Uses of *Saccharomyces cerevisiae* in the fight against COVID-19.

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Abstract. COVID-19 affected human health, the economy, and politics worldwide. In that context, many alternative diagnostic, immunization, and therapy methods applying genetic engineering in yeast were developed. *Saccharomyces cerevisiae* was proven a valuable model for many biotechnological and pharmaceutical applications. *S. cerevisiae*-based tools can enable more efficient, and faster vaccination and decrease inequalities in access to health resources. This review presented some research developed during the COVID-19 pandemic where S. cerevisiae is used as a vaccine carrier, diagnostic tool, antibody biological factory, and a study model for SARS-CoV-2.

Keywords. SARS-CoV-2. Genetic Engineering. Saccharomyces cerevisiae. Biosensor. Vaccine.

1. Introduction

The coronavirus disease 2019 (COVID-19) pandemic caused profound impacts on the economy, health, politics, and lifestyle globally; which led to numerous research projects seeking new ways to develop vaccines, therapies, and diagnostic tools [1].

COVID-19 is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The emergence of multiple variants in the word population causes mutations that can escape from the antibodies' activity and lead to greater transmission and vaccine resistance [2, 3]. Those variants may be selected in the presence of antibodies and eventually become prevalent. [4].

Another issue is that the COVID-19 symptoms not only can be shared by many other diseases but can also be quite inconsistent. Besides that, asymptomatic cases are frequently observed. Thus, numerous attempts have been made in order to discover high-precision diagnostic methods [5].

Nucleic acid-based diagnostics became used widely for their precision. On the other hand, they depend on RNA extraction - which exposes researchers to the virus -, expensive reagents and equipment, and may not detect new variants [5].

Biosensors are alternatives for the diagnosis of many diseases due to the low cost of production and the possibility of applying genetic engineering to enhance sensibility and accuracy [5].

The use of recombinant yeast as biosensors, vaccine carriers, and producers of heterologous products like bacterial, viral, protozoan, nematodes, or cancer antigens [6] offers several advantages such as easy genetic manipulation, non-pathogenicity, high stress-tolerance, molecular processes well conserved in high eukaryotic organism, and the ability to resist non-refrigerated storage conditions. The large amounts of information as a model organism and a resourceful genetic toolkit make *Saccharomyces cerevisiae* one of the best candidates as a model in the study of pathogens and the development of biotechnological solutions to disease.

In the current work, is presented an overview of *S. cerevisiae* uses in the study, prevention, diagnosis, and treatment of COVID-19.

2. Research Methods

The current paper presents a literature review of the applications of *S. cerevisiae* in the characterization of the SARS-CoV-2 virus, diagnosis of COVID-19 cases, and the development of vaccines, antibodies, and microbodies.

The MESH terms "*Saccharomyces cerevisiae*", "SARS-CoV-2", and "COVID-19" were combined on PubMed Advanced Search Builder.

3. Applications of *S. cerevisiae* during the COVID-19 outbreak

3.1 SARS-COV-2 characterization

During the early phases of an outbreak, having

isolates available to scientists and health authorities on a large scale is essential to develop appropriate responses, such as diagnostic tools, vaccines, and antivirals, in a short time [7].

Yeast systems were proven robust reverse-genetics platforms for RNA viruses owing to the ability to recombine overlapping DNA fragments in vivo. This technique, called transformation-associated recombination (TAR) cloning, makes it possible to generate large sequences of cDNA, which are often unstable in *Escherichia coli* systems due to the toxicity of the fragments in this organism [7]. TAR in yeast has been employed from pathway engineering to the construction of synthetic bacterial and viral genomes [8].

In 2020, Thao et al. [9] reconstructed SARS-CoV-2 in a week using a *S. cerevisiae* strain. Singh et al. [8] also obtained a synthetic attenuated SARS-CoV-2 genome in *S. cerevisiae* using multi-copy yeast vectors.

The SARS-CoV-2 RNA single strand encodes four main structural proteins: the spike, membrane envelope, and nucleocapsid proteins. The type I viral fusion protein spike (S) is involved in invasion processes. One of its components, the receptor binding domain (RBD) recognizes angiotensin-converting enzyme 2 (ACE2) on the surface of host cells from the human respiratory tract [9].

Greaney et al. [10] developed a mapping method with a yeast-display system to identify escape mutations to the SARS-CoV-2 RBD and applied it to 10 human monoclonal antibodies. The yeast cell displays RBD with a fluorescent label on its surface to quantify RBD expression and antibody binding by flow cytometry. The generated escape maps predict which mutations are selected in the presence of neutralizing antibodies and can inform the design of antibody cocktails.

Francino-Urdaniz et al. [11] also developed an S RBD yeast surface display (YSD) platform by integrating high-throughput screening platforms with deep sequencing to predict RBD escapability.

Genetic alterations in the ACE2 receptors can affect virus transmission and vaccine effectiveness. Heinzelman et al. [12] designed a yeast surface display-based model to understand how ACE2 mutations evolve and identified many mechanisms to increase spike biding.

S. cerevisiae moreover can be used in mutation prediction systems. Ou et al. [13] created a non-pathogenic yeast-based system to predict mutations in SARS-CoV-2 main or 3C-like protease (Mpro or 3CLpro) gene. Mpro is fundamental for virus replication and a drug target to medicines such as Paxlovid. It is highly conserved among all coronaviruses and has no human homologs, minimizing the possibility of side effects [14].

However, the intensive use of inhibitors acts as an evolutionary pressure that drives modifications which can grant major drug resistance [13].

Flynn et. al. [14] performed a mutational analysis without drug pressure of SARS-CoV-2 Mpro to provide information to design inhibitors. The obtained results indicate mutation-sensitive positions - ideal sites for inhibitors -, and mutation-tolerant positions that must be avoided.

Huisman et. al [15] and Liu et al. [16] developed high throughput yeast display approaches to assess peptide-MHC binding for large collections of peptide antigens and identified SARS-CoV-2-derived MHC binders. Major histocompatibility complex (MHC) proteins act in adaptive immunity by presenting antigen fragments. Although the use of computational algorithms is the fastest way to predict MHC-II ligands, it presents high false-positive rate and incomplete coverage of ligands, which makes experimental trials still necessary [16].

3.2 COVID-19 diagnosis

Biosensors are tools that use biological recognition elements and generate a detectable signal when in contact with the target [5]. Two popular yeast biosensing strategies are the signaling pathway coupled to G Protein-Coupled Receptors (GPCRs) and the Yeast Surface Display (YSD). CORONAYEAST has been developed by Maneira et al. [5] as a biosensor that combines the GPCRs with YSD.

He et al. [17] produced a whole-cell biosensor with llama-derived single-domain antibodies (nanobodies) for colorimetric detection of SARS-CoV-2 spike proteins, enabling the concentration and purification of SARS-CoV-2.

3.3 Development of vaccines and antibodies production

Peptide-based vaccines are a popular immunization method, yet they present stability-related issues and require special storage and transport conditions. Whole-cell vaccines represent a more suitable alternative to long-term storage and environmental conditions [6].

In nucleic acid vaccines, the DNA or RNA responsible for encoding target antigens is delivered to immune system cells. Thus, vaccine carriers should promote protection from those antigens. Yeasts like *S. cerevisiae* represent an attractive alternative for having a GRAS (Generally Recognized as Safe) status for the production of therapeutic proteins, and being more viable economically than other delivery mechanisms, such as nanoparticles and liposomes, besides promoting immunostimulation due to the properties of their wall cell composition [18].

Many yeast species are used to make whole yeast vaccines (WYV), with *S. cerevisiae* being the most used one. Furthermore, some yeast cell components

may act as adjuvants to stimulate or modulate immune response [6], improving WYV performance. Even though *S. cerevisiae* is considered non-pathogenic, its cell wall components can stimulate antigen presentation by interacting with dendritic cells and macrophage receptors [19, 20].

Yeast surface display (YSD) strategies are also used for vaccine production and allow them to be orally administered [6]. Using yeast as a delivery platform allows oral administration and other administration routes that wouldn't be possible with naked nucleic acids [18].

An *S. cerevisiae*-based oral vaccine developed by Gao et al. [21] showed promising mucosal, humoral, and immune responses in mice. RBD was expressed on the surface of yeast. Xing et al. [22] also made a similar mRBD vaccine, however, the first group kept the gal promoter in *S. cerevisiae*, while the second knocked out gal80. That way, gene expression could be induced in glucose medium, reducing time and costs in production.

Attenuated virus variants and vaccines might also be obtained by targeting viral enzymes. Ornelas et al. [23] developed a synthetic yeast tool for the identification and targeting of SARS-CoV-2 RNA capping enzymes.

Developing antibodies might be a slow process. Therefore, Wellner et al. [24] created the autonomous hypermutation yeast surface display (AHEAD), an antibody generation synthetic system that mimics the process of vertebrate somatic hypermutation using yeast.

4. Conclusion

Novel yeast-based strategies are valuable resources in diagnostic, vaccine development, and medical treatments. The low costs and rapidity make them a viable alternative to reduce the immunization gap and the inequitable access to health products between rich and low-income countries.

The SARS-CoV-2, among SARS-CoV, MERS-CoV, Ebola, showed us the importance of understanding evolution and transmission of diseases and creating quick responses to stop the spreading of RNA viruses. The mentioned approaches and technologies in this review can also be used in future viral outbreaks.

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6. References

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