ORIGINAL ARTICLE

Volume 20 Issue 2 2025

DOI: 10.21315/aos2025.2002.OA03

ARTICLE INFO

Submitted: 12/06/2025
Accepted: 29/10/2025
Online: 22/12/2025

Identification of Yeast Species Isolated from Dental Plaque of Periodontitis Patients and Healthy Subjects Using TYI-S-33 Medium

Nurin Jazlina Nor-Azmi[a], Haslina Taib[a], Zeehaida Mohamed[b,c]*, Rosmaniza Abdullah[b], Suharni Mohamad[a]*

[a]*School of Dental Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia*

[b]*Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia*

[c]*Hospital Pakar Universiti Sains Malaysia, Health Campus, Jalan Raja Perempuan Zainab 2, 16150 Kubang Kerian, Kelantan, Malaysia*

*Corresponding author: zeehaida@usm.my; suharni@usm.my

To cite this article: Nor-Azmi NJ, Taib H, Mohamed Z, Abdullah R, Mohamad S (2025). Identification of yeast species isolated from dental plaque of periodontitis patients and healthy subjects using TYI-S-33 medium. *Arch Orofac Sci*, **20**(2): 143–150. https://doi.org/10.21315/aos2025.2002.OA03

To link to this article: https://doi.org/10.21315/aos2025.2002.OA03

ABSTRACT

Yeast is a ubiquitous microorganism commonly found in various parts of the human body, including the oral cavity. In healthy individuals, they typically exist as commensals; however, under specific conditions, they can transition to a pathogenic state, contributing to the onset and progression of diseases such as periodontitis. This study aimed to compare the composition and density of yeast species in the subgingival dental plaque of periodontitis patients and healthy subjects. Subgingival dental plaque samples were collected from 15 periodontitis patients and 15 healthy subjects. The samples were cultured in TYI-S-33 medium, and 16 viable isolates were analysed. Species identification was conducted using MALDI-TOF mass spectrometry, and yeast density was estimated using the McFarland method. Six species of yeast were identified among the isolates. A comparative analysis revealed no statistically significant differences in yeast species composition and density between the two groups. These findings highlight the diversity, prevalence, and density of yeast species in the oral cavities of periodontitis patients compared to those of healthy subjects, although their specific role in the pathogenesis of periodontitis remains inconclusive. Future research should focus on exploring the interactions between yeasts and other components of the oral microbiome to better understand their role in oral health and disease progression.

Keywords: *Candida; dental plaque; oral yeast; periodontitis; TYI-S-33 medium*

http://aos.usm.my/ ©Penerbit Universiti Sains Malaysia. 2025
This work is licensed under the terms of the Creative Commons Attribution (CC BY) (http://creativecommons.org/licenses/by/4.0/).

INTRODUCTION

The oral cavity is a complex ecosystem housing a diverse microbiome of bacteria, viruses, fungi, and protozoa, such as *Trichomonas tenax* that work synergistically to maintain oral health (Zhang *et al.*, 2020). Yeast species are prominent commensals in healthy individuals, contributing to microbial balance. However, under conditions of dysbiosis, yeast may transition into opportunistic pathogens, forming biofilms, interacting with virulent bacterial communities, evading host immune responses, and exacerbating disease progression (Slazhneva *et al.*, 2022; Kashyap *et al.*, 2024).

Periodontitis, a chronic inflammatory disease involving the progressive destruction of periodontal tissues and supporting structures, is a multifactorial condition closely linked to microbial imbalance (Tsuchida *et al.*, 2017; Deo & Deshmukh, 2019). Dysbiosis often promotes the colonisation and proliferation of yeast species, particularly *Candida* spp., further aggravating the disease. Periodontal pockets, a defining feature of periodontitis, provide an anaerobic, nutrient-rich environment that is highly conducive to yeast proliferation (Bosshardt, 2017).

Among the yeast species identified in the oral cavity, *Candida albicans* is the most extensively studied, recognised for its significant role in both oral health and disease. Other *Candida* species have also been isolated, such as *C. tropicalis*, *C. glabrata* and *C. dubliniensis*, highlighting the diversity of yeast species within the oral microbiome (Suresh *et al.*, 2019; Slazhneva *et al.*, 2022). Despite this, the diversity and density of yeast species within the biofilms of periodontal pockets, known as subgingival dental plaque, in periodontitis patients compared to individuals with clinically healthy gingiva remain underexplored.

This study aimed to identify and quantify yeast species in subgingival dental plaque samples from both groups, following the unexpected observation of yeast growth in Diamond's TYI-S-33 medium, originally used for *T. tenax* culture (Nor Azmi *et al.*, 2025). Yeast isolates were identified through Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS), and their density was estimated using the McFarland method.

Understanding the fungal component of the oral microbiome is essential for elucidating its roles in health and disease. As the oral cavity serves as a primary entry point for systemic infections, its microbial composition offers valuable insights into overall health status. Identifying differences in yeast composition between healthy and diseased states can advance our understanding of the potential contribution of yeasts to the aetiopathogenesis of periodontitis.

MATERIAL AND METHODS

Sample Collection

Subgingival dental plaque samples were obtained from 30 individuals with informed consent, consisting of 15 periodontitis patients and 15 healthy subjects with clinically healthy gingiva, allowing dental biofilm without attachment loss or periodontal pockets. Periodontitis was defined according to the 2017 classification criteria outlined by Caton *et al.* (2018), with study inclusion requiring periodontal pocket depths (PPD) greater than 4 mm at more than one tooth site and clinical attachment loss (CAL) greater than 3 mm. Individuals who had taken antibiotics or undergone dental treatment in the past three months were excluded from the study.

The sample collection was conducted at the Dental Clinic of Hospital Pakar Universiti Sains Malaysia (Hospital Pakar USM), Kelantan, Malaysia. This study involving human participants was approved by the Human Research Ethics Committee (HREC) of Universiti Sains Malaysia, with reference number USM/JEPeM/22090602.

Culture in TYI-S-33 Medium

The collected samples were centrifuged at 2,000 × g for 5 minutes. The resulting pellets were inoculated into pre-warmed Diamond's TYI-S-33 medium, following the protocol of El Sibaei *et al.* (2012). The cultures were incubated for 7 days with daily observations. To monitor growth, a drop of the inoculum was smeared onto a glass slide, stained with Giemsa and examined under the light microscope. On the seventh day, the cultures were transferred into cryogenic vial and stored at –80°C for further analysis.

MALDI-TOF MS

The viable isolates (n = 16) from the revived culture samples were subjected to MALDI-TOF MS (Bruker Daltonics-MALDI Biotyper® Sirius System, Biomed Global, Germany) analysis according to standard protocols. The protein spectra obtained from each isolate were compared against a reference database to identify the species at both the genus and species levels.

McFarland Method and Statistical Analysis

Each of the 16 yeast isolates was prepared and adjusted to match 0.5 McFarland standard, which corresponds to an optical turbidity of approximately 1.5×10^8 CFU/mL. A densitometer was used to directly measure the turbidity of the yeast suspension. The McFarland values of yeast suspensions from periodontitis patients and control subjects were compared to assess significant differences between the two groups using the Mann-Whitney U test. A significance level of $p < 0.05$ was considered statistically significant. All analyses were conducted using IBM SPSS Statistics version 28.0.1.

RESULTS

Identification and Prevalence of Yeast Species

Yeast growth was observed in 16 out of 30 revived dental plaque samples cultured in TYI-S-33 medium, with 10 samples originating from periodontitis patients and 6 from healthy subjects with clinically evident dental biofilm. Fourteen samples showed no growth, indicating non-recoverable isolates. Three genera – *Candida*, *Pichia* and *Diutina*, comprising six species which are *Candida tropicalis*, *Candida glabrata*, *Candida dubliniensis*, *Candida albicans*, *Pichia kudriavzevii* (formerly known as *C. krusei*), and *Diutina rugosa* (formerly known as *C. rugosa*) were identified from the isolates using MALDI-TOF MS. Fig. 1 illustrates notable differences in budding patterns and the presence of pseudohyphae as observed under microscopic examination of the isolates.

Among the identified species, *P. kudriavzevii* and *C. tropicalis* were present in isolates from both periodontitis and healthy subjects, with a prevalence rate of 31.2% (5/16) and 25.0% (4/16), respectively. *C. glabrata* and *C. albicans* were exclusively detected in isolates from periodontitis patients, whereas *C. dubliniensis* and *D. rugosa* were observed only in healthy subjects. Notably, *C. dubliniensis* was the most prevalent species among healthy subjects, accounting for 50.0% (3/6) of the isolates, while *D. rugosa* was identified in a single sample, representing the only instance of this species among all isolates (6.3%, 1/16). In contrast, among isolates from periodontitis patients, *P. kudriavzevii*, *C. tropicalis* and *C. glabrata* were evenly distributed, each with a rate of 30.0% (3/10), whereas *C. albicans* was the least prevalent species, with a rate of 10.0% (1/10).

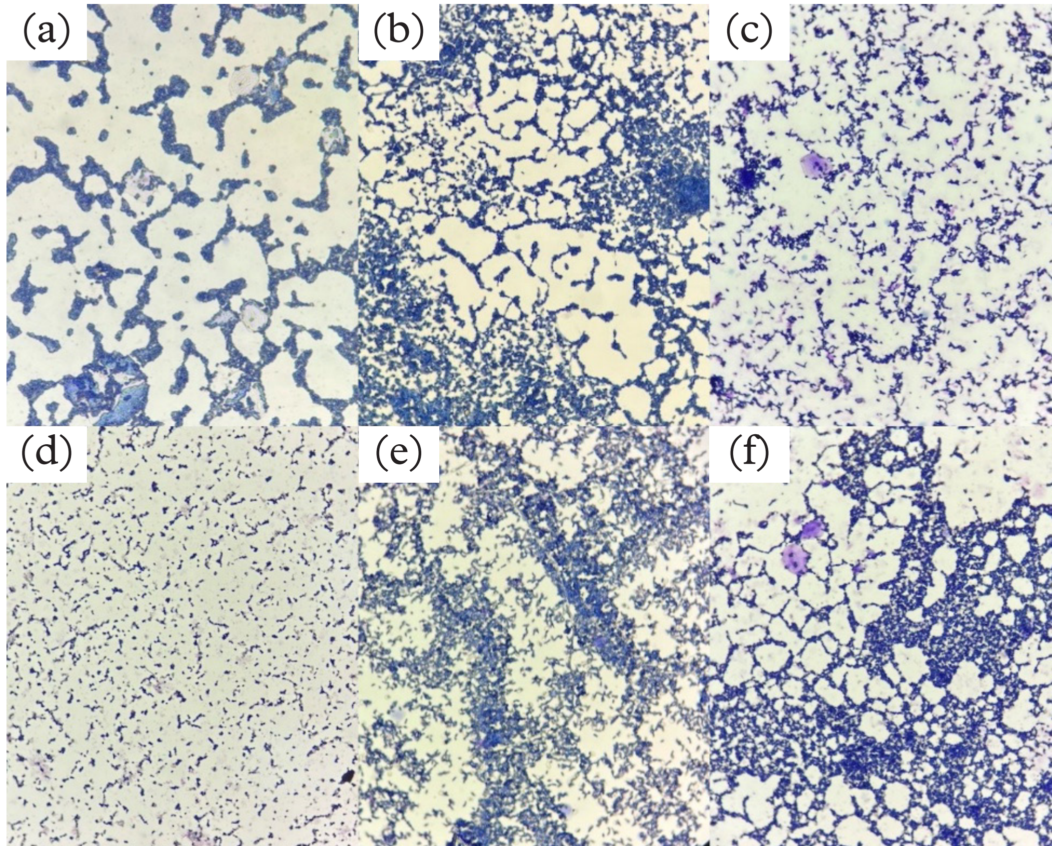

Fig. 1 Microscopic examination of yeast isolates stained with Giemsa and observed under 10× magnification using a light microscope. The species identified are as follows: (a) *C. albicans*; (b) *C. dubliniensis*; (c) *C. glabrata*; (d) *C. tropicalis*; (e) *P. kudriavzevii*; and (f) *D. rugosa*.

Yeast Density between Periodontitis Patients and Healthy Subjects

The density of yeast in isolates from periodontitis patients and healthy subjects was assessed using the McFarland method. Isolates from periodontitis patients exhibited higher McFarland values, ranging from 1.92 to 2.20 (SD = 0.089), while those from healthy subjects showed a slightly broader range, from 1.14 to 2.19 (SD = 0.385). As the McFarland values were not normally distributed, the Mann-Whitney U test was used to compare the two groups. The results indicated no significant difference between the groups (Z = 0.597, p = 0.562), suggesting that the McFarland values were similar across both groups as summarised in Table 1.

Table 1 Comparison of the median McFarland values between periodontitis patients and healthy subjects

Variable	Median (IQR)		Z- statistics	*p*-value*
	Periodontitis patients	Healthy subjects		
McFarland values	2.065 (0.14)	2.025 (0.37)	0.597	0.562

Note: *Normality assumption was not fulfilled. Mann-Whitney U test was applied.

DISCUSSION

Several yeast species have been identified as colonisers of the oral cavity, particularly within periodontal pockets, which provide a humid, nutrient-rich and anaerobic environment conducive for their survival and proliferation (Lasserre *et al*., 2018; Slazhneva *et al*., 2022). These species are capable of adhering to and penetrating the epithelial lining, potentially triggering inflammatory responses under conditions of dysbiosis (Sardi *et al*., 2010; 2013; Suresh *et al*., 2019). Such disruption to the oral microbiome may contribute to the development and progression of periodontitis (Bhuyan *et al*., 2022).

In this study, six species of yeast comprising of *C. tropicalis*, *C. glabrata*, *C. dubliniensis*, *C. albicans*, *P. kudriavzevii* and *D. rugosa* were identified from subgingival dental plaque of periodontitis patients and healthy subjects. These species were isolated using Diamond's TYI-S-33 medium, a selective growth medium originally designed for anaerobic protozoa such as *Entamoeba* and *Trichomonas* species (Diamond *et al*., 1978).

P. kudriavzevii (31.2%, 5/16) and *C. tropicalis* (25.0%, 4/16) were present in both periodontitis patients and healthy subjects, suggesting their role as commensal microorganisms in the oral cavity. However, their ability to act as opportunistic pathogens under specific conditions cannot be disregarded. In contrast, *C. glabrata* (18.8%, 3/16) and *C. albicans* (6.3%, 1/16) were exclusively detected in periodontitis patients, indicating their possible association with the development and progression of periodontal diseases. Conversely, *C. dubliniensis* (18.8%, 3/16) and *D. rugosa* (6.3%, 1/16) were only found in healthy subjects, which might indicate a non-pathogenic role in maintaining oral health.

Previous studies have consistently identified *C. albicans* as the most prevalent yeast species in the oral cavity, followed by *C. glabrata*, *C. tropicalis*, *P. kudriavzevii* and *C. dubliniensis* (Sardi *et al*., 2010). This aligns with the findings of De-La-Torre *et al*. (2018), who used oral rinse and periodontal pocket samples cultured on chromogenic agar plates and reported a high detection rate of *C. albicans* among patients with moderate chronic periodontitis (MCP) and severe chronic periodontitis (SCP), with prevalence rates of 55.8% and 91.0%, respectively. Other species reported included *C. glabrata* (0.12%, MCP only), *C. tropicalis* (3.2% MCP, 1.5% SCP), and *C. dubliniensis* (0.07% MCP, 0.5% SCP). In control subjects, *C. albicans* were detected at 66%, followed by *P. kudriavzevii* (15%), *C. glabrata* (7.4%) and *C. dubliniensis* (7.1%). Likewise, Jabri *et al*. (2022) reported detection rates of 37.0% for *C. albicans* and 9.0% for *C. dubliniensis* in periodontitis patients.

Slazhneva *et al*. (2022) reported that *C. albicans* exhibited the highest detection rates among chronic periodontitis patients, ranging from 7.5% to 100%, followed by *C. dubliniensis* (4.8% to 100%), *C. glabrata* (0% to 90.0%), *C. tropicalis* (0% to 40.0%) and *P. krudriavzevii* (3.3% to 18.3%). Similarly, among subjects with clinically healthy periodontium, *C. albicans* remained the most prevalent with detection rates ranging from 9.1% to 47.8%, followed by *P. krudriavzevii* (0% to 10.0%), *C. dubliniensis* (0% to 8.7%), *C. tropicalis* (0% to 6.7%) and *C. glabrata* (0% to 2.0%).

Our study, however, revealed different patterns of yeast detection. Among periodontitis patients, *P. kudriavzevii* was identified in 40.0% (4/10) of the isolates, followed by *C. tropicalis* and *C. glabrata* both at 30.0% (3/10), and *C. albicans* at 10.0% (1/10). Among healthy subjects, *C. dubliniensis* was the most frequently detected species with a rate of 50.0% (3/6), followed by *P. kudriavzevii* and *C. tropicalis* at 16.7% (1/6). Neither *C. albicans* nor *C. glabrata* was detected in the healthy subjects of our study.

Interestingly, *D. rugosa*, which was identified in 6.3% (1/16) of the isolates has rarely been reported in the oral cavity. None of the studies included in the systematic review documented its presence. Nevertheless, previous reports by Pires-Gonçalves *et al.* (2007) and De-La-Torre *et al.* (2018) have documented *D. rugosa* in the oral cavity. Al-Manei *et al.* (2023) reported its detection in the oral cavity of patients with head and neck cancer, while Esquivel *et al.* (2023) identified it in veterinary contexts.

The discrepancies between previously reported detection rates and our findings could be attributed to our limited sample size and variations in study populations, diagnostic criteria of periodontitis used for study inclusion, sampling methods, and geographical factors, that may influence the prevalence of these yeast species in the oral cavity. The unexpectedly low prevalence of *C. albicans* observed in this study could be linked to the use of TYI-S-33 medium, which may not provide the optimal conditions for the growth of *C. albicans*. This species is a well-known dominant commensal of the oral cavity and is commonly associated with inflammatory periodontal diseases, due to its adaptability and ability to coexist with both commensal and pathogenic bacteria, including *Porphyromonas gingivalis*, *Prevotella intermedia* and *Aggregatibacter actinomycetemcomitans* (De-La-Torre *et al.*, 2018; Slazhneva *et al.*, 2022).

Quantitative analysis of yeast density using the McFarland method revealed that isolates from periodontitis patients had a narrower range of McFarland values (1.92 to 2.20), with median of 2.065 (IQR = 0.14), indicating an increased yeast density in the periodontitis group, likely due to the favourable conditions within the periodontal pockets. In contrast, isolates from healthy subjects had a broader range of McFarland values (1.14 to 2.19) with median of 2.025 (IQR = 0.37), suggesting greater variability in yeast load. This variability could be influenced by individual factors such as oral hygiene, medication and dietary habits.

Despite these trends, the Mann-Whitney U test (Z = 0.597, p = 0.562) showed no significant difference in McFarland values between periodontitis patients and healthy subjects, suggesting that the overall yeast density was similar between the two groups. An outlier was also observed in a single isolate from the healthy subjects harbouring *D. rugosa*, with a significantly lower McFarland value of 1.14 compared to other isolates. This anomaly may represent either a reduced yeast load or potential contamination during the culturing process, as the presence of *D. rugosa* in the oral cavity is uncommon. Therefore, molecular confirmation is necessary to ensure accurate identification, particularly for rare isolates.

CONCLUSION

This study is the first to demonstrate the diversity, prevalence, and density of yeast species between periodontitis patients and individuals with clinically healthy gingiva using subgingival dental plaque cultured in TYI-S-33 medium. Despite the limited sample size and use of culture medium not optimised for yeast growth, the consistent finding of a higher yeast load observed in periodontitis patients, along with the distinct species distribution, highlights the significance of this study. These observations warrant the need for further research into the role of yeast in shaping the oral microbiome. Additionally, exploring the potential association between yeast species and the development or progression of periodontal diseases is essential. Future studies should employ larger cohorts and optimised culture conditions to investigate biofilm formation, interactions between yeast and other microorganisms within the oral cavity, and their influence on inflammatory pathways. The comprehensive understanding of the dynamics and synergistic interactions within the oral microbiome remains a mystery waiting to be unveiled.

ACKNOWLEDGEMENTS

The authors extend their heartfelt gratitude to Associate Professor TS. Dr. Wan Muhamad Amir bin Wan Ahmad from the School of Dental Sciences, Universiti Sains Malaysia, for his valuable guidance and expertise in statistical analysis. The authors also sincerely thank all participants for their contributions, as this study would not have been possible without their involvement. Furthermore, the authors would like to acknowledge the Ministry of Higher Education Malaysia for awarding the MyBrainSc scholarship to Nurin Jazlina Nor Azmi, which provided essential financial support for her studies and research. This manuscript was financially supported by Ministry of Higher Education Malaysia for Fundamental Research Grant Scheme (FRGS) with project code: FRGS/1/2022/SKK0/USM/02/7.

REFERENCES

Al-Manei K, Sobkowiak MJ, Nagadia RH, Heymann R, Sällberg Chen M, Özenci V (2023). Mycobiota profile of oral fungal infections in head and neck cancer patients receiving radiotherapy: A 6-year retrospective MALDI-TOF mass spectrometry study. *Oral Oncol*, **146**: 106556. https://doi.org/10.1016/j.oraloncology.2023.106556

Bhuyan R, Bhuyan SK, Mohanty JN, Das S, Juliana N, Juliana IF (2022). Periodontitis and its inflammatory changes linked to various systemic diseases: A review of its underlying mechanisms. *Biomedicines*, **10**(10): 2659. https://doi.org/10.3390/biomedicines10102659

Bosshardt DD (2017). The periodontal pocket: Pathogenesis, histopathology and consequences. *Periodontol 2000*, **76**(1): 43–50. https://doi.org/10.1111/prd.12153

Caton JG, Armitage G, Berglundh T, Chapple ILC, Jepsen S, Kornman KS *et al.* (2018). A new classification scheme for periodontal and peri-implant diseases and conditions—Introduction and key changes from the 1999 classification. *J Clin Periodontol*, **45**(Suppl 20): S1–S8. https://doi.org/10.1111/jcpe.12935

De-La-Torre J, Quindós G, Marcos-Arias C, Marichalar-Mendia X, Gainza ML, Eraso E *et al.* (2018). Oral *Candida* colonization in patients with chronic periodontitis. Is there any relationship? *Rev Iberoam Micol*, **35**(3): 134–139. https://doi.org/10.1016/j.riam.2018.03.005

Deo PN, Deshmukh R (2019). Oral microbiome: Unveiling the fundamentals. *J Oral Maxillofac Pathol*, **23**(1): 122–128. https://doi.org/10.4103/jomfp.JOMFP_304_18

Diamond LS, Harlow DR, Cunnick CC (1978). A new medium for the axenic cultivation of *Entamoeba histolytica* and other *Entamoeba*. *Trans R Soc Trop Med Hyg*, **72**(4): 431–432. https://doi.org/10.1016/0035-9203(78)90144-x

El Sibaei MM, Abdel-Fattah NS, Ahmed SA, Abou-Seri HM (2012). Growth kinetics, antigen profiling, and proteinase activity of Egyptian *Trichomonas tenax* isolates derived from patients having oral infections. *Exp Parasitol*, **130**(4): 416–422. https://doi.org/10.1016/j.exppara.2012.01.018

Esquivel BD, Rouse Salcido EM, Schweiker AM, Holder BL, KuKanich B, KuKanich KS *et al.* (2023). Fungal diversity and drug susceptibility of the oral mycobiome of domestic dogs. *Front Vet Sci*, **10**: 1281712. https://doi.org/10.3389/fvets.2023.1281712

Jabri B, Iken M, Ait-Ou-Amar S, Rida S, Bouziane A, Ennibi OK (2022). *Candida albicans* and *Candida dubliniensis* in periodontitis in adolescents and young adults. *Int J Microbiol*, **2022**: 4625368. https://doi.org/10.1155/2022/4625368

Kashyap B, Padala SR, Kaur G, Kullaa A (2024). *Candida albicans* induces oral microbial dysbiosis and promotes oral diseases. *Microorganisms*, **12**(11): 2138. https://doi.org/10.3390/microorganisms12112138

Lasserre JF, Brecx MC, Toma S (2018). Oral microbes, biofilms and their role in periodontal and peri-implant diseases. *Materials*, **11**(10): 1802. https://doi.org/10.3390/ma11101802

Nor Azmi NJ, Taib H, Mohamed Z, Zakaria NZ, Nik Mohd Noor NZ, Mohamad S (2025). Exploring the prevalence of oral trichomonas in patients with periodontitis at a tertiary teaching hospital. *Acta Trop*, **268**: 107696. https://doi.org/10.1016/j.actatropica.2025.107696

Pires-Gonçalves RH, Miranda ET, Baeza LC, Matsumoto MT, Zaia JE, Mendes-Giannini MJ (2007). Genetic relatedness of commensal strains of *Candida albicans* carried in the oral cavity of patients' dental prosthesis users in Brazil. *Mycopathologia*, **164**(6): 255–263. https://doi.org/10.1007/s11046-007-9052-5

Sardi JC, Duque C, Mariano FS, Peixoto IT, Höfling JF, Gonçalves RB (2010). *Candida spp.* in periodontal disease: a brief review. *J Oral Sci*, **52**(2): 177–185. https://doi.org/10.2334/josnusd.52.177

Sardi JCO, Scorzoni L, Bernardi T, Fusco-Almeida AM, Mendes Giannini MJS (2013). Candida species: Current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options. *J Med Microbiol*, **62**: 10–24. https://doi.org/10.1099/jmm.0.045054-0

Slazhneva E, Tikhomirova E, Tsarev V, Orekhova L, Loboda E, Atrushkevich V (2022). Candida species detection in patients with chronic periodontitis: A systematic review and meta-analysis. *Clin Exp Dent Res*, **8**(6): 1354–1375. https://doi.org/10.1002/cre2.635

Suresh UA, Nisha KJ, Shruthi S, Guru S, Batra P (2019). Candida species in periodontal disease: A literature review. *IP Int J Periodontol Implantol*, **4**(4): 124–129. https://doi.org/10.18231/j.ijpi.2019.027

Tsuchida S, Satoh M, Takiwaki M, Nomura F (2017). Ubiquitination in periodontal disease: A review. *Int J Mol Sci*, **18**(7): 1476. https://doi.org/10.3390/ijms18071476

Zhang X, Sadowski P, Punyadeera C (2020). Evaluation of sample preparation methods for label-free quantitative profiling of salivary proteome. *J Proteomics*, **210**: 103532. https://doi.org/10.1016/j.jprot.2019.103532