Procedia of Engineering and Medical Sciences

VOLUME 9, ISSUE 2 | 2024

*Article*

# Inhibition Biofilm Producer *Candida albicans* Causing Thrust Candidiasis by Purified Invertase from *Saccharomyces Cerevisiae*

**Zainab Salim Hussin**[1]

1. Department of Microbiology, College of Science, Al-Karkh University of Science, Iraq
* Correspondence: zainabsalim88@kus.edu.iq

**Abstract:** In invasive oral candidiasis, a serious health issue in developing countries, *Candida albicans* is the most often isolated species. Thus, invertase was isolated from *Saccharomyces cerevisiae*, refined using acetone as an organic solvent, and then highly recovered by chromatographic techniques. One of the main manufacturers of biofilm is *Candida albicans*, which is the cause of oral candidiasis. After 72 hours of incubation, the percentage of biofilm inhibition reached a range of 78–89%. This indicates that invertase has a growing inhibitory impact on *Candida* biofilm development when concentration and incubation period are increased. Thus, the potential use of invertase as an antibiofilm agent against multi-drug resistance *Candida albicans*.

**Keywords:** *Candida albicans*, invertase, *Saccharomyces cerevisiae*

## 1. Introduction

The composition, metabolic activity, and pathogenicity of the incredibly diverse oral microbiota are influenced by a multitude of internal and external variables [1]. A variety of bacteria species occupy the oral cavity. One of the main factors contributing to infections in humans is thought to be Candida species [1, 2].

There are four different major kinds of oral candidosis that are regarded as discrete clinical entities [2]. These consist of two acute erythematous and pseudomembranous candidosis, which are temporary forms, and two long-term hyperplastic and erythematous candidosis, which are persistent forms [3].

Oral candidosis is a common fungal illness that affects the mucosa of the mouth. It comes in several forms. Oral candidosis, also referred to as "a disease of the diseased," is common in "the young, the old, and the sick" [2, 3]. Conventionally, the overabundance of Candida albicans on the mucosal surface is considered to be the most common cause of oral candidosis.

In addition to enriching colonization, *C. albicans* becomes more virulent when it interacts with oral bacteria. Greater *C. albicans* hyphae proportion and less tissue penetration are characteristics of multispecies oral bacterial and *Candida albicans* infections [4].

The carbohydrase known as invertase (β-fructofuranosidase, E.C. 3.2.1.26) converts sucrose into glucose and fructose by a catalytic process in an equimolar ratio, which is what is known as invert sugar [5]. One of the most common industrial uses of invertase is

**Citation:** Hussin, Z. S. Inhibition Biofilm Producer *Candida albicans* Causing Thrust Candidiasis by Purified Invertase from *Saccharomyces Cerevisiae*. Procedia of Engineering and Medical Sciences 2024, 9(2), 58-65.

Received: 10th April 2024
Revised: 17th April 2024
Accepted: 24th April 2024
Published: 1st May 2024

the generation of commercial invert sugar, which is chosen over acid hydrolysis for financial reasons. Additionally, microbial invertase has been used to synthesize short chain fructooligosaccharides, which are crucial for probiotics [6].

The possibility that organisms will be eliminated by antifungal medications and human defense mechanisms is decreased when candidal biofilms are present. Therefore, proper biofilm control is crucial [7]. Consequently, the primary objectives of this study were to isolate and purify invertase from Saccharomyces cerevisiae and examine its potential as an antibiofilm agent against *Candida albicans*, which causes thrust candidiasis.

## 2. Materials and Methods

### *2.1. Extraction of Yeast Invertase*

Five grams of lyophilized yeast cells were floated in one part water (deionized) to extract invertase from the yeast (1:1 w/w). Sodium carbonate (1%) and toulene (3%) were added. Cell autolysis was allowed to occur over the four days that the suspension was cultured at room temperature. At 4000 rpm and 4°C, the crude extract was centrifuged. After adding 1M sulfuric acid to bring the supernatant's pH to 4, it was let to settle overnight at 4°C. The same centrifugation method was used to remove the silt [8].

### *2.2. Invertase activity and protein content*

Using bovine serum albumin as a standard, the method by [9] was used to determine the protein content. According to [10], the reaction between 3,5-dinitrosalicylic acid and sugar reduction with transmittance at 490 nm was used to measure the activity of the invertase enzyme. A final reactive combination containing 40 mM sucrose was produced by mixing 120 mM sucrose solution as the substrate, 1.0 mL of extract, and 1.0 mL of acetate buffer, pH 5.0 in the reaction medium. In the blank test, the substrate was replaced with 1.0 milliliter of water. The reaction was conducted at 25 °C and pH 5.0. After ten minutes, 3,5-DNS was used to determine the content using glucose as the reference. An enzyme activity unit is the quantity of enzyme that causes a one-gram rise in glucose concentration of one μg $RS.min^{-1}$.

### *2.3. Purification of invertase*

Invertase was purified using a modified version of the procedure outlined by [10]. When the cold 96% ethanol was added to reach 50% (v/v) saturation, the isolated invertase precipitated at 4°C. The invertase-containing pellets were dialyzed overnight at 4°C against deionized water after being fully dissolved in deionized water. By centrifuging, material that precipitated during dialysis was eliminated. The resulting extract was placed onto column of DEAE-cellulose with (2 x30 cm), and a 0-0.6 M NaCI gradient was used for elution. On the column of Sephadex G-100 with (1.5 x 80 cm), the concentrated and pooled fractions which indicated that the most active levels were loaded. The column was eluted using the acetate pH 5.0 buffer at a flow rate of 0.5 ml/min. The fractions with maximum activity were then kept for later use after the measurement of protein concentration at 280 nm then invertase were measured.

### *2.4. Isolation and identification of Candida albicans*

24 clinical specimens of oral candidiasis were obtained from infected people. The specimens were then cultivated on CHROMagar Candida and Sabouraud dextrose agar, incubated at 35°C, and monitored every day for seven days. The Gram stain method was used to confirm that there was no bacterial contamination when yeast colonies were growing. The Vitek 2 System's carbohydrate absorption profiles were used to identify the yeasts.

*2.5. Screening of biofilm production*

The colony of each fungal isolate was grown for 24 hours at 37°C in Sabouraud dextrose broth. After the incubation time, fresh Sabouraud dextrose broth was used to prepare successive two-fold dilutions for each isolate, which were then incubated for 24 hours at 37°C. After that, the microtiter was empty, wiped with purified water, then flipped over to dry. 200 µl of 1% crystal violet was added to each well, and it was left to stand for 15 minutes before being washed with distilled water. Each well received about 200 µl of ethanol:acetone (80:20) mixture, and an Elisa reader was used to test the absorbency at 450 nm. The absorbency of the well containing sterile Sabouraud dextrose broth was used as a cut-off value. According to, samples are classified as positive if their absorbance is more than the cutoff value and negative if their absorbance is less than the cutoff value [11].

*2.6. Invertase as inhibitor for biofilm formation*

Invertase activity against the formation of biofilms is measured. By using a microtiter plate technique, 100 µl of chosen cell suspension was mixed with 100 µl of pure invertase in 50 mM acetate buffer, pH 5.0. As previously noted, the biofilm experiment was conducted and incubated for 24, 48, or 72 hours at 37°C, the percentage of biofilm inhibition was computed [12]. Biofilm inhibition percentage (%) = [O.D. control - O.D. treatment] / O.D. control x 100.

## 3. Results and Discussion

*3.1. Extraction of Saccharomyces cerevisiae invertase*

*Saccharomyces cerevisiae* invertase was isolated using the autolysis method and obtained as a crude extract with an activity of 2.12 U/ml. Within the tiny layer of space between the yeast's outer cell wall and plasma membrane, invertase is a glycoprotein classed as extracellular in nature [13]. Between sucrose's C (2) and O, the glucosidic bond is hydrolyzed by the enzyme invertase, also known as β-D-fructofuranosidase [14].

*3.2. Purification of invertase*

*Saccharomyces cerevisiae* crude enzyme solution containing isolated invertase was precipitated using 50% acetone saturation and had a specific activity of 11.3 U/mg. The goal of the dialysis procedure was to remove any leftover contaminants. A DEAE-Cellulose column was filled with the generated solution. Two protein peaks were seen following the elution with a 0-0.6 M NaCl gradient; the second protein peak included invertase activity, with 44.6 U/mg as a specific activity (Figure 1). The sephadex G-100 column was loaded with the active fractions, and following the elution, a single protein peak of dextranase activity of 92.1 U/mg protein, a yield of 69%, and a 34.7-fold purification as indicated in Figure 2 and Table 1.

**Table 1**. Invertase purification from *S. cerevisiae*

| Purification step | Size (ml) | Invertase activity (U/ml) | Protein conc. (mg/ml) | Specific activity (U/ mg) | Total activity | Purification fold | Yield (%) |
|---|---|---|---|---|---|---|---|
| **Crude extract** | 35 | 6.12 | 0.8 | 2.65 | 214.2 | 1 | 100 |
| **Acetone precipitation** | 14 | 9.5 | 0.6 | 11.3 | 152 | 4.26 | 70.9 |
| **DEAE-Cellulose** | 11 | 13.4 | 0.3 | 44.6 | 147.4 | 16.8 | 68 |
| **Sephadex G-100** | 11 | 15.9 | 0.19 | 92.1 | 174.9 | 34.7 | 69 |

In an aqueous solution, the selective precipitation of a protein is one of the most well-known, widely used, and simple to comprehend isolation procedures in protein purification [14].

In this purifying operation, yeast invertase has multiple benefits. First, with careful and selective application of conditions that injure the cell wall while maintaining the plasma membrane, the enzyme can be isolated from yeast cells ; second, The extracted enzyme is stabilized by the substantial oligosaccharide content, which either inhibits protein aggregation or reduces its susceptibility to protease attack and other undesirable events ; and third, changes in each subunit's sugar content create a smeared band to migrate, making the subunits easy to locate during SDS-PAGE examination [13].

The acetone precipitation method produced 56% recovery of the purified invertase from Candida guilliermondii, while chromatography produced 44.8% [10]. As shown by [15] enzymatic proteins can be separated from other analytes in the original extract using precipitation, dialysis, and chromatographic separation. Using the acetone: water method, [16] also recovered an invertase from S. cerevisiae, attaining an 89.9% recovery rate and a 2.1-fold purification factor.

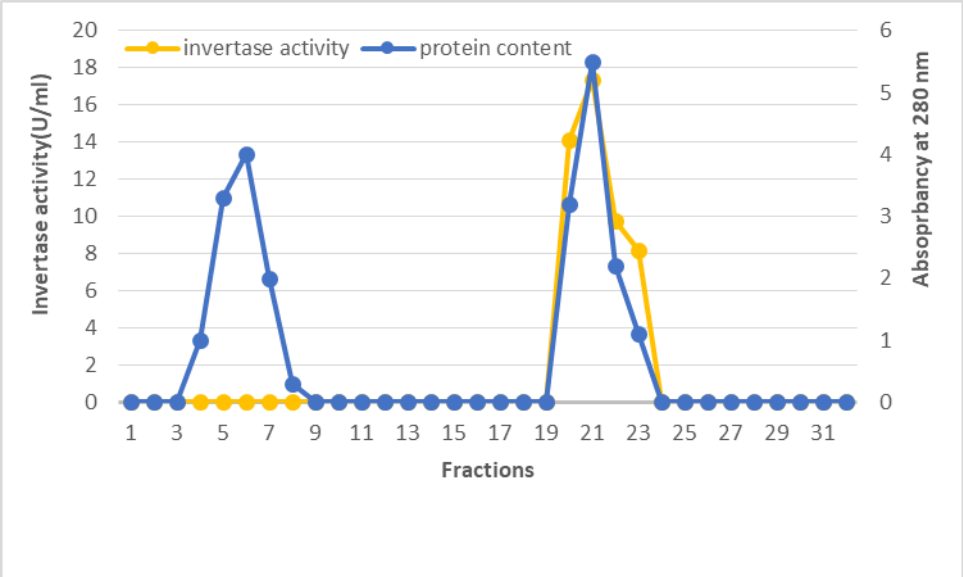


**Figure 1**. Purification of invertase from *Saccharomyces cerevisiae* by DEAE-Cellulose column

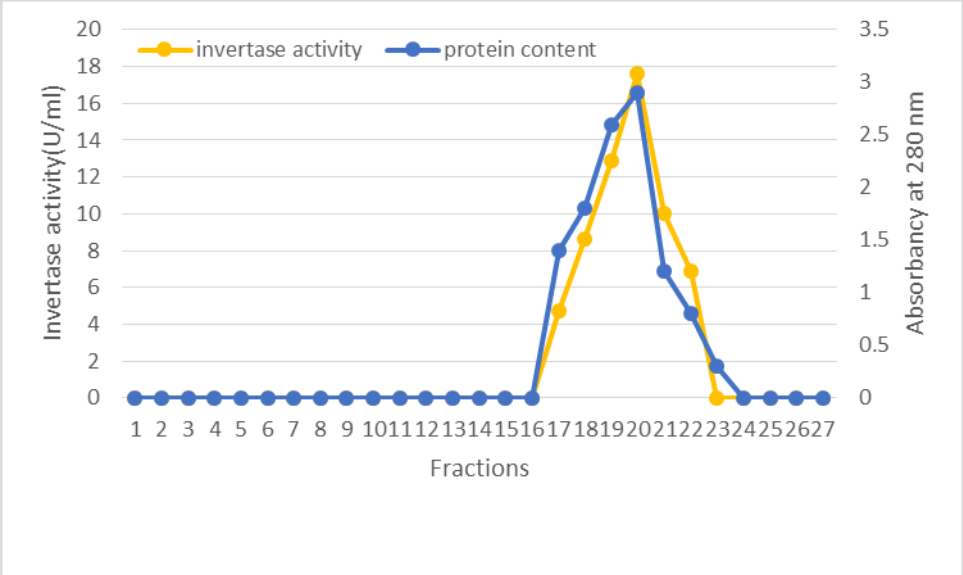


**Figure 2**. Purification of invertase from *Saccharomyces cerevisiae* by sephadex G-100 column

*3.3. Isolation and identification of Candida albicans*

In 24 cotton swab samples, seven isolates of Candida albicans were discovered in thrust candidiasis from affected individuals. They had colonies that were light greenish on chromogenic media, were oval to spherical in shape, and were gram positive.

The yeast that is most commonly isolated from individuals with oral cavity infections is *Candida albicans*. *Candida* species, particularly *Candida albicans*, *Candida glabrata*, *Candida krusei*, and *Candida tropicalis*, can be directly identified and isolated using CHROMagar *Candida* [17]. The most significant risk factor for Candida species colonization of the mucosa surface is prolonged denture wear, which may be enough to cause oral candidiasis [18]. The conditions that lead to oral candidiasis include fungal infections (mostly *Candida albicans*), poor dental hygiene, aging dentures, inadequate denture fit, and mucosal trauma [19].

*3.4. Screening of biofilm production*

As indicated in Table 2, every isolate of Candida albicans exhibited varying degrees of biofilm production capacity.

**Table 2**. Levels of production of Biofilm formation in *Candida albicans*

| **Fungal Isolates** | **Optical density at 450 nm** | **Density Level** |
|---|---|---|
| *Candida albicans1* | 0.59 | ++ |
| *Candida albicans2* | 0.73 | +++ |
| *Candida albicans3* | 0.57 | ++ |
| *Candida albicans4* | 0.72 | +++ |
| *Candida albicans5* | 0.19 | + |
| *Candida albicans6* | 0.67 | +++ |
| *Candida albicans7* | 0.25 | ++ |

One of *Candida albicans'* main virulence traits is its capacity to create biofilms, which are tightly packed cell communities stuck to a surface [20]. The ability of *Candida albicans* to infect a variety of host niches is made possible by several different virulence traits and fitness characteristics. The ability to alter morphologically between yeast and hyphae, adhesions and invasions on the cell surface, biofilm growth, phenotypic exchange, and the release of hydrolytic enzymes are examples of virulence factors [21].

*3.5. Invertase as biofilm inhibitor*

Utilizing all of the isolates of *Candida albicans*, invertase activity was examined in relation to biofilm formation. It was discovered that the saccharomyces cerevisiae purified invertase had antibiofilm activity against a range of isolates of *Candida albicans*. The inhibition of biofilm rose with increasing doses of pure invertase relative to the control group after a 24-hour period. At 225 μg/ml, the percentage of inhibition varied between 42 and 5% (Table 3). For the isolates of *Candida albicans*, over time, the biofilm inhibition percentage increased and, as shown in Figure 3, reached a range of 78-89% after 72 hours.

**Table 3**. Invertase as inhibitor for formation of biofilm by *Candida albicans*

| Isolate | Reduction of biofilm (%) | | |
|---|---|---|---|
| | At 75 µg/ml | At 150 µg/ml | At 225 µg/ml |
| *C. albicans1* | 35 | 41 | 49 |
| *C. albicans2* | 24 | 37 | 43 |
| *C. albicans3* | 22 | 29 | 42 |
| *C. albicans4* | 33 | 41 | 45 |
| *C. albicans5* | 35 | 43 | 55 |
| *C. albicans6* | 41 | 49 | 58 |
| *C. albicans7* | 37 | 46 | 51 |

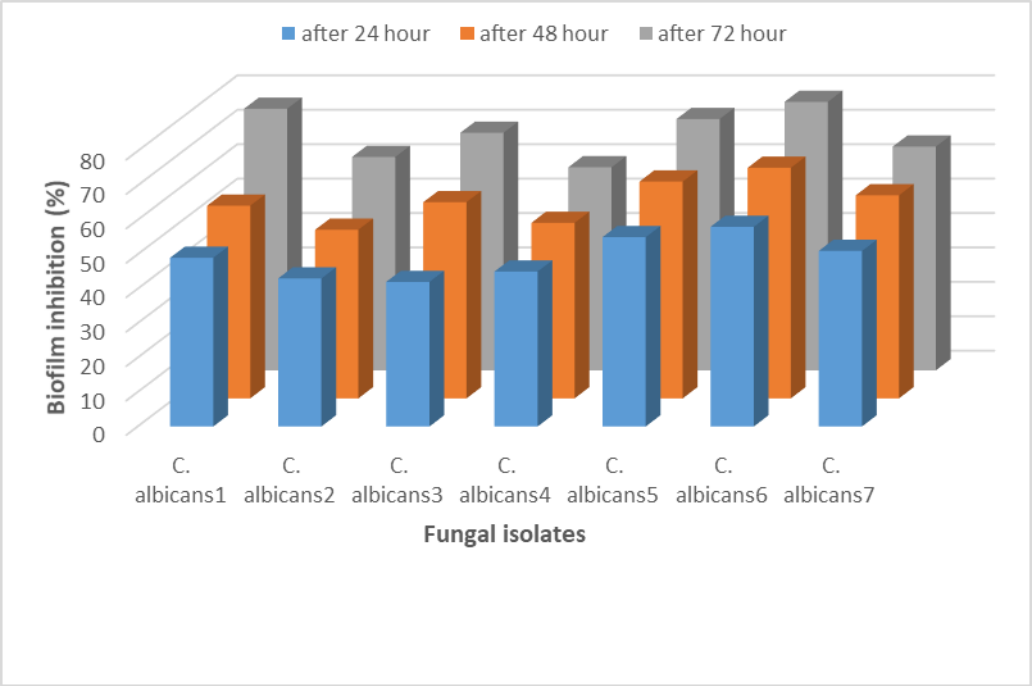


**Figure 3**. An inhibition of *Candida albicans* biofilm formation by invertase in different incubation periods

## 4. Conclusion

*Candida albicans* is the fungus that is most frequently linked to oral thrush, also known as oral candidiasis. The capacity of *Candida albicans* to create biofilms, which shields them from outside influences including the host immune system and antifungal medications, is one unique characteristic of their pathogenicity. As concentration and incubation duration are raised, the invertase's inhibitory effect on the formation of *Candida* biofilms grows. Consequently, invertase may be used as an antibiofilm agent to combat *Candida albicans* that is resistant to many drugs.

## REFERENCES

[1] S. H. Al-Amad, B. Rahman, N. Khalifa, and M. A. Awad, "Oral candidal carriage and its association with dental carious lesions in asymptomatic adults: a cross-sectional study from the UAE," *BMC Oral Health*, 2021, doi: 10.1186/s12903-021-01559-3.

[2] M. H. Arzmi, S. Dashper, and ..., "Polymicrobial interactions of Candida albicans and its role in oral carcinogenesis," *Journal of Oral Pathology …*, 2019, doi: 10.1111/jop.12905.

[3] A. Babatzia, W. Papaioannou, A. Stavropoulou, and ..., "Clinical and microbial oral health status in children and adolescents with type 1 diabetes mellitus," *International dental …*, 2020, [Online]. Available: https://www.sciencedirect.com/science/article/pii/S0020653920313757

[4] W. H. Bowen, R. A. Burne, H. Wu, and H. Koo, "Oral biofilms: pathogens, matrix, and polymicrobial interactions in microenvironments," *Trends Microbiol*, 2018, [Online]. Available: https://www.cell.com/trends/microbiology/fulltext/S0966-842X(17)30213-5

[5] J. Liu, J. Cheng, M. Huang, C. Shen, K. Xu, and ..., "Identification of an invertase with high specific activity for raffinose hydrolysis and its application in soymilk treatment," *Frontiers in …*, 2021, doi: 10.3389/fmicb.2021.646801.

[6] S. Kulshrestha, P. Tyagi, V. Sindhi, and K. S. Yadavilli, "Invertase and its applications–a brief review," *Journal of pharmacy …*, 2013, [Online]. Available: https://www.sciencedirect.com/science/article/pii/S0974694313002934

[7] Y. W. Cavalcanti, D. J. Morse, W. J. da Silva, and ..., "Virulence and pathogenicity of Candida albicans is enhanced in biofilms containing oral bacteria," *Biofouling*, 2015, doi: 10.1080/08927014.2014.996143.

[8] A. A. A. Aburigal, E. A. Elkhalifa, E. A. M. Sulieman, and ..., "Extraction and partial kinetic properties of invertase from Schizosaccharomyces pombe," *Int. J. Food Sci. Nutr …*, 2014, [Online]. Available: https://www.researchgate.net/profile/Abdel-Moneim-Sulieman-2/publication/265731926_Extraction_and_Partial_Kinetic_Properties_of_Invertase_from_Schizosaccharomyces_pombe/links/5652b40208ae4988a7af249e/Extraction-and-Partial-Kinetic-Properties-of-Invertase-from-Schizosaccharomyces-pombe.pdf

[9] B. Mm, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding," *Anal Biochem*, 1976, [Online]. Available: https://cir.nii.ac.jp/crid/1572543024174951680

[10] T. L. Avila, R. P. Toralles, E. T. Jansen, and ..., "Extraction, purification and characterization of invertase from Candida guilliermondii isolated from peach solid wastes," *Revista Brasileira de …*, 2022, [Online]. Available: https://www.scielo.br/j/rbf/a/FVLTxkyPNCZ5kcnfF47dLJp/

[11] M. B. Marak and B. Dhanashree, "Antifungal Susceptibility and Biofilm Production of Candida spp. Isolated from Clinical Samples," *Int J Microbiol*, 2018, [Online]. Available: https://www.hindawi.com/journals/ijmicro/2018/7495218/abs/

[12] K. J. Ryan, "Sherris Medical Microbiology, ed 4, 2004," *McGraw Hill*, 2004.

[13] A. P. Timerman, "The isolation of invertase from baker's yeast–an introduction to protein purification strategies," *Protein purification*, 2012, [Online]. Available: https://books.google.com/books?hl=en&lr=&id=YiyaDwAAQBAJ&oi=fnd&pg=PA29&dq=the+isolation+of+invertase+from+baker%27s+yeast+an+introduction+to+protein+purification+strategies&ots=Bd7DCCYf0M&sig=8orCTUqwcGsCGbUUgpr7dWyVKhU

[14] L. Cantarella, F. Alfani, and M. Cantarella, "β-D-fructofuranoside fructohydrolase," in *Whitaker, J.R., Voragen, A.G.J.; Wong, D.W.S. (ed.). Handbook of food enzymology*, New York: Marcel Dekker, 2013, pp. 787–804.

[15] U. Andjelković, S. Pićurić, and Z. Vujčić, "Purification and characterisation of Saccharomyces cerevisiae external invertase isoforms," *Food Chem*, 2010, [Online]. Available: https://www.sciencedirect.com/science/article/pii/S0308814609013053

[16] M. V Ferreira, T. L. AVILA, C. R. Kuhn, and ..., "Identifying yeast isolated from spoiled peach puree and assessment of its batch culture for invertase production," *Food Science and …*, 2016, [Online]. Available: https://www.scielo.br/j/cta/a/G8XLCSvv9LZrhBrZfzmVDbQ/?lang=en

[17] C. Manikandan and A. Amsath, "Isolation and rapid identification of Candida species from the oral cavity," *Int J Pure App Biosci*, 2013, [Online]. Available: https://www.academia.edu/download/85862405/isolation-and-rapid-identification-of-candidaspecies-from-the-oral-cavity.pdf

[18] B. H. Şen, K. E. Safavi, and L. S. W. Spångberg, "Colonization of Candida albicans on cleaned human dental hard tissues," *Arch Oral Biol*, 1997, [Online]. Available: https://www.sciencedirect.com/science/article/pii/S0003996997000265

[19] T. Klinke, B. Guggenheim, W. Klimm, and T. Thurnheer, "Dental Caries in Rats Associated with Candida albicans," *Caries Res*, 2011, [Online]. Available: https://karger.com/cre/article-abstract/45/2/100/85798

[20] Q. Du, B. Ren, X. Zhou, L. Zhang, and X. Xu, "Cross-kingdom interaction between Candida albicans and oral bacteria," *Front Microbiol*, 2022, doi: 10.3389/fmicb.2022.911623.

[21] F. Haque, M. Alfatah, K. Ganesan, and M. S. Bhattacharyya, "Inhibitory Effect of Sophorolipid on Candida albicans Biofilm Formation and Hyphal Growth," *Sci Rep*, 2016, [Online]. Available: https://www.nature.com/articles/srep23575