ROLE OF MICRO ORGANISMS AS AN ALTERNATIVE ANIMAL MODEL IN THE BIOMEDICAL RESEARCH

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. As these microbial models have the 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.

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

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I. INTRODUCTION

Caenorhabditis elegans, a soil nematode worm, has only been utilized as a central model organism since the early 1960 in biological research. *C. elegans* offers a great alternative model organism for biomedical research studies. It was chosen to be a model organism in the late 1970s when Sydney brought to investigate the development of neurons in the laboratory [1]. A free-living soil nematode inhabiting organic matter rich environments. *C. elegans* was the first eukaryotic multicellular organism used as an alternative to animal model organism in the research studies. Early in 1998, the entire *C. elegans* genome sequence was published and the genome sequence comprises of 100 million bp long and has about 20,500 genes as in humans. Due to its short life cycle, modest adult size (1.5 mm), and simplicity of handling in a laboratory setting, this soil nematode presented good promise model organism for genetic studies (Fig 1). It is nematode organism that resembles an almost featureless tube that moves in either a forward or reverse direction like a simple sine ‘S’ wave fashion. *C. elegans* life cycle is just about as short as two to three weeks. The hermaphrodite nature of the nematode can able to do self fertilization but they 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 compared to humans and other animal models, as they don’t have heart and bones, but they share many similar genes and pathways with humans. Each *C. elegans* is made up of about 1000 somatic cells that is ability of the cell forming the whole body of an organism. Mutant strain of *C. elegans* in which specific genes are altered, can be generated easily to study the functions of genes. *C. elegans* is a highly useful alternative animal model organism since many of the genes in its genome have functional human counterparts. *C. elegans* has been used as an alternative animal model to study the variety of diseases of humans includes age related Parkinson’s disease and mitochondrial diseases [3, 4].

Figure 1: Soil Nematode *Caenorhabditis Elegans*

II. SPECIAL FEATURES MAKES AS AN ALTERNATIVE ANIMAL MODEL

The anatomical examination of *C. elegans* is simpler 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). Its transparent body is made up of 3 layers consists of an epidermal layer, intestinal layer, and muscular layer. The Nervous and reproductive forms are found between the three layers. Unique feature of *C. elegans* is that their development is very specific, so that each cell can be traced back to embryo life stage. Eventhough, *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* bacteria as their natural diet. Cultures of *C. elegans* can be preserved as frozen and revived as per need. *C. elegans* is a small microscopic organism so it is convenient to keep in the laboratory and easy to maintain. The worm is clearly transparent because of this advantage the entire life cycle stage help us to study the behavior of each and every individual cell of the organism. *C. elegans* genetic map containing more than 100 genetic loci dispersed over six chromosomes, 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 worms can be frozen in liquid nitrogen at -196 ºC the procedure followed same for preserving the cell lines. Stored stocks have retained their live viability at -80 ºC for the past 12 years.

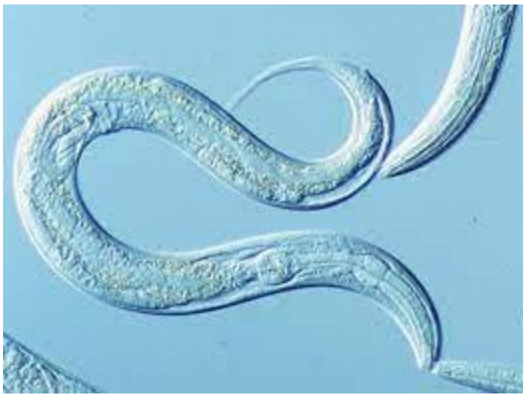

Figure 2: Transparent Microscopic Structure of *C. Elegans*

III. RESEARCH DOCUMENTATION

- *C. elegans is* used as a model organism for Parkinson's disease which is a neurological disorder that affects the movement related with age [3]. Moreover, *C. elegans* mutant strain provides models for many human diseases like innate heart disease, kidney related disease and neurological disorders.

- Research studies using *C. elegans* proved that genes responsible for a range of mitochondrial diseases in humans found very similar to genes present in *C. elegans* makes the alternative model organism for studying the mitochondrial diseases [4].

- As a model organism *C.elegans* 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 to study the innate immune response to numerous pathogens[5].

- *C. elegans* mutant strain can be used to screen thousands of drugs for many important human diseases.

- Studying apoptosis in C. *elegans* could be the key to counteracting the effects of ageing in humans.

- *C. elegans* was also used as a model organism in research studies to examine bacterial behavior within the host during infection. For instance, research studies found that *S. typhimurium* Phop/phoQ and SPI-1 virulence factors are get expressed once the organism has colonised *C. elegans* and these virulence factors are essential for starting the source of infection in the organism [5].

IV. YEAST ORGANISM

Single-celled yeast called *Saccharomyces cerevisiae* is widely utilized 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 awarded and 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 counterparts. It plays a primary model organism for many molecular pathways. 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. Research 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 biochemical pathways that regulate important aspects of cell biology of eukaryotes, such as the cell cycle, programmed cell death, protein folding, quality controls, and degradation, are significantly similar in yeast and humans.

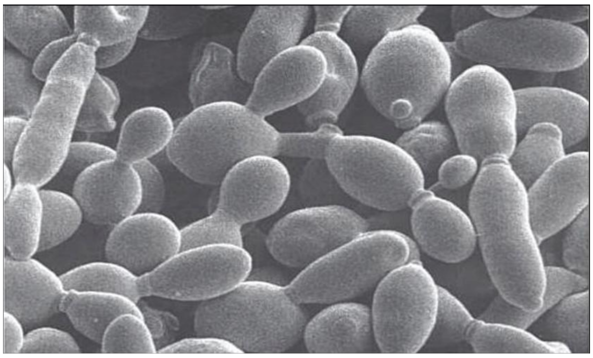

Figure 3: Single Celled Budding Yeast Cell

V. BENEFITS OF YEAST ORGANISM AS A MODEL ORGANISM

Saccharomyces yeasts are commonly used as a probiotic and for treating intestinal ailments. Belonging the probiotic group of bacteria; these yeasts have several imperative 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 intestinal absorptive cells (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 toxicity drugs can be tested on yeast cells containing mutated human genes to see whether the drug can restore the normal function. In yeast, the genetic manipulation is very easy and affordable when compared to similar research studies carried out in vertebrate animals such as laboratory mice and minuscule laboratory 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. Nearly, 20% of human genes have known to play a role in disease counterparts with yeast model organism.

Figure 4: *Saccharomyces Cerevisiae* on Culture Plates

VI. 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. These genes are mainly responsible for causing human hereditary non-polyposis colorectal cancer. The study of these genes will also 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 model for the development of commercially significant proteins such hepatitis vaccine, virus-like particles, 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 involved in cancer and neurodegenerative diseases [8].

- *S. cerevisiae* may be a crucial organism for the generation of recombinant DNA 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].

- *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 reported study [11].

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

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 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,

- Ames test
- Test for Chromosomal Aberration
- 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. In order to develop on their own, the *Salmonella typhimurium* strains employed in the Ames test are those that are unable to generate the amino acid histamine. Salmonella 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. In this test, *Salmonella typhimurium* that has been specifically exposed to the test substance or chemical is transferred to a specific medium that devoid of histidine, and the level of genotoxicity can be assessed by counting the colonies that emerge after the reverse mutation of the histidine synthesis genes has taken place and these colonies can now synthesize histidine amino acid [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, fertilizer, pesticides and medications, are mutagenic.

VII. CONCLUSION

Need for alternatives in 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 sentient animals were to be used in research experiments, every attempt should be made to replace them with non sentient alternative models. Russel and Burch, developed the 3R strategy which includes refinement means the experimental study procedure to minimize the unnecessary pain and suffering to the animals. Reduction refers to reduce the total number of animals used in the experiments. Replacement involves replace animal models with the use of model organisms as an alternative to animal usage in testing and research provides better and reliable results. On considering, the above reasonable facts use of animals for research and testing purpose can be altered as per needs. The micro organisms are with maximum restricted genetic similarity to humans that could be successfully applied to minimize 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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