**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 research studies. It has been adopt to use as a model organism in late 1970s when Sydney Brenner to study the neuronal development in the laboratory [1]. A free-living nematode inhabiting organic matter rich environments like rotting fruits and vegetables. *C. elegans* was the first eukaryotic multicellular organism used as an alternative animal model 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 great potential for genetic analysis, partly because of its rapid life cycle, small size (1.5mm long adult) and ease of management under laboratory condition (Fig 1). It is an 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 basic molecular biology processes. The worms are very much simpler to handle than humans, for e.g., it doesn’t have bones, heart/ a circulatory system, but it does share many 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 body of an organism. Mutant forms of *C. elegans*, in which specific genes are altered and can be produced 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 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* as 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 organism so it is convenient to keep in the laboratory and easy to maintain. The worm is clearly transparent throughout its life stage which helps to study the behavior of individual cells. An important reason *C. elegans* was chosen for study was that high quality electron micrographs had 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 genetic manipulation. The viable stocks of nematodes can be frozen in liquid nitrogen to those used for mammalian cell lines. Stored stocks have retained their viability for 25 years 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* mutants provide models for many human diseases including neurological disorders, congenital heart disease and kidney 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 *C. elegans* makes the alternative model organism for studying the mitochondrial diseases [4].
* *C. elegans* as a model organism in the immunology studies the organism has only the innate immune response this includes antimicrobial molecules like lectins, lyzozyme and other antibacterial molecules this make the *C .elegans* as a model organism to study the innate immune response to pathogens[5].
* *C. elegans* mutants can be used to screening thousands of potential 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.
* Research studies used *C. elegans* as a model organism to study the behavior of bacteria in the host during infection; for example studies revealed that *S. typhimurium* virulence factors includes Phop/phoQ and SPI-1 are expressed once *S. typhimurium* colonizes *C. elegans* , and these virulence factors are crucial for establishing infection in *C. elegan*s [5].

**Yeast organism:**

Yeast *Saccharomyces cerevisiae* is a single celled yeast organism most commonly used in the bread making industry (fig 3). It is the first eukaryotic organism, where the full length genome was sequenced in the year 1996. Its genome size is 12,157,105 bp in length and contains 6,692 genes. In 2001, three scientists shared the Nobel Prize for their independent work establishing the role of different genes in controlling the cell cycle and investigating the link between the cell cycle in yeast and that in humans. These three scientists were Leland Hartwell, Paul Nurse and Tim Hunt. Budding yeast *S. cerevisiae* is widely used as an animal model due to its ease of manipulation in both haploid and diploid state. This form of state makes it easy to isolate the recessive mutation strains. Use of an alternative to animal model and then with continuous development of new experimental studies for manipulating various aspects 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 thrive under laboratory condition. This has remained at the forefront of genetics research because it is quick and easy to grow with an average of 90 minutes generation time. The maturity of yeast provides good information in the field of genetic and molecular tools. It has turn to be positioned as the primary platform for development of many high-throughput technologies includes transcriptome, proteome and metabolome. Studying the biology of yeast has enabled scientists to work out the connections between genes and proteins and the functions they carry out in our cells. Yeast and humans share a significant characteristic of their functional pathways that control key aspects of eukaryotic cell biology, cell cycle, programmed cell death, protein folding, quality control and degradation.



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 that, like our cells, yeast cells have a nucleus that contains DNA packaged in chromosomes. The yeast saccharomyces has been accepted as the model organism for several metabolisms such as cell cycle, biogenesis, protein folding, genetic manipulation and recombination etc., (7) *S. cerevisiae* is a unicellular micro organism that grows very fast tolerates several chemicals and cultured easily. It was reported that this yeast could able to discover the process of diseases because of the conservation of molecular interactions from yeast to humans. Yeast cells share many biological properties with our cell system. Yeast shares some genes with the human beings so it can also be used to test new drugs for their toxicity. 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. In addition to this yeast organism can able to grown in acidic and high sugar conditions (fig 4). These conditions may prevent the growth of bacteria thus preventing bacterial contamination and thereby conflicting the 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 include MSH2 and MLH1 are the genes found to be the most shared similarities with the humans and yeast organism. These genes are involved in the hereditary non-polyposis colorectal cancer in humans. Research in these genes will help the scientists to learn more about the genes involved in 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).
* It is also used as a cell factory to produce commercially important proteins such as human serum albumin, virus like particles for vaccination and hepatitis vaccine. About 20% of biopharmaceuticals are produced by yeast, the advantages being that as a eukaryotic model enables production and proper folding of many 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* can be an essential organism for recombinant protein production in the pharmaceutical industry (Table 1). It has full cellular components and membrane compartments that produce many eukaryotic proteins, including humans [9].
* Initially, the essential biopharmaceuticals like insulin and its analogs produced by *S.cerevisiae*
* Rosenfeld and Racaniello [10] reported that hepatitis C virus was demonstrated in *S. cerevisiae*, and all proteins for the virus were encoded. Another study reported that *S. cerevisiae* can safely express the hepatitis B surface antigen in prophylactic vaccines [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 harmone | 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:**

On great, discovery of ames test reduced the use of laboratory animals in the biomedical research studies especially genotoxicity and mutagenecity tests. Most of the genotoxicity studies and mutagenecity agents can be determined by combining one or more of the alternative methods. Using microorganisms as a model organism in the mutagenecity and toxicity research studies which relatively replaces the use of laboratory animals thus improves the animal welfare. Therefore, according to the International and domestic guidelines for agricultural fertilizers and its toxicity studies it is mandatory to conduct and obtain an evaluation of level of toxicity from the following three tests which includes,

1. Ames test

2. Chromosomal Aberration test

3. Micronucleus test using rodents

On the other hand, for evaluation of general chemicals the industrial safety and Health law specifies the Ames test is as an important mandatory test to perform. Generally, the Ames test is conducted to detect the potential for mutagenesis; although this test uses bacteria the mechanism behind the bacterial mutagenesis is very similar to that of higher organisms. The test found to be relatively simple, and the results can be obtained in a short period of time at reliable cost. 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]. Ames test is used to identify the reverse mutations which are present in strains and also to detect the mutagenecity of environmental samples such as drugs, dyes, reagents, cosmetics, waste water, fertilizers and other pesticides.

**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.

**References:**

[1]. Garcia-Sancho M (2012) From the genetic to the computer program: the historicity of ‘data’ and ‘computation’ in the investigations on the nematode worm *C .elegans* (1963-1998). Stud Hist Philos Biol Biomed Sci C 43(1):16-28

[2].Apfeld, J. and Alper, S. (2018) what can we Learn About Human Disease from the Nematode C.elegans? Disease Gene Identification- Methods in Molecular Biology, Vol1706. Human Press, New York, NY doi:10.1007/978-1-4939-7471-9\_4.

[3].Harrington, A.J., et al. (2010) *C. elegans* as a model organism to investigate molecular pathways involved with parkinsons disease.

[4]. Maglioni, S. and Ventura, N. (2016) *C .elegans* as a model organism for human mitochondrial associated disorders.

[5].Marsh, E.K., and May, R.C. (2012) *Caenorhabditis elegans*, a Model organism for investigating Immunity. Applied and Environmental Microbiology DOI: 10.1128/AEM.07486-11.

[6]. Chen X, Kelly CP. Saccharomyces. In Versalovic J, Wilson M, editors. Therapeutic microbiology. Washington: ASM Press; 2008. p. 51-60 DOI: 10.1128/9781555815462.

[7]. Pereira C, Countinho I, Soares J, Bessa C, Lea˜o M, Saraiva L. New insights into cancer-related proteins provided by the yeast model. FEBS Journal. 2012;279:697-712. DOI: 10.1111/j.1742-4658.2012.08477.x

[8]. Galao RP, Scheller N, Alves-Rodrigues I, Breinig T, Meyerhans A, Diez J. Saccharomyces cerevisiae: a versatile eukaryotic system in virology. Microb Cell Fact. 2007;6:32. doi: 10.1186/1475-2859-6-32

 [9]. Nielsen J. Production of biopharmaceutical proteins by yeast: advances through metabolic engineering. Bioengineered. 2013;4(4):207-211. DOI: 10.4161/bioe.22856

[10]. Rosenfeld AB, Racaniello VR. Hepatitis C virus internal ribosome entry site-dependent translation in Saccharomyces cerevisiae is independent of polypyrimidine tract-binding protein, poly(rC)-binding protein 2, and La protein 1. J Virol. 2005;79:10126-10137. DOI: 10.1128/JVI.79.16.10126-10137.2005

[11].Valenzuela P, Medina A, Rutter WJ, Ammerer G, Hall BD. Synthesis and assembly of hepatitis B virus surface antigen particles in yeast. Nature. 1982;298:347-350. DOI: 10.1038/298347a0

[12].Payne T, Finnis C, Evans LR, Mead DJ, Avery SV, Archer DB, Sleep D. Modulation of chaperone gene expression in mutagenized Saccharomyces cerevisiae strains developed for recombinant human albumin production results in increased production of multiple heterologous proteins. Appl Environ Microbiol. 2008;74(24):7759-7766. DOI: 10.1128/AEM.01178-08

[13]. Huang M, Bao J, Nielsen J. Biopharmaceutical protein production by Saccharomyces cerevisiae: current state and future prospects. Pharmaceutical Bioprocessing. 2014;2(2):167-182. DOI: 10.4155/pbp.14.8

[14]. Ferrer-Miralles N, Domingo-Espin J, Corchero JL, Vazquez E, Villaverde A. Microbial factories for recombinant pharmaceuticals. Microb Cell Fact. 2009;8:17. DOI: 10.1186/1475-2859-8-17

[15]. Ma JK, Drake PM, Christou P. The production of recombinant pharmaceutical proteins in plants. Nat Rev Genet. 2003;4(10):794-805. DOI: 10.1038/nrg1177

[16]. Cino J. High-yield protein production from Pichia pastoris yeast: a protocol for benchtop fermentation. Am Biotechnol Lab. 1999;17:10-21

[17]. Martinez JL, Liu LF, Petranovic D, Nielsen J. Pharmaceutical protein production by yeast: towards production of human blood proteins by microbial fermentation. Curr Opin Biotechnol. 2012;23(6):965-971. DOI: 10.1016/j.copbio.2012.03.011

[18]. Egel-Mitani M, Andersen AS, Diers II, Hach M, Thim L, Hastrup S, Vad K. Yield improvement of heterologous peptides expressed in yps1-disrupted Saccharomyces cerevisiae strains. Enzyme Microb Technol. 2000;26(9-10):671-677. DOI: 10.1016/s0141-0229(00)00158-7

[19]. Song GY, Chung BH. Overproduction of human parathyroid hormone by fed-batch culture of a Saccharomyces cerevisiae mutant lacking yeast aspartic protease 3. Process Biochem. 1999;35(5):503-508. DOI: 10.1016/S0032-9592(99)00097-7

[20]. Hadiji-Abbes N, Martin M, Benzina W, Karray-Hakim H, Gergely C, Gargouri A, Mokhad-Gargouri R. Extraction and purification of hepatitis B virus-like M particles from a recombinant Saccharomyces cerevisiae strain using alumina powder. J Virol Methods. 2013;187:132-137. DOI: 10.1016/j.jviromet.2012.09.023

[21]. Zhang L, Liu J, Lu J, Yan B, Song L, Li, L, Cui F, Zhang G, Wang F, Liang X, Xu A. Antibody response to revaccination among adult non-responders to primary Hepatitis B vaccination in China. Hum Vaccin Immunother. 2015a;11:2716-2722. DOI: 10.1080/21645515.2015.1045172

[22].King TH, Shanley CA, Guo Z, Bellgrau D, Rodell T, Furney S, Henao-Tamayo M, Orme IM. GI-19007, a novel Saccharomyces cerevisiae-based therapeutic vaccine against tuberculosis. Clin Vaccine Immunol. 2017;24:e00245–e00217. DOI: 10.1128/CVI.00245-17

[23]. Kaslow DC, Shiloach J. Production, purification and immunogenicity of a malaria transmission-blocking vaccine candidate: TBV25H expressed in yeast and purified using nickel-NTA agarose. Biotechnology (N Y). 1994;12:494-499. DOI: 10.1038/nbt0594-494

[24]. Fazlalipour M, Keyvani H, Monavari SH, Mollaie HR. Expression, purification and immunogenic description of a hepatitis C virus recombinant CoreE1E2 protein expressed by yeast Pichia pastoris. Jundishapur J Microbiol. 2015;8:e17157. DOI: 10.5812/jjm.8(4)2015.17157

[25]. Jacobs E, Rutgers T, Voet P, Dewerchin M, Cabezon T, de Wilde M. Simultaneous synthesis and assembly of various hepatitis B surface proteins in Saccharomyces cerevisiae. Gene 1989;80:279-291. DOI: 10.1016/0378-1119(89)90292-8

[26]. Kim HJ, Kim SY, Lim SJ, Kim JY, Lee SJ, Kim HJ. One-step chromatographic purification of human papillomavirus type 16 L1 protein from Saccharomyces cerevisiae. Protein Expression Purif. 2010;70:68-74. DOI:

[27]. Kim HJ, Lee JY, Kang HA,Lee Y, Park EJ, Kim HJ. Oral immunization with whole yeast producing viral capsid antigen provokes a stronger humoral immune response than purified viral capsid antigen. Lett Appl Microbiol. 2014;58:285-291. DOI: 10.1111/lam.12188.1016/j.pep.2009.08.005

[28]. Malak A, Baronian K, Kunze G. Blastobotrys (Arxula) adeninivorans: a promising alternative yeast for biotechnology and basic research. Yeast. 2016;33:535-547. DOI: 10.1002/yea.3180

[29]. van Ooyen AJ, Dekker P, Huang M, Olsthoorn MMA, Jacobs DI, Colussi PA, Taron CH. Heterologous protein production in the yeast Kluyveromyces lactis. FEMS Yeast Res. 2006;6:381-392. DOI: 10.1111/j.1567-1364.2006.00049.x

[30]. Vieira Gomes AM, Souza Carmo T, Silva Carvalho L, Mendonça Bahia F, Skorupa Parachin N. Comparison of yeasts as hosts for recombinant protein production. Microorganisms. 2018;6(2):38. OI: 10.3390/microorganisms6020038

[31]. D.M. Maron and B.N. Ames, Mutat.Res., 113,173(1983).