Chapter

Saccharomyces: Is a Necessary Organism or a Biological Warrior?

Nilay Seyidoglu and Cenk Aydin

Abstract

Saccharomyces is a eukaryotic organism that possesses approximately 6,000 known genes since 1996. It has long been used for food, bakeries, drinks, and therapeutics due to its many ingredients and its role in several mechanisms. Saccharomyces can be used as an experimental organism for medicinal products in the pharmaceutical industry. Particularly in public health, the use of Saccharomyces in the production of vaccines is remarkable. It has been alleviated that this yeast helps clarify the function of individual proteins in pathogenic viruses. To clarify virus life and host interactions, virus replication systems in Saccharomyces were interested in scientists. The new antiviral strategies with yeasts suggest the biological mechanism of a pathogen virus. Due to the variety of diseases and current epidemic conditions, these organisms play an essential role in prevention and treatment. This chapter will try to update Saccharomyces' scientific discoveries with the most recent and up-to-date literature.

Keywords: Saccharomyces, pandemic diseases, experimental organisms, public health, antiviral strategies

1. Introduction

Besides poor treatment and vaccination programs, a healthy immune system and antioxidant mechanism are the essential defenders considering the current viral diseases. The viral diseases hosted in a body has several impacts on organs and systems. Also, long-term drug use or vaccination programs can cause some acute side effects on the body, such as gut microbiota, immunity, lung tissue, etc. Therefore, probiotics, prebiotics, vitamins, natural antioxidants have been generally recommended over the years. Probiotics named live microorganisms have beneficial effects on systems, and they have been used successfully. Prebiotics are non-digestible foods that stimulate intestinal tissue growth and modulate immunity. Vitamins, minerals, and natural antioxidants have been used to enhance immune activity and health in viral diseases. It can be said that all these supplements are essential for adequate homeostasis.

Today, evaluate the most effective, economical, and safe vaccines is a significant challenge. Thus, some crucial organisms have been interested in vaccine production as well as nutrition. Among the different vaccination process, yeasts have a broad interest in the scientific area (**Figure 1**). These unicellular and saprotrophic fungi have been used as a biological model. They have also been accepted as critical models for experiments due to their cellular structure, components, and rapid growth. Yeast also can be cultured easily and manipulated genetically. These features showed that

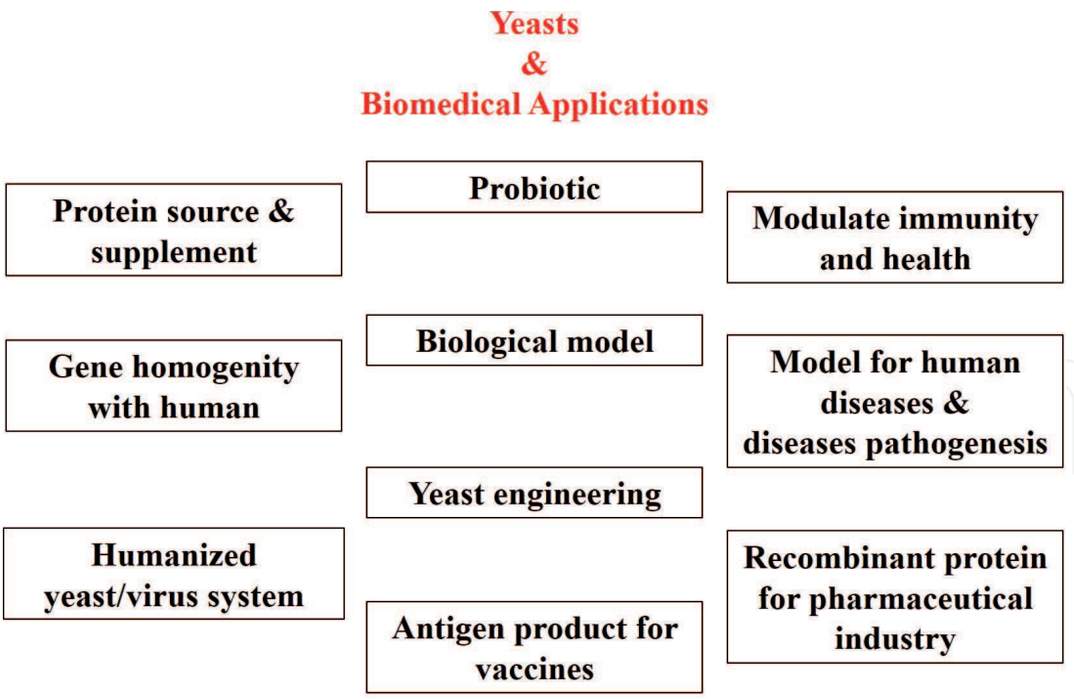

Figure 1.
Biomedical applications of yeasts.

yeasts are beneficial to identify the cellular mechanism of virus and vaccine programs safely [1, 2].

The yeast Saccharomyces, the essential eukaryotic organism, have been used as a biological model. Nevertheless, there is a notable gene homology in this yeast with human genes. In this chapter, we try to identify the Saccharomyces yeast as a useful model for biological experiments and observe the importance of viruses, viral diseases, and vaccines.

2. Saccharomyces

Saccharomyces cerevisiae is a model organism extensively used to investigate the biology of eukaryotic cells. It is widely used as a cell factory for producing pharmaceuticals, chemicals, and biofuels [3].

Saccharomyces, which is a genus belonging to the Saccharomyces fungus kingdom, includes many yeast species. The name of Saccharomyces is derived from the Latin words saccharo- (sugar) and - Greek mikes (mushrooms). These yeasts were initially suggested in 1680, and named Saccharomyces in 1837. A successful systemic concept on these higher eukaryotes was designed by Mayr [4]. Yeasts' cultured forms have been used for thousands of years due to rapid reproduction and essential components. Typical features of Saccharomyces are the usage of nitrate and ability for the fermentation of carbohydrates. Saccharomyces have an excellent capacity for ethanol production, and they are suitable yeasts for large-scale fermentation [5]. These important yeasts can be used for the food industry to produce several foods such as bread, beer, wine, distilled spirits, and industrial alcohols. The most knows are *S. cerevisiae, S. boullardii, S. pombe, S. pastorianus*, and *S. paradoxus*, mostly used for food and treatments. Nevertheless, these yeasts have a small nucleus and central vacuole and have glucan and mannoproteins on their cell walls. Saccharomyces include a single linear double-stranded DNA, ribosomal proteins, and non-ribosomal molecules, like other eukaryotes. It was suggested that their genetic structure is beneficial for the model organism, especially *S. cerevisiae* [6].

S. cerevisiae a single celled organism that is used as a model organism. These yeasts have been studied to understand the concept of cell cycle regulation, DNA repair, and other cellular mechanisms. It was also reviewed that a model to identify the mutations in the cell cycle in cancer and some diseases, especially neurodegenerative diseases [7]. However, a form of *S. cerevisiae* called *S. boulardii* had been observed in clinical trials for treatment such as inflammation and diarrhea. Mc Farland and Bernasconi reported that *S. boulardii* is a wild type of Saccharomyces, a pharmaceutical agent [8]. The action of *S. boulardii* has been described by releasing trypsin-like protease, which inhibits the toxins in inflammations [9].

Schizosaccharomyces pombe is a fission yeast that was isolated in 1893 by Paul Lindner from East African millet beer. It is a model organism for eukaryotic cell biology and molecular biology as well as *S. boulardii* and *S. cerevisiae*. In 1590, Mitchison was firstly studied with this yeast in an experimental organism. Eser et al. reported that it could be used to treat diabetes and other diseases [10]. This fission yeast has been studied for eukaryotic RNA metabolism due to its gene expression.

S. bayanus (S. eubayanus), S. paradoxus, and *S. pastorianus* have similar genome size with *S. cerevisiae*. They all have been studied for DNA reassociation studies [11]. *S. pastorianus* is a lager yeast, an interspecific hybrid between *S. cerevisiae* and another *S. bayanus (S. eubayanus)* [12]. It is also a synonym of *S. carlsbergensis* and closely related to genus *S. cerevisiae*. Another wild type of yeast, *S. paradoxus*, can be isolated from nature, especially tree exudates or oils. It is an essential type of yeast for genetic and genomic studies. A yeast named *S. bayanus (S. eubayanus)*, which was isolated from the tree, is related to *S. cerevisiae* and *S. pastorianus* [13]. *S. bayanus* has been used for genomic studies, expression patterns, and nucleosomes profiles [14–16].

Saccharomyces yeasts focus on the dietary field as a probiotic and the process of treating the disease. Belong the probiotic action; these yeasts have several vital roles on mechanisms such as bacterial adhesion, enhancement of immune cells and responses, modulation of the signaling pathways of the host, and improvement of the strengthening of enterocytes [17]. Nevertheless, Saccharomyces are used as model organisms in biological studies, particularly chemicals and pharmaceuticals.

3. Experimental organism for pharmaceutical industry

Over the last fifty years, remarkable progress in our ability to produce advanced drugs has improved people's health and longevity. Pharmaceutical proteins are one of the fastest-growing groups of medicines and are currently critical to treating many diseases [18].

Proteins have a catalyzer role in several metabolic reactions as well as an essential responsibility for cellular mechanisms. There are unique systems that can be used to produce proteins for the pharmaceutical industry from a single cell to multiple organisms, including eukaryotes, especially yeasts. Dozens of pharmaceutical proteins, including insulin, vaccines, and blood factors, produced by *S. cerevisiae*, have been commercialized. It was reviewed that yeasts are essential for biological activities, mainly producing the purified product due to its cost-effective, fast production like bacteria and high density of cell cultures [19]. In recent years, indeed, as a model organism, yeasts have been provided to identify the pathogenesis and role for diseases, especially *S. cerevisiae* and *S. pombe.*

The yeast Saccharomyces has been accepted as the significant organism for several metabolisms such as cell cycle, biogenesis, protein folding, genetic manipulation, recombination, etc. [20]. *S. cerevisiae* is a unicellular microbial organism that grows fast, tolerances to chemicals, and cultured easily. It was reported that

this yeast could discover the process of diseases because of the conservation of molecular interactions from yeast to humans [21, 22]. On the other hand, *S. cerevisiae* can be an essential organism for recombinant protein production for pharmacy. It has full cellular organelles and membrane compartments that produce many eukaryotic proteins, including humans' [23]. Initially, the essential biopharmaceuticals insulin and its analogs have been produced by *S. cerevisiae*. Researchers have reported other important biopharmaceuticals such as the human serum albumin, hepatitis vaccines, and virus-like particles for vaccination (**Table 1**). Also, several medicines have been produced with *S. cerevisiae* until 2012 reported by the European Medicines Agency [18]. Furthermore, current studies showed that metabolic engineering pathways and optimization procedures of *S. cerevisiae* are essential for producing recombinant proteins for pharmaceuticals and biomedical areas [18, 19]. *S. cerevisiae* carries out human-like glycoprotein that is efficient for producing recombinant proteins. Protein secretion of *S. cerevisiae* is complex processing that follows as transcription, translation, translocation, post-translational modifications, folding, peptide cleavage, glycosylation, sorting, and secretion. This important organism enables genetic modifications. It was reported that the first eukaryotic organism sequenced DNA in *S. cerevisiae* [41]. Due to the protein misfolding and aggregation, *S. cerevisiae* has been used as a model organism.

Nevertheless, *S. pombe* has been accepted as a model organism together with *S. cerevisiae*. This fission yeast is used as a successful host. It was reviewed that *S. pombe* and generated strains have significant facilitation for producing drug glucuronides [42, 43]. The classical yeast genetics approaches can be described for *S. pombe*. It has been accepted as the most ancient yeast molecule. However, *S. pombe* has been more advanced evolutionarily than other yeasts. *S. pombe* has become a model organism until 2002 [44, 45].

Recombinant proteins are recognized as an important part of the drug industry. Among these proteins, Saccharomyces has greater attention than others due to their eukaryotic properties, easy genetic manipulation, and capable of modifications. *S. cerevisiae* emerges as the most common host to express heterologous genes and therapeutic proteins [46]. This organism may provide a simple background for isotype expressions, and thereby drug metabolism studies can be easily associated with genome screens, underlying toxicity, and encoded genomes.

Bioparhamaceutical products	**Category**	**References**
Human serum albumin	Blood factors	Payne et al. [24]
Recombinant proteins	Protein	Huang et al. [18], Ferrer-Millares et al. [19], Ma et al. [25], Cino [26]
Insulin	Hormone	Martinez et al. [27]
Glucagon	Hormone	Egel-Mitani et al. [28]
Human parathyroid hormone	Hormone	Song et al. [29]
Purified protein for vaccines	Protein	Hadiji-Abbes et al. [30], Zhang et al. [31], King et al. [32], Kaslow and Shiloach [33], Fazlalipour et al. [34].
Virus like particles	Protein	Jacobs et al. [35], Kim et al. [36], Kim et al. [37].
Gene expression systems	Gene	Malak et al. [38], van Ooyen et al. [39], Vierira Gomes et al. [40].

Table 1.
Examples of bioparhamaceutical products of Saccharomyces.

4. Antiviral strategies

While the vaccines currently available have proven invaluable in the fight against infectious diseases and eradicating viruses, there are many drawbacks to the current vaccine preparation or application regimen despite these successes. Certain limitations of conventional vaccines require multiple adjuvants and injections to induce a necessary or optimal immune response. Another reason is the constant increase in the number of post-vaccination allergic reactions or hypersensitivities in a specific group of people [47, 48].

Today, there are several critical viral diseases such as human hepatitis B and C, immunodeficiency virus (HIV), severe acute respiratory syndrome coronavirus (SARS), coronavirus-disease 2019 (COVID19), etc. Due to the inadequacy of treatment options for these infections, new antiviral strategies and model organisms, particularly yeast, were of interest to the researchers.

Yeasts have a delivery system for nucleic acids, and thus they can be an alternative for virus description. Besides, a humanized yeast system was identified for yeast/virus systems to study diseases [49]. Yeasts are used for subunit vaccine formulations with producing antigens against viruses. It was reviewed that yeast can be used for vaccine development in such strategies; whole recombinant yeast, virus-like particles, yeast display, and purified protein immunogens [50]. Among yeasts, *S. cerevisiae* has been accepted as a versatile model organism for viruses' research, from the wire of public health to vaccine production.

Rosenfeld and Racaniello [51] reported that hepatitis C virus (HCV) was demonstrated in *S. cerevisiae*, and all proteins for the virus were encoded. Another study reported that *S. cerevisiae* could safely express the hepatitis B surface antigen in prophylactic vaccines [52]. Researchers observed that yeast could help clarify the function of viruses' proteins with dissection of RNA viruses' life cycle [53, 54]. Nevertheless, several protein immunogens can be purified from Saccharomyces. These immunogen proteins derived from yeasts are associated with virus-like particles. Virus-like particles can provide an alternative for viruses, and FDA approved this vaccine for hepatitis B and papillomavirus [55]. Also, the circumsporozoite protein derived from *S. cerevisae* is an immunodominant antibody of malaria. This preparation increased the antibodies and thereby neutralized the sporozoites [56]. Due to the yeast membrane permeability, *S. cerevisiae* enables entry to the chemical compounds and provides virus-host interactions. Some researchers showed that beta-glucan of the yeast cell wall could provide the immune response that important for vaccine development [57].

All things considered, the yeast-based carrier system can be a potential model to develop the vaccine insights of virus-host interactions. The yeast strategies can improve the recognition of pathogen antigens peptides, activate the immune response, and also modulate the yeast-based vaccines. Researchers for further pioneering findings have still endured the studies.

5. Future perspectives

There have been many illnesses that have not been controlled by vaccination and new ones as well. Mutation, genetic exchange, environmental and interspecific transference, or human contact are the most emerging diseases. However, new scientific technologies, model organisms and a number of researchers have proven beneficial to vaccination strategies. In this respect, it is possible to observe yeasts for the upcoming vaccines for several diseases.

Yeast engineered to the virus has been accepted as an ideal therapeutic approach. This vaccine's strategy is improving humoral immunity due to the ability of yeast to the generation of immune responses.

There is a numerous increasing study to obtain the vaccine strategy of yeasts. Studies in yeast proteins and cell wall components, including beta-glucan, may become more critical for vaccine strategies under different phases of clinical trials on animals or humans. According to the essential features of yeast, the yeast-based vaccine strategy is being necessary for vaccine development. It has foreseen that diversity of yeast strains will improve in the future.

6. Conclusion

The yeast system provides invaluable antiviral strategies. Significant studies have been conducted on yeast progression in the identification of viral diseases and antiviral strategies. Based on a better understanding of yeast protein and viruses, the search for new vaccines and medications for viral or pandemic diseases is safer and more effective. However, experiments with animal models and human cells are still underway in many types of yeast. Knowledge of these new biological systems and technologies, models, and organisms will open up new science avenues.

Conflict of interest

The authors declare no conflict of interest.

Acronyms and abbreviations

HIV	Human immunodeficiency virus
SARS	Severe Acute Respiratory Syndrome
COVID-2019	Coronavirus-disease 2019
FDA	U.S. Food and Drug Administration
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
S. cerevisiae	*Saccharomyces cerevisiae*
S. boullardii	*Saccharomyces boullardii*
S. carlsbergensis	*Saccharomyces carlsbergensis*
S. bayanus	*Saccharomyces bayanus*
S. pastorianus	*Saccharomyces pastorianus*
S. paradoxus	*Saccharomyces paradoxus*
S. ebayanus	*Saccharomyces ebayanus*
S. pombe	*Schizosaccharomyces pombe*

Appendices and nomenclature

Yeast	The most important eukaryote; Saccharomyces.
Single celled organism	*Saccharomyces cerevisiae*
Nucleosomes	DNA, RNA
Biopharmaceuticals	insulin and its analogs
Eukaryotes	The organisms whose cells have a nucleus enclosed within a nuclear envelope

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References

[1] Wickner RB, Fujimura T, Esteban R. Viruses and prions of *Saccharomyces cerevisiae*. Adv Virus Res. 2013;86:1-36. doi:10.1016/B978-0-12-394315-6.00001-5

[2] Walch B, Breinig T, Schmitt MJ, Breinig F. Delivery of functional DNA and messenger RNA to mammalian phagocytic cells by recombinant yeast. Gene Therapy.2012;19:237-245. DOI: 10.1038/gt.2011.121

[3] Nielsen J, Keasling JD. Engineering cellular metabolism. Cell. 2016;164(6):1185-1197. DOI: 10.1016/j.cell.2016.02.004

[4] Mayr E. Systematics and the Origin of Species. New York: Columbia University Press; 1942.

[5] Zhao XQ, Bai FW. Mechanisms of yeast stress tolerance and its manipulation for efficient fuel ethanol production. J Biotechnol. 2009;144:23-30. DOI: 10.1016/j.jbiotec.2009.05.001.

[6] Del Giudice L, Massardo DR, Pontieri P, Wolf K. Interaction between yeast mitochondrial and nuclear genomes: Null alleles of RTG genes affect resistance to the alkaloid lycorine in rho(0) petites of *Saccharomyces cerevisiae*. Gene. 2005;354:9-14. DOI: 10.1016/j.gene.2005.03.020

[7] 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.

[8] Mcfarland LV, Bernasconi P. Saccharomyces boulardii: a review of an innovative biotherapeutic agent. Microbial Ecology in Health and Disease. 1993;6:157-171. DOI: 10.3109/08910609309141323

[9] Pothoulakis C, Kelly CP, Joshi MA, Gao N, O'Keane CJ, Castagliuolo I, Lamont JT. *Saccharomyces boulardii* inhibits Clostridium difficile toxin A binding and enterotoxicity in rat ileum. Gastroenterology. 1993;104:1108-1115. DOI: 10.1016/0016-5085(93)90280-p

[10] Eser P, Wachutka L, Maier KC, Demel C, Boroni M, Iyer S, Cramer P, Gagneur J. Determinants of RNA metabolism in the *Schizosaccharomyces pombe* genome. Molecular Systems Biology. 2016;12(2):857. DOI: 10.15252/msb.20156526

[11] Stewart GG. Saccharomyces, Introduction. In: Batt CA, Tortorello ML, editors. Encyclopedia of Food Microbiology. 2nd edition. Amsterdam: Elsevier; 2014. p.297-301.

[12] Boynton PJ, Greig D. The ecology and evolution of non-domesticated Saccharomyces species. Yeast. 2014: 31(12):449-462. DOI: 10.1002/yea.3040

[13] Libkind D, Hittinger CT, Vale'rio E, Gonçalves C, Dover J, Johnston M, Gonçalves P, Sampaio JP. Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast. Proc Natl Acad Sci USA. 2011;108:14539-14544

[14] Guan Y, Dunham M, Caudy A, Troyanskaya O. Systematic planning of genome-scale experiments in poorly studied species. PLOS Computational Biology. 2010;6(3):e1000698. DOI: 10.1371/journal.pcbi.1000698

[15] Guan Y, Yao V, Tsui K, Gebbia M, Dunham MJ, Nislow C, Troyanskaya OG. Nucleosome-coupled expression differences in closely-related species. BMC Genomics. 2011;12:466. DOI: 10.1186/1471-2164-12-466. PMC 3209474. PMID 21942931

[16] Guan Y, Dunham MJ, Troyanskaya OG, Caudy AA. Comparative gene expression between two yeast species. BMC Genomics. 2013;14:33. doi:10.1186/1471-2164-14-33.

[17] Chen X, Kelly CP. Saccharomyces. In Versalovic J, Wilson M, editors. Therapeutic microbiology. Washington: ASM Press; 2008. p. 51-60 DOI: 10.1128/9781555815462.ch5

[18] 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

[19] 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

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

[21] Botstein D, Fink GR. Yeast: an experimental organism for modern biology. Science. 1988;240:1439-1443. DOI: 10.1126/science.3287619

[22] Smith MG, Snyder M. Yeast as a model for human disease. In: Curr Protoc Hum Genet. 2006. Chapter 15:Unit 15.6. DOI: 10.1002/0471142905.hg1506s48

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

[24] 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

[25] 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

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

[27] 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

[28] 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

[29] 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

[30] Hadiji-AbbesN,MartinM,BenzinaW, 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

[31] 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

[32] 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

[33] 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

[34] 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

[35] 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

[36] 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: 10.1016/j.pep.2009.08.005

[37] 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

[38] 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

[39] 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

[40] 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

[41] Goffeau A, Barrell BG, Bussey H, Davis RW, Dujon B, Feldmann H, Galibert F, Hoheisel JD, Jacq C, Johnston M, Louis EJ, Mewes HW, Murakami Y, Philippsen P, Tettelin H, Oliver SG. Life with 6000 genes. Science. 1996;274(5287):546, 563-547. DOI: 10.1126/science.274.5287.546

[42] Giga-Hama Y, Kumagai H. Expression system for forein genes using the fission yeast *Schizosaccharomyces pombe*. Biotechnology and Applied Biochemistry. 2000;30(3):235-244. DOI: 10.1111/j.1470-8744.1999.tb00776.x

[43] Drăgan CA, Buchheit D, Bischoff D, Ebner T, Bureik M. Glucuronide production by whole-cell biotransformation using genetically engineered fission yeast

Schizosaccharomyces pombe. Drug Metab Dispos. 2010;38(3):509-515. doi: 10.1124/dmd.109.030965

[44] Wood V, Gwilliam R, Rajandream MA, Lyne M, Lyne R, Stewart A, Sgouros J, Peat N, Hayles J, Baker S, Basham D, Bowman S, Brooks K, Brown D, Brown S, Chillingworth T, Churcher C, Collins M, Connor R, Cronin A, Davis P, Feltwell T, Fraser A, Gentles S, Goble A, Hamlin N, Harris D, Hidalgo J, Hodgson G, Holroyd S, Hornsby T, Howarth S, Huckle EJ, Hunt S, Jagels K, James K, Jones L, Jones M, Leather S, McDonald S, McLean J, Mooney P, Moule S, Mungall K, Murphy L, Niblett D, Odell C, Oliver K, O'Neil S, Pearson D, Quail MA, Rabbinowitsch E, Rutherford K, Rutter S, Saunders D, Seeger K, Sharp S, Skelton J, Simmonds M, Squares R, Squares S, Stevens K, Taylor K, Taylor RG, Tivey A, Walsh S, Warren T, Whitehead S, Woodward J, Volckaert G, Aert R, Robben J, Grymonprez B, Weltjens I, Vanstreels E, Rieger M, Schäfer M, Müller-Auer S, Gabel C, Fuchs M, Düsterhöft A, Fritzc C, Holzer E, Moestl D, Hilbert H, Borzym K, Langer I, Beck A, Lehrach H, Reinhardt R, Pohl TM, Eger P, Zimmermann W, Wedler H, Wambutt R, Purnelle B, Goffeau A, Cadieu E, Dréano S, Gloux S, Lelaure V, Mottier S, Galibert F, Aves SJ, Xiang Z, Hunt C, Moore K, Hurst SM, Lucas M, Rochet M, Gaillardin C, Tallada VA, Garzon A, Thode G, Daga RR, Cruzado L, Jimenez J, Sánchez M, del Rey F, Benito J, Domínguez A, Revuelta JL, Moreno S, Armstrong J, Forsburg SL, Cerutti L, Lowe T, McCombie WR, Paulsen I, Potashkin J, Shpakovski GV, Ussery D, Barrell BG, Nurse P. The genome sequence of *Schizosaccharomyces pombe*. Nature. 2002;415:871-880. DOI: 10.1038/nature724

[45] Hoffman CS, Wood V, Fantes PA. An ancient yeast for young geneticists: A primer on the *Schizosaccharomyces pombe* model system. Genetics. 2015;201:403-423. DOI: 10.1534/genetics.115.181503

[46] Darby RA, Cartwright SP, Dilworth MV, Bill RM. Which yeast species shall I choose? *Saccharomyces cerevisiae* versus Pichia pastoris (review). Methods Mol Biol. 2012;866:11-23. DOI: 10.1007/978-1-61779-770-5_2

[47] Shams H. Recent developments in veterinary vaccinology. Vet J. 2005;170(3):289-299. DOI: 10.1016/j.tvjl.2004.07.004

[48] Fang Y, Liu MQ, Chen L, Zhu ZG, Zhu RZ, Hu Q. Rabies post-exposure prophylaxis for a child with severe allergic reaction to rabies vaccine. Hum Vaccin Immunother 2016;12:1802-1804. DOI: 10.1080/21645515.2016.1143158

[49] Mager WH, Winderickx J. Yeast as a model for medical and medicinal research. Trends Pharmacol Sci. 2005;26:265-273. DOI: 10.1016/j.tips.2005.03.004

[50] Kumar R, Kumar P. Yeast-based vaccines: New perspective in vaccine development and application. FEMS Yeast Research. 2019;19. DOI: 10.1093/femsyr/foz007

[51] 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

[52] 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

[53] Blanco R, Carrasco L, Ventoso I. Cell killing by HIV-1 protease. J Biol Chem.

2003;278:1086-1093. DOI: 10.1074/jbc.M205636200

[54] Kapoor P, Lavoie BD, Frappier L. EBP2 plays a key role in Epstein-Barr virus mitotic segregation and is regulated by aurora family kinases. Mol Cell Biol. 2005;25:4934-4945. DOI: 10.1128/MCB.25.12.4934-4945.2005

[55] Zhang X, Xin L, Li S, Fang M, Zhang J, Xia N, Zhao Q. Lessons learned from successful human vaccines: delineating key epitopes by dissecting the capsid proteins. Hum Vaccine Immunotherapeutics 2015;11:1277-1292. DOI: 10.1080/21645515.2015.1016675

[56] Nussenzweig V, Barr P, inventors; New York University, Chiron Corporation, assignee. Vaccine against the sporozoite stage of malaria. Patent US4997647A. 1991.

[57] Karumuthil-Melethil S, Gudi R, Johnson BM, Perez N, Vasu C. Fungal βglucan, a Dectin-1 ligand, promotes protection from type 1 diabetes by inducing regulatory innate immune response. J Immunol 2014;193:3308-3321. DOI: 10.4049/jimmunol.1400186