopy is useful. The stained yeast cells are better for viewing but the stained cells become unsuitable for physiological studies. Fluorescence microscopy is helpful to highlight the features of yeast cell using fluorescent dyes such as Calcofluor, DAPI, and Rhodamine etc. Calcofluor helps to visualize white bud scar and it interacts with chitin in scar and it fluoresces. The 4, 6-diamidino-2-phenylindole (DAPI), specifically, causes fluorescence in DNA of the nucleus. DAPI causes the mitochondria to fluorescence and these organelles appear pink and white. Rhodamine also helps to visualize mitochondria. Neutral red stains vacuoles appear purple red [3-5]. The application of mono-specific antibodies technique against structural proteins in combination with fluorescent dyes like fluorescein isothiocyanate (FITC) or Rhodamine-B facilitate the visualization of more cellular features of yeast cell [6]. The iodine stains glycogen and its deposits get stained as red-brown. Methylene blue stains non-viable

whole cells as blue. Aminoacridine and F-C ConA stain cell walls and indicate specific mannan present [5]. Electron microscopy, scanning electron microscopy, transmission electron microscopy, and atomic force microscopy facilitate the investigations related to ultrastructure of organelles and molecular architectural aspects [5,7,8].

Generalized Structure of Yeast

The generalized cell structure of a yeast cell is dependent on the physical and chemical fluctuations occurring during their growth. Their size varies between 2 mm to 50 mm in length while width varies between 1 to 10 mm depending on the species. These appear to be ovoid, ellipsoid, hemispherical, filamentous-as septate hyphae or sometimes cylindrical in shape. On an average the larger diameter is around 5 to 7mm and smaller diameter is about 1 to 7mm. The cell size increases during brewing process while in lab conditions the size is smaller. Each yeast cell is encased with thick cell wall (Figure 1).

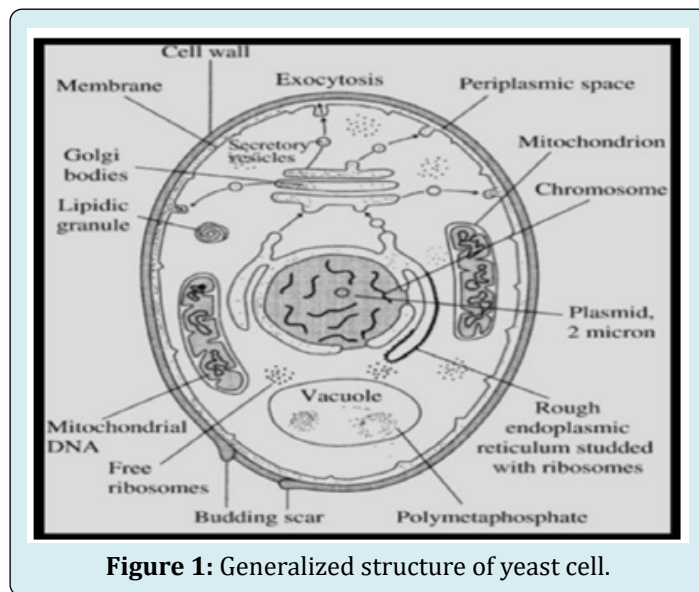


Figure 1: Generalized structure of yeast cell.

Topography of Cell Surface

Topography of cell surface reflects on the nature and the morphology of cell wall of yeast and it appears different during its growth, and during budding phase. There is a distinct birth scar and it is well marked as circular ridge like structure or raised ridge like structure while the scar appears as convex depression or plate like with or without raised annulus. In some species, the cell surface shows many protrusions in case of *Leucosporidium* yeast and in some species like *C. slooffii*, the surface is smooth [9]. The scanning electron microscopy illustrates such features in case of young

bud, yeast cell and a grown-up yeast cell.

Cell Wall of Yeast

The cell wall is structurally and functionally dynamic cell organelle, and it secures shape and size of a yeast cell. The cell wall participates in the physiological, biochemical and molecular processes in addition to growth, budding, and cell division. Cell wall also maintains the specific functional mechanical strength, osmotic pressure, and other forms of stresses; these stresses are due to ambient environment [10]. The cell wall of yeast is stiff and hard layer having 100

to 200 nm thickness. It comprises about 25% of dry mass of the cell. The basic structural components of cell wall are highly glycosylated glycoproteins—the mannoproteins, β -glucans and chitin. It consists of glucomannoprotein which associates with β -1.3 glucan and β -1.6 glucan. The molecules of glucomannoprotein link via disulphide bonds. The mannoproteins get bound with chitin β -1.4 polyN-acetylglucosamine, these in turn, get associated with plasma membrane. The enzyme invertase acts as marker and is associated with cell wall of yeast. There are three membrane bound synthetases such as Csh-1 (a repair enzyme), Csh-2 (participate in the formation of septum), and Csh-3 (Cds) helps in cell wall maturation and also in the formation of bud ring) [5]. The composition of cell wall exhibits variations in different conditions of growth and the synthesis of the required macromolecules. The space and time factors also play an effective role in maintaining its composition. The yeast cell wall can be removed if it is treated with lytic enzymes under the influence of osmotic stabilizers [9]. During this process mostly, no harm is done to its structure and functionality. The resultant cellular product is called 'spheroplast' or commonly 'naked cell'. This state of yeast is suitable to study the regeneration and development of cell wall, and also to investigate intergeneric and intrageneric cellular fusion. The glycosylphosphatidylinositol (GPI) is one of the main constituents of cell wall protein and its attachment site refers to as Pst1p, and it is produced during regeneration of protoplast. Commonly, yeast cell exhibit aggregation and due to the aggregating behavior among the cells and it is called asexual aggregation [5]. The ability to adhere reversibly to each other results in macroscopic flocculation/flocculation of yeast cells. This feature is commonly observed during brewing process. The probable explanation for the mechanism of flocculation is not well understood and it is believed that specific lectins (also named as flocculins), are present in cell wall of yeast. These surface glycoproteins directly bind with mannoproteins of the neighboring yeast cells, and result in flocculation. This feature may participate in sexual aggregation. The subunit of agglutination like A-galp is O-glycosylated protein and has N-terminal producing signals and C-terminal signal to glycosylphosphatidylinositol (GPI) protein that ensures anchorage. Agglutinin proteins help in differentiating a- and b-cells [5,9].

Periplasmic Space

The periplasmic space is an enclosure bounded by cell wall externally and plasma membrane internally. In this space, mostly, proteins like invertase and acid phosphatase are present which do not permeate via cell wall. These enzymes catabolize those substrates which do move across plasma membrane. These enzymes act as markers for the functionality of cell wall and plasma membrane. The signal

sequence of invertase and phosphatase are very helpful in the recombinant DNA technology [5].

Plasma Membrane

This membrane is basically a lipid bilayer harboring specific proteins that anchor cytoskeleton components, enzymes needed for synthesis of cell wall, signaling pathways, and cellular transportation. Its thickness is around 7.5 nm and at places it invaginates forming protrusions in cytoplasm. The lipid components of this membrane include phospholipids like phosphatidylcholine, phosphatidylethanolamine, and minor proportions of phosphatidylinositol, phosphatidylserine, and phosphatidylglycerol, along with sterols such as ergo sterol and zymosterol. The composition of plasma membrane exhibits fluctuations in its structure and functionality in accordance to growth and the ambient conditions [5]. There is a total seizure of phosphatase activity in the presence of ammonium molybdate (0.1 mol/ml) and sodium vanadate (1.0 mol/ml) at 6.0 pH. There exists vanadate sensitive ATPase activity (pH 6.0) in presence of KCl (as activator). This behavior of plasma membrane reflects on the proton flux activity [11]. Vanadate sensitive ATPase activity is a useful parameter to characterize plasma membrane in a bio system [5].

Cytoskeleton of Yeast

A cell has to perform integrated and coordinative functions that ensure its supportive, flexibility, cellular rigidity, normal spatial and life sustaining functionalities. Cytoskeleton facilitates appropriate functional performances depending on the types, location, and assigned functions of the cells. This component is also helpful in the delivering cellular responses to the intracellular, intercellular, biochemical, and molecular fluctuations within a biosystem, and provides survival 'robust conditions'. Cytoskeleton plays essential and primary role in maintaining cellular shape, size, and elaborate intracellular consortium [12,13]. Cytoskeleton participates in an effective coordinative role with plasma membrane to accomplish diverse functions like development, control of cellular morphology, polarity, adhesive ability for anchorage, and membrane stability in a cell and yeast cells are no exception. It also cooperates during localizing growth area, budding, development of mutant phenotypes, and maintenance of polarized growth in yeast cell and its buds [14]. The patches of cortical actin are present more in the zone of active cell growth, and deposition of cell wall within the bud. Cable or thick strands of actin are seen within mother and bud yeast cell specifically along the 'mother-daughter bud axes'. During the early phase of cell cycle patches of ring of actin come to the surface of mother yeast cell, thus, marking the spot for the emergence of new daughter bud. This actin ring is persistent and demarcates

the neck of baby bud [15]. This ring disappears as the baby bud grows but cortical actin patch develops and locates at the tip and the lateral sides of the developing bud. During the late phase of development the actin patches disperse randomly on the surface of mother yeast cell and matured yeast bud [14].

The cytoskeleton is very important cellular component, and deals with structural and mechanical dynamic cellular functionalities. This ability is attained due to the assembly and disassembly of the compositional protein sub-units of the components of cytoskeleton. For example, microtubules and microfilaments participate in the physiological processes like mitosis, meiosis, mobility of cell organelles, and formation of septum. The cytoskeleton is asymmetrical in organization, and these aspects become very obvious during vegetative growth of yeast cell [5]. There are about 100 accessory proteins which are conserved, and these proteins coordinate the spatiotemporal assembly and disassembly of the actin protein of cytoskeleton. This represents the mechanism of dynamics of functions of actin. Investigations based on molecular genetics, genome-analysis, real time imaging technique, and ultrastructure are very helpful in understanding the molecular dynamics and physiological functionality [15].

Intracellular Transportation in Yeast

Components of cytoskeleton play significant roles in the processes of intracellular transport, thus, maintain the appropriate distribution of molecular cargo between cytoplasm, cell organelles and non-membranous organelles [5]. Yeast cells exhibit actin cellular based transport, this cellular transport system uses the mechanism which regulates polarized cell growth and separation of its cell organelles before cell division sets in. During bud formation assembly of actin cable starts from bud, and undergoes reorientation of actin cables. This process focuses towards growth and secretion of tip of proposed bud. These cables function as polarized molecular path that resemble the delivery system made of type-V myosin and this set up is the need of formation of daughter yeast cell [5]. In yeast cell, proteins like, myosin, kinesins and dynein are the three major motor proteins which bring about the intracellular transportation. The myosins and kinesins proteins link with polarized cytoskeletal filaments. This process meets its energy requirements from ATP-hydrolysis that enable the movements. These molecules carry the molecular-cargo to the distant site in one direction within a cell. There are motor proteins that make the filaments to slide against each other thereby, creating a driving force; this driving force is similar to that which brings about the migration of nucleus and cell division [5,16,17]. There are two isoforms of tropomyosin proteins in yeast cell, namely, Tmp1p and Tmp2p. These are

present in addition to myosins. Tmp1p is more pronounced isoform tropomyosin, and it binds with actin cable and also with filaments giving them the required stability. The stabilized actin cables and filaments participate in cellular growth and carries metabolic molecular cargo including many organelles. The acylated form of these proteins exhibit better binding efficiency of actin. The Tmp2p is a minor isoform of tropomyosin and it facilitates the working of the major isoform tropomyosin [5].

Cytoplasm of Yeast Cell

Cytoplasm is the fluid component of any cell and yeast cell is no exception. This medium is the major stage for all the metabolic, biochemical, and physiological transformations. It holds the membrane bound cell organelles, and it is bounded by plasma membrane [5]. The pH is of great physiological significance. This is slightly acidic (pH 5.2) aqueous colloidal medium and lodges micro and macromolecules. Some of the prime macromolecules are proteins, glycogen along with larger macromolecular entities like ribosomes, proteasomes, lipid particles; these inclusions give the suspension appearance to the cytoplasm of yeast cell. In addition to these constituents, some cytosolic non-organellar inclusions like glycolytic enzymes, fatty acid synthase complex, along with these components and enzymes involved in protein metabolic system are also present in cytoplasm. The functional entities relates to the formation and regulation of rostrum of cytoskeleton [5]. Under stress-state, the cytoplasm of yeast cell undergoes down-regulation. The life processes like cell division, growth, and the overall metabolic states function at the lowest ebb during the stress period. But the viability of the yeast cell is retained until the return of the favourable conditions. The mechanism involved during this stress-state is still ambiguous and offers a field to venture. The non-membrane organelle regenerates as a result of reversible polymerization of translation of 2B-initiation factor. This factor is an important enzyme that brings about the synthesis of protein those results in the formation of filaments in the form of large bundles. The correlative light and electron microscopic techniques illustrate this observation. Further, energy requirements, degradation of protein, and functions of proteins get restricted [18].

Nucleus

In yeast cell, the nucleus is spherical, centrally or sometimes excentral in position with diameter around 1.5 μ m. A well-defined double membrane encloses the nuclear contents and it distinguishes nucleus from the cytoplasm. This envelop is perforated at intervals, each perforation is called nuclear membrane pore and has diameter within the range of 50 to 100 nm [5]. This set up appears to be channel like and facilitates the transportation of metabolites

between nucleoplasm and cytoplasm. The outer membrane is in continuation with that of endoplasmic reticulum. (This feature is similar to the other eukaryotes). This link is retained even during mitotic division. Regeneration of pores in nuclear membrane appears as an important process [5,19]. The spindle pole body ensures anchorage of nucleus with both continuous and discontinuous microtubules. The spindle pole body is present on the outer phase of outer nuclear membrane. The spindle pole body divides into two poles and helps in doubling the number of chromosomes; this happens prior to isolation of two daughter cells. The nucleolus appears denser in comparison to the remaining nucleoplasm within nucleus. It is the site of location of r-RNA gene, and synthesis of r-RNA. It facilitates the assembling the subunits of ribosomes and also helps in processing of pre-m-RNA [5,20].

Chromosomes in Yeast

Chromosomes are congestedly arranged within nucleoplasm as chromatin material. Mostly, the nucleosome in case of yeast is around 145 bp of DNA in length. There are variations in the numbers and sizes of chromosomes in different species of yeast. The pulse-field gel electrophoresis is a suitable technique to study their karyotypes [5,21].

Endoplasmic Reticulum

The endoplasmic reticulum is well organized and is highly branching tubular cell-organelle, constituting around 10% of the volume of a cell. A double lipid layer membrane encapsulates this cell-organelle, and the same layer communicates intimately with the nuclear membrane [5]. Cellular stability, modifications, protein transport, and other regulatory processes are under the control of endoplasmic reticulum. The transmission electron microscopic and dual-axis electron tomographic techniques reveal that endoplasmic reticulum comprises of three primary domains, namely, the plasma membrane associated endoplasmic reticulum (pmaER), the central region central-cisternal endoplasmic reticulum (cecER) and the tubular endoplasmic reticulum (tuber) [22,23]. The reticulon family members like Rtn1p and Rtn2p present in budding yeast and interact with members of family of protein like Yop10 and DP1 resulting in the stabilization of sheet and tubules present in the region of membrane that exhibit high curvature [23-25]. The three primary classes of membrane lipids are sphingolipids, phospholipids, and sterols [23,26,27]. The endoplasmic reticulum also participates in the storage of calcium, homeostasis and maintenance of concentration of about 10 μ M free Ca⁺⁺, at least in wild variety of yeast. The synthesis of calcium sensitive photoprotein i.e., aequorin-a specific targeted protein, is also produced in endoplasmic

reticulum [23,28]. The Golgi apparatus, lysosomes (vacuoles in yeast), endosomes, secretory vesicles, plasma membrane are the product of endoplasmic reticulum, including the endoplasmic reticulum itself. Thus, endoplasmic reticulum is the production site of the most of the transmembrane proteins and lipid of cell-organelles. The membrane of endoplasmic reticulum transports the proteins produced in polyribosomes from cytosol into its lumen. The folding of protein due to chaperon and partly the protein glycosylation occur within endoplasmic reticulum. It is very important that protein must get correctly folded for their effective transfer from the endoplasmic reticulum. The process of packing of proteins takes place in the endoplasmic reticulum within vesicles. These vesicles bud-off from the membrane of endoplasmic reticulum and move to the Golgi apparatus and here these vesicles fuse with its membrane [5].

Golgi Apparatus: (Golgi Complex)

The morphological aspects of Golgi apparatus exhibit variations in different species of yeast. Basically, this cellular organelle is made of membranous stacks arranged in parallel pattern or as dispersed cisternae. The proximal region is cis-Golgi network while distal or internal region is trans-Golgi network. The synchronized process of proteins gains access in Golgi apparatus through cis-face and emerges from trans-face. This synchronized processing of proteins involves formation of complex oligosaccharide chains of N-glycosylated proteins, phosphorylation of oligosaccharide which will be reaching the vacuole [5]. The processes like synthesis of proteoglycan (O-linked glycosylation of proteins), alteration of lipids, sulfation of tyrosine, etc., come in this category [5]. In spite of the existence of varied morphological patterns of Golgi apparatus, the mechanism related to the molecular transport in different patterns is basically similar. The concerned functional aspects like maturation of cisternae, to and fro protein movements, retention of secretory cargo proteins also follow similar mode. Phosphatidylinositol-4-phosphate and phosphatidyl-4-kinase both play major roles in the isolation of multiple proteins within Golgi apparatus. The hydrolysing enzyme Sac-1, phosphatidylinositol-4-kinase and phosphatidylinositol-4-phosphate together regulate the amount of phosphatidylinositol-4-phosphate under the state of glucose deficiency. This indicates that the level of carbon source possibly acts as an external signal to maintain molecular traffic in the Golgi body of yeast cell [29-32]. The Golgi vesicles carry the molecular cargo to and fro within the parts of Golgi apparatus and endoplasmic reticulum. The endoplasmic reticulum associates the degradation system, thus prevents the transportation of defective molecular cargo. The defective molecular cargo includes misfolded proteins, and any molecule that disrupts functionality of endoplasmic reticulum and Golgi apparatus [5,29].

Transport Vacuole System

In yeast cell, intracellular transport is one of the vital significant functions. The intracellular distribution of metabolites results in appropriate supply and drainage of biomolecules for successful functioning of yeast cell. The vacuole system of yeast cell carries out this responsibility efficiently. The plasma membrane and other cellular membranes participate in the formation of vacuole system. Mostly, the membrane of different organelles takes part in formation of vesicle and the vesicle gets detached as a result of budding process. The nature of vesicles formed depends on the nature of cargo it has to carry and the cellular target. The membrane of these vesicles has basic bilayer nature but gets additional coating of specific molecules depending on their specific function. Based on the special coating the vesicles are grouped as COP-I, COP-II, and clathrin coated vesicles [5]. COP-I coated vesicles have coating of multimeric protein complex along with GTP-binding protein Arf1p (an ARF). This coating is coatomers. These vesicles are responsible for retrieval (reclaiming) process and starts with cis-Golgi network extending to trans-Golgi network [5,33]. The three biomolecules are present in endoplasmic membrane, namely, small GTP-binding protein (Sar1p), the Sec1p, and the Sec23/24p complex. These help in the formation of coating of second group of transport vacuoles- the COP-II. The COP-II coated vesicles carry their cargo in forward direction, i.e., anterograde movements from endoplasmic reticulum towards Golgi apparatus [34]. The outward movement gets counter balanced involving inward movement (retrieval move); this mechanism helps in bringing back the selected molecules to their place of origin and these molecules are available for reuse from endoplasmic reticulum. The loading of cargo is not random but this process is systematically selective [35-37]. As and when there is a need for COP-II vesicles then the enzymes like small GTPase Sar1p and GEF Sec12p which are present in endoplasmic reticulum get activated and these cause in the induction of COP-II vesicle in the transportation of biomolecules [38]. The third group of the vesicle is clathrin coated vesicles. These vesicles bud off from the membranes of cell organelles and plasma membrane. Clathrin coated vesicles participate in direct molecular transport and act as late secretory pathway ensuring endocytic transportation of biomolecules. These vesicles carry cargo between trans-Golgi network and secretory vesicles return them to Golgi network procured back [5,39]. Drainage and degradation of biomolecules appear to be two primary functions. The drainage vacuoles acts as receptors of proteins from plasma membrane, internalized protein via endocytosis, proteins which need to be weeded out away from secretory process at Golgi apparatus. The drainage vacuoles also receive proteins emerging or representing cataclysmic molecular interactions

and organelles remains to be eliminated from cell [5,40]. The vacuoles in which enzymatic molecular degradation is carried are termed as 'vacuole degraders' and the process is referred to as vacuolar degradation. The varied intravacuolar hydrolases like endopeptidases, aminopeptidases, carboxypeptidases, neuleases, glycosidases, lipases, phospholipases, phosphatases etc., bring about the molecular degradation in vacuoles [5,40,41]. The specific secretory pathway ensures the delivery of the hydrolysing enzymes and the respective substrates in the concerned vacuoles [5,40]. Generally hydrolytic activities take place around 5.0 pH while the pH of cytoplasm is around 7.2, this indicates that the vacuole concerned with molecular degradation regulate the pH. These vacuoles are also concerned with some of the physiological processes such as storage for basic amino acids, polyphosphates, and some metallic cations. The membrane of such vacuoles regulates the cytosolic ionic concentrations via homeostatic regulatory process [5,40].

Peroxisomes in Yeast

The other name of these cell organelles is microbodies, and is present as an encapsulated in single double lipid layered body. These cellular components have hydrogen peroxide forming oxidase and detoxifying catalase. These metabolize the hydrogen peroxide molecules. Peroxisomes metabolize β -oxidation of fatty acids. The number and structural aspects vary depending on the conditions for growth. These are very important for establishing cellular viability [5,42,43].

Mitochondria in Yeast Cell

The mitochondria of yeast cell is similar to those found in other eukaryotes, and has been a target of investigation due to its significant role during fermentation process. Two types of lipid bilayers encapsulate it, outer membrane and inner membrane. These two layers have a space in between; the inner membrane encloses the matrix of mitochondria. The outer layer acts as a harbor which holds enzymes which metabolize lipids. The inner membrane has cytochromes concerning respiration, ATP synthase to facilitate functioning of respiratory chain, and transport proteins needed for trafficking of components with lower-molecular-weight. The matrix of mitochondria contains mitochondrial DNA and acts as a site of tricarboxylic acid cycle; molecular gadgetry for protein synthesis, and mitochondrial ribosomes. The proteins required in mitochondria are either synthesized or imported. The size and the morphologies of mitochondria depend on the conditions for growth of yeast cell. An appropriate mode of aerobic respiration is ensured as aerobic conditions and suitable amount of oxygen and carbon source is ensured [5].

Nature and Behavior of Yeast

Yeast is able to tolerate higher concentrations of sugars, salts, acetic acid, and under these conditions maintains appropriate rate of growth. Such stress tolerant traits are useful for biotechnological point and enhances the efficacy of the industrial processes and declines the possibilities of contamination due to unwarranted microbes. At the same time the tendency of yeast to resist extreme condition may result in spoilage of food in spite of presence of common preservatives and higher osmotic ambient environment [1,44,45]. Yeast is also very effective osmotolerant and is able to maintain its growth under higher osmotic pressure. Molecular mechanism is related to the concentrations of sugar and salts; these two components individually affect the overall degree of osmotolerance of yeast [46-48]. The process of permeability in yeast is dependent on the rate-limited due to the passage of water through plasmalemma and/or cell wall. These two are the first structural and functional barriers for permeability. This hydrophysical process is temperature dependent. The coefficient for hydraulic water permeability is $1 \text{ to } 2 \times 10^{-13} \text{ cm}^3/\text{dyne}/\text{sec.}$, at 20°C [49].

Yeast and its Genomics

Yeast- unicellular eukaryotic fungus is mostly omnipresent in any type of biomes and this organism exhibits relatively more genetic diversity as compared to other angiosperms and chordates. Yeast is a member of sub-phylum Saccharomycotina (sometime also called Hemiascomycota). These biosystems can be easily cultured and have small genome having around 10 to 20 Mbp. These features make them one of the most suitable experimental organisms to investigate molecular genetics, biotechnology, and evolutionary genomics. The genetic related studies on yeast open new horizon in the taxonomic, genomic diversify functional metabolism, and ecological aspects. Some of the species of yeast are commensals (*Saccharomyces*) or pathogens (*Candida albicans*) [50]. Since, on the onset of genetic engineering large scale reading and writing of DNA along with understanding, modifications and alterations in the genetic code have been the prime aspect of investigations. These investigations have also involved software version Sc-1.0 and Sc-2.0. The Baker's yeast - *Saccharomyces cerevisiae* is one of the most favoured experimental organisms [51]. Yeast is useful model for studying genetic engineering. In organisms where inefficient homology-directed repair restricts CRISPR-Cas 9 genome editing, use of non-homologous end joining helps to secure DNA double strand breaks. There are efforts to reduce the application of non-homologous end joining technique. The successful deployment of linear precision editing and random intermediation of non-homologous end joining facilitates optimization of microbial production of (S)-norcoclaurine

is possible [52]. Norcoclaurine is a compound present in plant like fruits of *Nandina domestica*, roots of *Aconitum carnehaelii*, stems of *Galium divaricatum*, *Annona suamosa*, and *Nelumbo nucifera* (seeds of lotus). Norcoclaurine is also known as 'higenamine' and is a dietary ingredient for sports and weight loss schedules but it also causes health risks [53]. Thus, yeast is a valuable experimental model for such type of investigations and can be utilized to the maximum extent during antagonizing pair of DNA double strand break pathways for microbial industries [52].

Yeast and Senescence, Life Cycle and Cell Cycle

Specifically, in medical practices, prolonged life span of an individual is highly expected at least during or post treatment. Otherwise the long life span relates with the age of cells and mechanism of senescence. The most common parameters that relate with life-span of an individual are chronological lifespan and replicative lifespan. The process of longevity is well illustrated in yeast whose genome is well characterized. Other aspect of such studies relates directly or indirectly contributes to the estimation of replicative or chronological lifespan. The genome of human and yeast are well understood and amenable the genetic manipulation and screening of aging related diseases [54,55]. Chronological lifespan involves growth depending on the nutrients, number of cells per unit volume, metabolic interactions that involve growth factors and attainment of specific level prior to reproductive phase. The replication lifespan is the duration in which a cell or an individual forms daughter cell or offspring and after this phase there is a decline in the replicative abilities [54,55]. It is possible to regulate the process of longevity in case of yeast and link with metabolic genome [56]. Among eukaryotes and mammalian cells the quantum of food intake i.e., caloric dependent food, is one of the prime parameter that influences the life span, physiological aging, and senescence associated pathogenesis but the mechanism involved is not well understood [57-59]. The mechanism involved during the utilization of food in the process of aging is not well understood. The metabolites such as alpha-ketoglutarate inhibit the synthesis of ATP-synthase and target of rapamycin (TOR) involve during increase of life span of yeast [60]. Quasi-programmed aging in budding yeast relates with cell proliferation, differentiation, stress-response, survival and death [61]. The scarcity of nutrient like carbon, phosphate and nitrogen influence the cell cycle, like arresting the G1 phase and entry of dividing yeast cell in to G0 (a quiescent phase). The target of rapamycin (TOR) and protein kinase-A (PKA) pathway of signaling impacts the protein kinase Rim-15 and this regulates the initiation of G0 phase of cell cycle [62]. The Pho80-Pho85 cyclin and dephosphorylation of cyclin dependent kinase (CDK) complex play a major role in controlling the entry of cell in the G0 phase [63]. Adversely

affected signaling pathways related to cellular proliferation, physico-biochemical status of cell, like, osmotic, oxidative or replicative stress influence the progress of cell cycle. These parameters indicate the significance of nutritional factors in maintaining a specific rate of cell proliferation [56,57]. There exists a temporal relationship between the parameters affecting cell cycle and cyclin dependent kinases and other cyclins. Their quantum of cyclin-3 proteins remain fairly constant during the progress of cell cycle. The yeast is one of the most suitable experimental models to investigate the mechanisms that regulate life span and cell cycle [55,60,61].

Genetic Engineering and Yeast

The ability of DNA to write and read is the bases of genetic engineering and its applications are reaching new technological horizons in the current scenario of genetic engineering and biotechnology. These offshoots of biological sciences involve changes in the genetic code of a biosystem using molecular tools and applications in the industries. The disciplines of synthetic biology concerned with chemical synthesis of genome and chromosome and replace their natural counterparts. The investigations related to their functions are of great significance in biological sciences and industries [52]. The genetic engineering and epigenetic investigations are primarily based on the two experimental models, i.e., *Escherichia coli* and *Saccharomyces cerevisiae*. Among these two, *Saccharomyces cerevisiae*, enjoys preference over the *E. coli*, because this eukaryote is generally regarded as safe and has very efficient homologous recombination system, its genome is completely sequenced [64,65]. The third generation sequencing technique is quite recent and has the benefit of both long sequencing reads and the massive parallel of the sequencing of second generation but this technique has low degree of accuracy [66-68].

Epigenetics and Yeast

A consolidated definition of epigenetics may be 'a phenomenon in which a trait gets inherited as phenotype without any changes in its DNA sequence'. This inheritance is the result of either mitosis or meiosis. The current attention relates with understanding the basic mechanism involving many distinct but closely knitted molecular pathways that control initiation, maintenance, and the degree of heritability of the epigenetic trait. Basically, there are three primary signaling parameters, namely, epigenator, initiators, and maintainers; these accomplish the process of epigenesis. The specific features of yeast that make it suitable for the epigenetic studies are short compact genome, well characterized genetic and reproductive cycle, short generation time, and the developed suitable technologies [69,70]. A DNA-binding protein, a noncoding RNA, or another structural unit acts as an initiator and these decide the future assembly of

chromatin structure [71]. The intracellular changes have an ability to stimulate the epigenetic phenotype. The changes are intracellular and in the ambient environmental niche, these are epigenator signals. These signals initiate epigenator signaling pathways that lead to the initiation and activation of the epigenetic process. The epigenator signals activate and pinpoint the precise location of epigenetic process, i.e., chromatin environment. This phenomenon maintains the chromatin environment during the first and the subsequent generations.

The process of transcriptional silencing has been well established in *Saccharomyces cerevisiae*. It takes place at genomic sites like, silent mating-type loci, telomeres and ribosomal DNA, and telomeres. Epigenetic silencing at these sites is because of the absence of most of the histone modifications and deficiency of histone H14 lysine 16 acetylation [72]. Silent loci in *Saccharomyces cerevisiae* (also referred as budding yeast) and *Saccharomyces pombe* (also called fission yeast) act as proxy during heterochromatin among higher eukaryotic. These species have been the model to provide enormous information in establishing the epigenetics and its applications. Firstly, the phenomenon of epigenetics helps to provide information about the structure of chromatin, this helps to modify the cellular phenotype that too without any alteration in the DNA sequence. Secondly, it also reflects on the possible inheritance due to the changed chromatin state (modified genetic memory), probably during S-phase of cell cycle [73]. Further, the simplest eukaryotic epigenome and the reductionist epigenomic version of yeast make it very easy to conduct the related studies, specifically in the *Saccharomyces cerevisiae*. The budding and fission yeast do not exhibit the process of DNA CpG methylation. The RNA interference mechanism and repressive histone H3K9 are absent in *Saccharomyces cerevisiae* but these do take place in fission yeast and most of the eukaryotes. The existence of methylation of gene body in eukaryotes and its absence in yeast (some forms) reflects on the lineage loss during evolution; thus, the role of histones modification acts as means of demarcating various epigenetic states [74].

Toxicology and Yeast

Yeast cells are suitable model for investigating toxicity because of its convenient genetic manipulation, short life cycle, and easy access to the genomic sequence and are relatively easy to culture. The parameters that act as the bases of modulating the sensitivity in relation to the toxic materials include cellular thiols, like glutathione, phytochelatin, labile sulphide and metallothioneins. The products of gene also participate in transference or partition or isolation of metallic or other toxic materials. These features and pathological conditions have been investigated and confirmed in genomic sequence of *Saccharomyces cerevisiae* genomic

sequence. Even, the molecular mechanisms that participate in regulating the ability of toxicity of metal or other toxic materials can be suitably investigated [75].

An organism encountering toxic or xenobiotic materials exhibits toxicological responses during this process; there are many variations in genetic and protein expression at molecular levels during the process of response. The traditional testing of toxicity has limitations like, slow nature, lower capacity, expensive and restriction to limited endpoints. Since the enormous number of toxic or xenobiotic materials are present in environment affecting the biota. Their toxicity data are inadequate, hence, it is imperative to look for an alternative and effective mode of such investigations. Toxicogenomics is a suitable technique that meets the major criteria. Toxicogenomics includes DNA microarrays, high throughput NMR and analysis of protein expression. *Saccharomyces cerevisiae* provides integrated observations concerning toxicity mechanisms. Such studies are based on the combined assessment of transcriptomics and quantitative proteomics and these facilitate in evaluating the probable 'genome-wide expressions' during toxicological responses. To identify and conclude meaningful conclusions the observations from metabonomics/metabolomics and chemogenomics there must be integration of these observations and suitable tools of bioinformatics [76-78].

Yeast and Nanotechnology

Nanotechnology is multifaceted, multidimensional, bio remedial engineering and has applications in almost all technologies. It encrypts the basic activities in living and non-living entities of earth and also explains the modes that provide the existence, sustenance, reuse, recycle, and replenish the matter and energy on our blue planet. The biotic and abiotic components show a synchronized and orchestrated mutual coordination. Yeast, a member of 'fungi', plays its role as a multidimensional unit as an agent in biosynthesis and delivery agent for nanoparticles, genetic materials, and pharmaceutical drugs involving principles of nanotechnology [79-82].

Nanomaterials exhibit self-assembly in layered pattern also, and electrostatic interactions hold these layers. This technique is applicable in the non-viral gene delivery process. The self-assembled DNA as nanomaterial (in nanoforms) can be readily delivered to the desired cell. The DNA-nanoparticles caged within 'hollow yeast cell wall particles', is successful mode to transfer the genetic material. The core component consists of t-RNA or polyethylenimine having layer of DNA layer, the final layer is of t-RNA or polyethylenimine. This form of transferring genetic material using yeast cell wall is useful for oral as well as systemic drug administration. The effective aspect is protection against

phagocytes. The tagged t-RNA, polyethylenimine, and DNA with fluorescein and or rhodamine are easy to detect or visualize involving fluorescent microscopy, fluorescence spectroscopy, and flow cytometry quantitatively. The DNA payload depends on the specific ratio between t-RNA and yeast cell wall particles [79,80]. Synthesis of nanoparticles has been one of the prime aims of nanotechnology to meet the demands of their applications but the cost effectiveness, eco-friendliness, and the environmental derogative implications of nanomaterials compel nanotechnologists to look for the alternative methods. The green biosynthesis is apparently a suitable choice. Nanoparticles like, organic and inorganic origin nanoparticles and quantum dots have been successfully procured from plants, bacteria, yeast, and lower organisms. Louis Pasteur is a prominent pioneer in this field and found that microorganism like yeast, is capable of chemical conversion in absence of oxygen, and results the formation of alcohol from sugars [83,84]. Yeast plays major role in the fermentation industry because of the presence of reducing enzymes in them; this process is either extracellular or intracellular in nature. Same concept is applicable for the green biosynthesis of nanoparticles [80]. During the synthesis of metallic nanoparticles, the source of the element of the nanoparticle in question should be present in the reaction mixture in a nontoxic range i.e., 1ppm to 1000 ppm. This amount is to be decided during early pilot experiments. In the normal course of experimentation, the presence of metabolites like carbonyl groups, phenols, amines, terpenoids, proteins, alkaloid and/or any suitable reducing metabolites is a need for the successful biosynthesis of metallic nanoparticles [85,86].

Yeast strains, like *Candida glabrata* and *Saccharomyces pombe*, are most suitable for intracellular synthesis of silver, selenium, titanium, gold, and cadmium sulfide nanoparticles [87,88]. Extracellular synthesis of silver and cadmium sulfide nanoparticles using these organisms is also feasible [89,90]. Probably, in yeast, the membrane bound enzymes, like oxidoreductases and quinones, participate in the synthesis of nanoparticles. These enzymes are pH sensitive [91], and become active during stress conditions [89]. Generally, the stress is the result of additional or reduced presence of metallic nutrients in the culture media that commences the stream of interactions resulting in the formation of bio-production of phytochelatin synthase and glutathione. These enzymes help to counteract the cellular stress [89]. Further, these enzymes have specific redox and nucleophilic properties and these facilitate bioreduction of metal ions; this aspect is the bases of formation of nanoparticles. The binding of metallic ions helps in the formation of nanoparticles, like cadmium, selenium, zinc, gold, nickel, copper and other metals [92]. In all probability the intracellular biosynthesis of metal nanoparticles is a mode of regulating cellular metallic ionic stress [90]. Yeast cells produce nanoparticles

intracellularly or extracellularly. Such cells are suitable sources for nanoparticles and such nanoparticles can be readily extracted and purified, using techniques, like centrifugation, dialysis of media in which yeast has been cultured for the biosynthesis. The yield of nanoparticles can be examined physically and chemically [93]. There are chances that nanoparticles exhibit aggregation or degradation; such behaviors relate with the technique involved and/or physicochemical characters of the reaction medium. The applications of such biosynthesized nanoparticles relate with their specific behavior like, antimicrobial, antiviral or anticancer [94-98]. The mechanism of such behaviors is due to the production of active oxygen species, damaging cell wall of bacteria, induction of inflammation or blocking the metabolic pathways of the concerned pathogen [98].

Yeast and Pharmaceutical Sciences

Unicellular yeast is unique organism that exhibits fundamental mechanisms relating with replication, cell division, recombination, and metabolism that are seen in eukaryotes and other prokaryotes. The species of *Saccharomyces cerevisiae* is one of the well-studied eukaryotic organisms as its complete genome is adequately established [99,100]. Further, its short life cycle, ability to be cultured easily, and fast growth rate make it more favorable model for investigations concerning with pharmaceutical sciences [101]. Baker's yeast (*S. cerevisiae*) is suitable material to synthesize antibiotic nanobiosomal peptide penicillin. Pharmaceutical products like insulin, human serum albumin, vaccines, and antibiotics like penicillin [101-103]. Some of the unconventional yeast species like *Hansenula polymorpha*, *Pichia pastoris*, *Yarrowialipolytica*, *Kluyveromyces marxianus*, *Kluyveromyces lactis* and *Scheffersomyces stipites* are utilized as sources for biopharmaceutical products [103]. In the recent times a trend is set relating yeast i.e., yeast has been the focal point for the biopharmaceutical science [104].

Yeast biotechnology provides products like crude glycerol, lignocellulose, corn starch, cane sugar, primary and secondary metabolites, and also recombinant proteins. Recombinant pharmaceutical proteins play prime role in the financial, production, and pharmaceutical aspects. Yeast is one of the most sought after cellular organism for this multi-billion dollar industry. The current advances in glycoengineering of yeast along with secretory mechanisms involved in yeast provide favorable and encouraging results. Two modes of investigations namely, strain and pathway engineering help in understanding the mechanism involved and provide appropriate homogeneity and higher production/yield. In this aspect tools of synthetic biology and omics technology are very helpful [105,106]. Some non-conventional species of yeast like, *Kluyveromyces marxianus*,

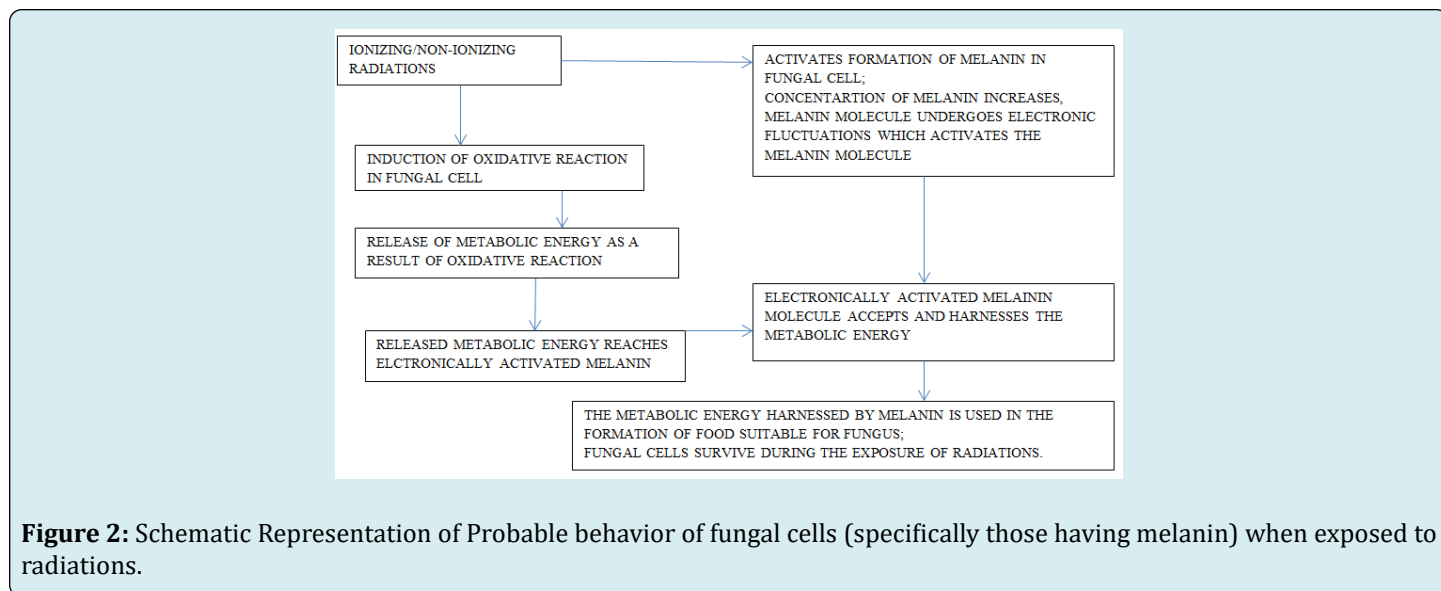
K. lactis, *Yarrowia lipolytica*, *Pichia pastoris*, *Scheffersomyces stipitis*, *Hansenula polymorpha*, and *Rhodotorula toruloides* show desirable phenotypes such as thermotolerance, ability to assemble carbon sources, and to secrete higher protein and lipid titers. There is a need to carry investigations in the field of advanced metabolic engineering and to explore the possible biological systems along with suitable methods [107].

Behavior of Fungi in Relation to Radiation

Fungi are able to take advantage of radiation and utilize their energy in synthesizing food for their survival. It is proposed that melanin plays significant role in utilizing energies present in these radiation as chlorophyll pigment does from solar radiations in green plants. Melanin is ubiquitous in biological world and most of the fungi possess this biomolecule if not all fungi. Its role in fungi is mysterious and need thorough investigation. Fungi are able to use the energy contained in these radiations during the synthesis of their food which, results in appropriate growth. This is the case specifically in fungi have melanin. The soil micro-organisms which are contaminated with either present in traces or otherwise, have melanin show this behavior indicating the probable role of melanin in the biochemical pathways. When the melanin present in fungi gets exposed to ionizing radiation and even other forms of electromagnetic radiations, it undergoes electronic modification. This feature is not seen in those organism in which melanin is absent. Thus, possibly melanin plays an important role in apprehending energy of electromagnetic radiations. The ability of fungi to exploit their oxidative reduction abilities and the efficacy of melanin enables them to trap energy of these radiations to gain and maintain its metabolic energy requirements. *Cryptococcus neoformans*, a fungal species not having natural melanin and *Wangiella dermatitidis*, a fungal species having natural melanin did show elevated concentration of melanin as a result of exposure to ionizing radiation with 500 times higher level than the background level and exhibit higher degree of growth. Melanin formation gets induced in *Cryptococcus neoformans* and its amount increases after the exposure. This feature is established based on the number of colonies formed and the gain in their respective dry-weights. The electronic fluctuation in the structure of melanin enables their ability harness energy from these radiations and exploits it for its metabolic needs (Figure- 2). One of the prime feature of fungal melanin is that it is similar to that present in mammalian skin [108]. Any bio-cell when provided with culture medium or any medium, like, the frequencies for different types of non-ionizing radiation causes some degree of stress and it is in response to the properties of the medium. The physiology of cellular response is in accordance to the fluctuations in the ambient environment and the ability of cell of a biological

system to adjust and reflects on its efficacy to survive in the changed circumstances [109,110]. The culture of *Serpula himantoides*, fungus, on exposure to Wi-Fi radiations exhibits elevated fatty acid contents and ergosterol.

Ergosterol plays significant role in vitamin D metabolism. Some form of modification takes place in their cell membrane and enhances antioxidative ability. This feature provides means to improve their dietary efficacy [111] Figure 2.



Responses of Yeast to the Non-Ionizing Radiations

The *Saccharomyces cerevisiae* on exposure to non-ionizing millimeter waves with range 85 to 105 GHz (as recommended by international commission on non-ionizing radiation protection) with non-thermal 20 mW/cm² (within safety limits) and power density around 1.0 mW/cm²SAR for 5 to 6 hours result in 62% decline in their growth rate. This impact is specifically within 85 to 105 GHz and depends on the parameter like energy and the density. The growth profile of colony of wild type and delta rad52 (DNA repair gene) cells show no impacts of these irradiations. This reflects that probably the non-thermal effect does not induce permanent changes in the genetic make-up. The horn antenna (~1.0 mW/cm² and compact waveguide (17.17 mW/cm²) emits radiations which causes complete stoppage of cell-division and this is due to non-thermal effect. This response is dose dependent. There is a possibility of its application in biomedical field [112]. The exposure of yeast cells to microwave radiations with 968MHz and power of 10dBm restricts collagenase and L-lactate dehydrogenase activities up to 26% while proliferation rate increases 15% [113]. Radiations from 905 MHz electromagnetic radiations closely match with the Global System for Mobile Communication (GSM) pulse modulation signals from mobile phone having specific absorption rate (SAR) OF 0.12W/kg. When *S. cerevisiae* strains FF18733 (wild-type), FF1481 (rad1mutant) and D7 (commonly used to detect reciprocal and nonreciprocal mitotic recombinations), do not induce

changes in the colony growth rate statistically. The exposed strain FF1481 is relatively more sensitive to such radiations [114]. Yeast cells of *Candida slooffii*, *Candida lipolytica*, and *Leucosporidium nivale* species can be readily cultured in laboratory and used for the appropriate investigations. For scanning electron microscopy these cells (in dry state) are fixed in buffered 3% glutaraldehyde for 1 hour, The coated dried cell of yeast coated with gold-are observed under scanning microscope using 10kV voltage. The cell wall of *Candida* species exhibits smooth surface but many protrusions are there on the surface of leucosporidium cells. There appears to be no relationship between the nature of the cell surface and thermophilic adaptive behavior of these species of yeast [115]. Radiations having low frequency magnetic field of 60 Hz and 500 mT could not induce growth rate and frequency of mutation among wild and radiation sensitive mutant species (rad or rev) of *S. cerevisiae*. The radiations from static and extremely low frequency magnetic field are not potent enough to affect the growth of yeast cells and even kill the model organism [116].

The microwave irradiations of 42 GHz and 50 mW power (the nonionizing millimeter-waves, MMW) cause no significant changes in the rate of growth of yeast cells [117,118]. The nonionizing millimeter-waves, MMW radiation having range 41.650 - 41.798 GHz for 4 h and 20 mW power result in frequency dependent sensitive effects like the growth rate elevates or declines in accordance to the frequency [119]. The exposure to radiation with 968 MHz and power of 17dBm for 7 hours induces higher rate of growth

in yeast cells [120]. The non-ionizing radiation are likely to induce changes non-thermal effects, modulation in cell membrane and its impacts on cell signalling, biomolecular resonance effects, these changes are dose and power/energy dependence [113]. The exposure (5 to 6 hours) form non-ionizing millimeter-waves, (MMW) (85-105 GHz; W-band with minimum density ~ 1.0 mW/cm² of 50cells/ μ l) causes 62% decline the growth rate in *Saccharomyces cerevisiae* yeast cells. The degree of the intensity of this effect varies in accordance to frequency, energy dose, and density of the model yeast cells. Such impacts are non-thermal in nature. The growth profile in case of wild type and delta rad52 (in which the repair mechanism of DNA is not present/or deleted) do not show any derogative effect on the respective stability of genomic set-up. The threshold parameters which favour inhibition of infection depend on the cell density specifically when exposed to the nonionizing millimeter-waves. The non-thermal impacts along with radiations for nonionizing millimeter-waves change the physical aspects of cell membrane resulting in disturbed cell signalling cascade and vesicular structure. These radiations absorb the water contents of yeast cell; this in turn changes the proteomics and conformational aspects of chromatin of the experimental cells. Modifications in genetic set-up can be directed to induce resistance for antifungal drugs in the test yeast cells. The super high frequency (SHF) 3 to 30 GHz bandwidth ensure the adaptability of the non-thermal techniques to modify the internal biochemical built up to overcome the burns developed during thermal effects as a result of conventional mode. Thus, there seems to be a possibility of retention of biochemical integrity of genetic set up of the experimental cells and induce a combined physical and bioelectromagnetic impact. This effect causes modifications in the proteome that is responsible for the behavior of the experimental yeast cells. Further, the most probable practical application of radiations from non-ionizing millimeter-waves, (MMW) involves the control of the targeted biological responses, and for this there is no need to modify activation of biochemical aspects or induce DNA damage to meet the fungal infection clinically [113,121-124].

Conclusion

The yeast organism has mostly similar cell organization and cell organelles to most of the eukaryotic cells. Generally, yeast cells have dimensions $5 \times 10 \mu\text{m}$, and their morphology, molecular aspects are readily observed. These biological entities are found in soil, plants, animals, and insects. Fungi have ability to exploit radiations to synthesize their food and able to tolerate higher concentrations of sugars, salts, and acetic acid and maintain their growth and budding processes. Their traits of stress tolerance are the bases of their applications and enhance the efficacy of different biotechnological and food processes. Yeast cells exhibit

an efficient propensity of resistance or tolerance, some of the extreme conditions like influence of preservatives and elevated osmotic ambient surrounding. The non-ionizing millimeter-waves modify the proteomes that regulate the behavior of the yeast cell. These radiations may be of practical importance during understanding the molecular mechanism in targeted cells without modifying the biochemical aspects and/or induction of DNA damage to investigate clinical aspects of fungal infection.

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Conflicts of Interest

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