**Role of Micro organisms as an Alternative Animal Model in the Biomedical Research**

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**Abstract:**

Significant limitations and rising animal ethical concerns towards welfare of laboratory animals especially using vertebrate animal model for research experimentation studies there is need for alternative methods in the biomedical research. Besides these, the animal experimentation studies involve time requirement, man power and technical support. In addition, animal may suffer from pain and stress throughout the study period. Recently, microorganisms are acceptable as models in metabolism, genetics and biochemistry and they can sometimes serves as models of more complex systems. The use of micro organisms includes *C. elegans,* Yeast and bacteria (*salmonella typhimurium*) as an alternative approach for testing and research to animal model achieves relevant advantages, results on both in vivo and in vitro assays. The distinctive characteristics such as microscopic, transparency, small size, short life cycle, makes more reliable to work with it when compared to animal model. Due to the fact, that some of its biochemical pathways are very similar to those of humans, it has been employed in the biomedical research studies. Alternative animal model may significantly reduce the usage of animals for experimentation which fulfill the 3R strategy formulated by Russel and Burch in 1959. Therefore, considering the above importance the alternative approach to animal model provides reliable results like animal models.

Key words: Animal welfare, Alternative animal model, *C. elegans*, yeast and Bacteria

**Introduction:**

The nematode worm (*Caenorhabditis elegans*) has only been used as a model organism since the early 1960. *C. elegans* is an excellent alternative model organism in the biomedical research studies. It has been adopt to use as a model organism in late 1970s when Sydney brought to study the neuronal development under laboratory condition [1]. A free-living soil nematode inhabiting organic matter rich environments like rotting fruits and vegetables. *C. elegans* was the first eukaryotic multicellular organism used as an alternative to animal model organism in the biomedical research. The complete genome sequence of the *C.elegans* was published in early 1998. The *C. elegans* genome is 100 million base pairs in length and contains an analogous number of genes as humans, about 20,500 genes. This soil nematode offered good potential for genetic analysis, because of its rapid life cycle, small size (1.5mm long adult) and ease of management under laboratory condition (Fig 1). It is a soil nematode organism is viewed almost featureless tube that moves forward or backward direction in a simple sine wave fashion. The life cycle of *C. elegans* is around only 2 to 3 weeks. The nematode worm *C. elegans* is either male/hermaphrodite (have both male and female reproductive organs) which are not female. The hermaphrodite can able to self fertilize but can also bred with males. *C. elegans* genome sequence share about 60% of human genes making it ideal to study the molecular biology processes. The worms are very much simpler and easy to handle than humans, for e.g., it doesn’t have bones and heart, but it does share many number of genes and molecular pathways with the humans. Each worm is made up of about 1000 somatic cells that is ability of the cell forming the whole body of an organism. Mutant strain forms of *C. elegans*, in which specific genes are altered and can be generated easily to closely study the gene functions. Many of the genes in the *C. elegans*, genome have functional counterparts with humans in which makes it an extremely model organism for many of the human diseases. *C. elegans* has been used as an alternative animal model to study the variety of human diseases includes Parkinson’s disease and mitochondrial diseases [3, 4].



**Figure 1.** Soil Nematode *Caenorhabditis elegans*

**Special features makes as an alternative animal model:**

The anatomy and development of *C. elegans* can be examined easily under a light microscope. The transparency body, the constant division of cell number and the constancy of cell position makes the most unique feature of this organism for the study of development stages (Fig. 2). Transparent body consists of three layers an epidermal layer, an intestinal layer, and a muscular layer. Nervous system and reproductive system are found between the three layers. A unique feature of *C. elegans* is that their development is very specific, so each cell can be traced back to embryo life stage. Although, *C. elegans* is a relatively simple organism, many of the molecular pathway signals controlling its development are also found in humans [2]. C. elegans can be grown easily and in large numbers on bacterial petridish plates containing *E. coli* bacteriaas their natural diet. Healthy cultures of *C. elegans* can be frozen and then deforested and revived as per need. *C. elegans* is a very small microscopic organism so it is convenient to keep in the laboratory and easy to maintain. The worm is clearly transparent throughout its life cycle stage which helps us to study the behavior of each and every individual cell. An important reason *C. elegans* was chosen for research study was that high quality electronic micrographs can been obtained*. C. elegans* genetic map containing more than 100 genetic loci dispersed over the six chromosomes, all of which are behavioral or morphological markers. On the other hand, *C. elegans* are easily amenable to various genetic manipulations. The viable fresh stocks of nematodes can be frozen in liquid nitrogen to those used for mammalian cell lines. Stored stocks have retained their live viability at -80**º**C for the past 12 years.



**Figure 2.** Microscopic transparent structure of *C. elegans*

**Research documentation:**

* *C. elegans* used as a model organism in the Parkinson’s disease it is an age related neurological disorder that affects the movement [3]. Moreover, *C. elegans* mutant strain provides models for many human diseases including neurological disorders, congenital heart disease and kidney related disease.
* Research studies using *C. elegans* reported that human genes responsible for a range of mitochondrial diseases found very similar to genes present in the soil nematode organism *C. elegans* makes the alternative model organism for studying the mitochondrial diseases [4].
* *C. elegans* as a model organism in the immunology related studies this organism has only the innate immune response this includes antimicrobial molecules like lectins, lyzozyme and other antibacterial molecules this makes the *C .elegans* as a model organism to study the innate immune response to many pathogens[5].
* *C. elegans* mutant strain can be used to screen thousands of drugs for many important human diseases.
* Studying the cell death/ Apoptosis in the *C. elegans* could hold the key to counteracting the effects of ageing in humans as well as providing information about cancer and diabetes diseases.
* C. elegans was used as a model organism in research studies to examine how bacteria behave within the host during infection. For instance, studies found that S. typhimurium's Phop/phoQ and SPI-1 virulence factors are expressed once the organism has colonised C. elegans and that these virulence factors are essential for starting an infection in the organism [5].

**Yeast organism:**

Single-celled yeast called Saccharomyces cerevisiae is widely utilised in the bread-making sector and its distinctive characters makes an alternative model in the biomedical research(fig 3). The whole length genome of this first eukaryotic organism was sequenced in 1996. About 6,692 genes are found within its 12,157,105 bp long genome**.** In 2001, three scientists shared the Nobel Prize for their independent work demonstrating the function of various genes in regulating the cell cycle and establishing the connection between the cell cycles of yeast and humans. These three researchers were Leland Hartwell, Tim Hunt, and Paul Nurse. Because it is simple to manipulate in both the haploid and diploid states, the budding yeast S. cerevisiae is frequently employed as a model organism. This form of state makes it easy to isolate the recessive mutation strains. Utilizing a non-animal model, followed by the ongoing creation of new experimental research investigations aimed at modifying different facets of its cellular machinery. It served as the primary model organism for molecular systems. As a single celled micro organism, it is able to reproduce quickly and grow under laboratory condition. Due to its rapid growth an average generation period of 90 minutes makes this trait has remained at the forefront of genetic research studies.

The mature yeast offers useful data for the development of genetic and molecular tools. It is now regarded as the main development platform for a variety of high-throughput technologies, such as transcriptome, proteome, and metabolome. Scientists have been able to determine the relationships between genes, proteins, and the actions carried out in our cells by studying the biology of yeast. The functional pathways that regulate important aspects of eukaryotic cell biology, such as the cell cycle, programmed cell death, protein folding, quality controls, and degradation, are significantly similar in yeast and humans.



Figure 3. Single celled budding yeast cell

**Benefits of Yeast organism as a Model organism:**

Saccharomyces yeasts mainly focus on the dietary field as a probiotic and the process of treating intestinal ailments. Belonging the probiotic action; these yeasts have several vital roles on mechanisms such as bacterial adhesion, enhancement of immune cells and responses, modulation of the signal pathways of the host, and improvement of the strengthening the enterocytes (6). Nevertheless, it has been found that yeast and humans have little in sharing properties because, yeast is a eukaryotic organism. This means, the yeast cells have a nucleus that contains DNA packaged in chromosomes. The yeast Saccharomyces is widely used as a model organism for a variety of metabolisms, including the cell cycle, biogenesis, protein folding, genetic engineering, and recombination (7). S. cerevisiae is a unicellular microorganism that can be easily cultivated, develops quickly, and tolerates a variety of substances. According to reports, this yeast could be used to understand how diseases work because molecular relationships between yeast and people are preserved. Yeast cells and human cells have a lot in common biologically. Because yeast and humans share some genes, it can be used to test the toxicity of new medications. For example, thousand of drugs can be tested on yeast cells containing mutated human genes to see whether the drug can restore the normal function. Genetic manipulation in yeast is easy and cheap when compared to similar experiments carried out in more complex animals such as laboratory mice and zebrafish. Additionally, the yeast organism may thrive in acidic and high-sugar environments (fig 4). These circumstances might inhibit bacterial development, which would prevent bacterial contamination and contradicting results. About 20% of human genes have known to play a role in disease counterparts with yeast organism.



**Figure 4.** *saccharomyces cerevisiae* on culture plates

**Research Documentation:**

* Yeast is the most important eukaryotic model organism; many important biological discoveries were made in the yeast, e.g., cell cycle.
* Genes comprise the genes that have been determined to have the most genetic overlap with yeast and humans are MSH2 and MLH1. Human hereditary non-polyposis colorectal cancer is caused by these genes. The study of these genes will aid researchers in their quest to understand the genes responsible for colon cancer.
* Research studies investigated and have also shown that yeast has receptors for estrogen found to be identical in affinity with those of the rat uterus (NRC, 1985b).
* Additionally, it serves as a cell factory for the production of commercially significant proteins such hepatitis vaccine, virus-like particles for vaccination, and human serum albumin. Yeast produces 20% of biopharmaceuticals, which has the benefit that it is a eukaryotic model that allows for the creation and appropriate folding of a large number of human proteins.
* Researchers reviewed that a yeast organism model can be used to identify the mutations in the cell cycle in cancer and some diseases, especially neurodegenerative diseases [8].
* S. cerevisiae may be a crucial organism for the generation of recombinant proteins in the pharmaceutical sector (Table 1). It produces several eukaryotic proteins, including those seen in humans, and contains complete cellular components and membrane compartments [9].
* At first, S. cerevisiae produced the necessary biopharmaceuticals like insulin and its analogues.
* According to Rosenfeld and Racaniello [10], the hepatitis C virus was found in S. cerevisiae, and all of the virus's proteins were encoded. In preventive vaccines, S. cerevisiae can safely express the hepatitis B surface antigen, according to another study [11].

Table 1**. Examples of Biopharmaceutical Products of *Saccharomyces cerevisiae***

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| --- | --- | --- |
| **Sl.No**. | **Biopharmaceutical Products** | **Category** |
| 1. | Human Serum Albumin | Blood factors [12] |
| 2. | Recombinant proteins | Protein [13,14,15,16] |
| 3. | Insulin | Hormone [17] |
| 4. | Glucagon | Hormone [18] |
| 5. | Human parathyroid hormone | Hormone [19] |
| 6. | Purified proteins for vaccines | Protein [20,21,22,23,24] |
| 7. | Virus like particles | Protein [25,26,27] |
| 8. | Gene expression Systems | Gene [28,29,30] |

**Ames test as an alternative approach to animal model in the biomedical research:**

The development of the ames test, particularly the genotoxicity and mutagenecity tests, greatly reduced the use of laboratory animals in biomedical research studies. By combining one or more of the alternative techniques, it is possible to determine the majority of genotoxicity studies and mutagenecity agents. By replacing laboratory animals with microbes as a model organism in mutagenicity and toxicity research investigations, the wellbeing of the animals is improved. Therefore, it is necessary to conduct the following three tests and acquire an assessment of their level of toxicity in accordance with international and domestic norms for agricultural fertilizers and their toxicity studies, which includes,

1. Ames test

2. Test for Chromosomal Aberration

3. A rodent micronucleus test

The Ames test, on the other hand, is a significant and required test that must be carried out according to the industrial safety and health law for evaluation of general chemicals. The Ames test is typically used to identify the possibility of mutagenesis; despite the fact that bacteria are used in this test; the process underlying bacterial mutagenesis is strikingly similar to that of higher animals. The test was discovered to be reasonably straightforward, and results could be obtained quickly and affordably. The Salmonella typhimurium strains used in the Ames test are those that are unable to produce the amino acid histamine that they need to develop on their own. Ames test uses the particular strains of *salmonella typhimurium* which cannot able to synthesize Aminoacid Histamine on its own which requires for their growth. In this test, the *salmonella typhimurium* particularly treated with the test compound/chemical is transferred to a particular medium that does not contain histidine and its evaluation of genotoxicity level can be determined by counting the emerged colonies that have become able to synthesize histidine amino acid as a result of reverse mutation occurred in the genes for histidine synthesis [31]. The Ames test is used to identify the reverse mutations that are present in strains and to determine whether environmental samples, including as waste water, fertiliser, pesticides, and medications, are mutagenic.

**Conclusion:**

Need for alternatives in the research studies may significantly reduce the number of animals sacrifice and pain during experimentation. The modified procedure to animal usage by Russel and Burch in 1959 proposed that when the animals were to be used in experiments, the every effort should be made to replace them with non sentient alternatives. Russel and Burch, developed the 3R strategy which includes refinement-refine the experimental procedure to decrease unnecessary pain and trauma to the animals. Reduction –reduce the number of animals used in the experiments. Replacement- replace the animal experiments with the use of model organisms as an alternative to animal usage in animal testing and research provides better and reliable results. On considering the above facts use of animals for research and testing purpose can be altered as per needs. The micro organisms are with maximum restricted genetical similarity to humans that could be successfully applied to minimize the animal usage. These microorganisms as a model organism are considerable and used in many ways as per need to get reliable study results.

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