

## Pilot Study: Anticandidal Probiotics Activity Against Oral Candida Isolates in Patients with Potentially Malignant Disorders

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### Abstract

Recently, probiotics have displayed benefits against oral potentially malignant disorders (PMDs) and oral cancer through various mechanisms including apoptosis and immunomodulation. However, the antifungal activity of probiotics against *Candida* isolates from PMDs has not yet been evaluated. The aim of this study was to evaluate the role of probiotics as potential antifungal agents against *Candida* associated with PMDs. Swabs were collected from 7 different outpatients visiting the Department of Oral Medicine and Radiology, Coorg Institute of Dental Sciences, Virajpet, who were diagnosed with oral PMDs. These swabs were subjected to microbiological studies. A semi-quantitative analysis of *Candida* inhibition by probiotics was performed using the agar overlay method. The isolates were identified based on colony color. The percentages of various grades of inhibition of different *Candida* species were calculated. In total, 14% of PMD isolates showed epithelial dysplasia, and *Candida* growth was observed among 71% of clinical isolates. The species isolated included *Candida albicans*, *Candida tropicalis*, and *Candida parapsilosis*. *C. albicans* and *C. tropicalis* showed 66% inhibition and *C. parapsilosis* showed 100% inhibition with probiotics. Overall, 71% of the clinical isolates were inhibited by probiotics. Probiotics could be a valuable adjunct or therapeutic agent in the management of *Candida*-associated PMDs.

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### Introduction

Oral cancer accounts for 3% of all malignancies worldwide. It is the 12<sup>th</sup> most common cancer in women and the 6<sup>th</sup> most common cancer in men. Oral squamous cell carcinoma accounts for 92–95% of all oral cancers.<sup>1</sup> Oral potentially malignant disorders (PMDs) are conditions that precede the onset of oral cancer. Lesions classified as PMDs include oral leukoplakia, erythroplakia, oral lichen planus, and oral submucous fibrosis.<sup>2,3</sup>

Several *in vitro* and clinical studies have suggested an association between *Candida* in PMDs and oral cancer with dysplasia and

increased malignant potential.<sup>4–11</sup> Antifungal resistance is one of the major concerns with the use of conventional antifungals. These agents are further limited by their adverse effects such as hypokalemia, hepatotoxicity, dysgeusia, gastrointestinal discomfort, neuromuscular effects, rhabdomyolysis, etc.<sup>12–16</sup> Hence; there is a need for potential alternatives.

Probiotics are live microorganisms that can provide health benefits to the host when administered in sufficient quantity.<sup>17</sup> They have been found to be beneficial in maintaining gastrointestinal and urinary tract health.<sup>18, 19</sup> Recent studies indicate their potential benefits in cancers including oral cancer. Their role in the prevention of oral cancer progression has been explained through mechanisms such as apoptosis and immunomodulation.<sup>20–22</sup>

The use of probiotics as potential antifungal agents against *Candida* isolated from PMDs has not yet been studied. Hence, this study was designed to evaluate whether probiotics inhibit clinical isolates of *Candida*

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obtained from patients with oral PMDs, and to explore the role of probiotics as an antifungal agent or adjunct in the management of PMDs considering its lower toxicity and other health benefits.

### Materials and methods

#### Source of Study:

The study group was comprised of patients who visited the outpatient department of Oral Medicine and Radiology, Coorg Institute of Dental Sciences, Virajpet, from April to July 2019, who had been clinically diagnosed with an oral PMD by an oral medicine specialist with more than 10 years of clinical experience. The oral lesions were confirmed histopathologically.

#### Inclusion Criteria:

- Patients older than 18 years of age.
- Patients who were clinically and histopathologically diagnosed with an oral PMD with positive *Candida* growth on CHROMagar.
- The following PMD categories were included:
  1. Oral leukoplakia
  2. Oral lichen planus

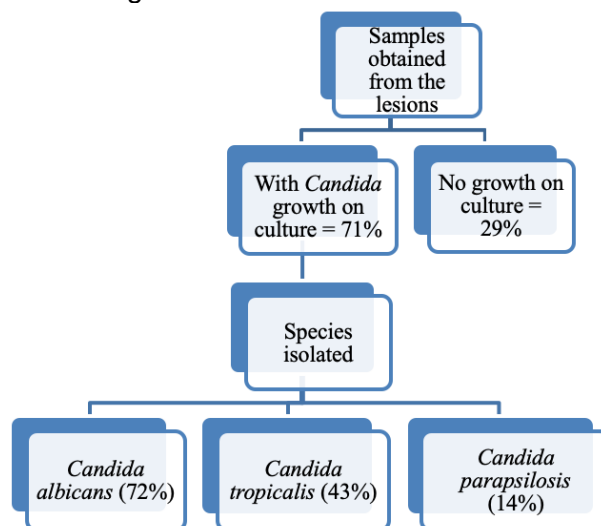
#### Exclusion criteria:

1. Patients who had been on antifungal therapy two weeks prior to sample collection.
2. Patients who were on systemic and intraoral topical steroids two weeks prior to sample collection.
3. Patients who stated a history of antibiotic intake and use of mouthwash in the last two weeks.
4. Patients with uncontrolled diabetes and systemic conditions such as bleeding and clotting disorders that contraindicate biopsy.
5. Patients diagnosed with immuno-compromised conditions.

### Results

Samples were obtained from 7 oral PMD lesions of 7 different patients. No growth was observed in samples obtained from 2 patients. Among the 5 oral lesions that displayed positive *Candida* growth, 4 were diagnosed as oral

leukoplakia and 1 as oral erosive lichen planus. The predominant species in both categories was *Candida albicans*. The percentages of various *Candida* species isolated from the samples are shown in figure 1.



**Figure 1.** Flowchart showing the quantified results of the isolated colonies.

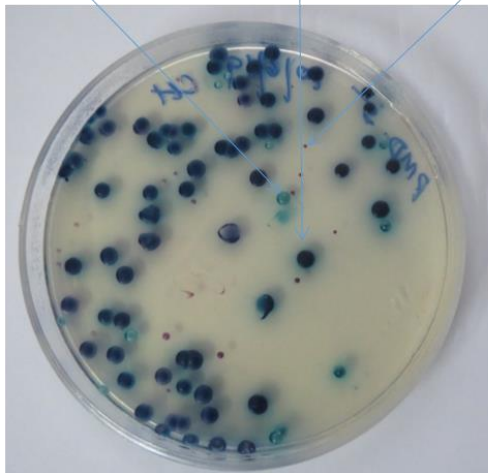
In the oral leukoplakia cases with positive *Candida* growth, *C. albicans* was observed in all cases, *Candida tropicalis* was observed in 75% (3 out of 4) of cases, and *Candida parapsilosis* was observed in 25% (1 out of 4) of cases. *C. albicans* was the only species observed in oral lichen planus cases.

The histopathological examination showed that 1 of the lesions with a clinical diagnosis of oral leukoplakia was diagnosed with severe dysplasia. No dysplasia was reported in the other lesions. The growth of multiple *Candida* species (*C. albicans*, *C. tropicalis*, and *C. parapsilosis*) was observed in the sample obtained from the severely dysplastic lesion (figure 2). The varying degrees of inhibition of these species are shown in table 1.

SPECIES OF CANDIDA % INHIBITION BY PROBIOTICS	<i>Candida albicans</i>	<i>Candida tropicalis</i>	<i>Candida parapsilosis</i>
NO INHIBITION (-)	33	33	0
MINIMAL INHIBITION (+/-)	33	0	0
PARTIAL INHIBITION (+)	0	33	0
TOTAL INHIBITION OVER STREAK (++)	0	0	100
TOTAL INHIBITION OVER AND BEYOND STREAK (++++)	33	33	0
<b>TOTAL INHIBITION</b>	<b>66%</b>	<b>66%</b>	<b>100%</b>

**Table 1:** Species-wise inhibition by probiotics.

*Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*



**Figure 2.** CHROMagar medium showing different *Candida* species.

The zone of inhibition from the probiotics is shown in figure 3 and was measured as follows:

(-) = no *Candida* growth inhibition over the probiotic culture.

(+/-) = minimal inhibition of *Candida* growth over the probiotic culture (clear zone observed over less than 50% of the total length of the streak).

(+) = partial inhibition of *Candida* growth over the probiotic culture (clear zone observed over more than 50% of the total length of the streak).

(++) = total inhibition of *Candida* growth over the probiotic culture (clear zone observed over the entire length of the streak).

(+++)= total inhibition of *Candida* growth over and beyond the probiotic culture (clear zone observed over the entire length of the streak and extending beyond the probiotic streak).

Probiotics inhibited 66% of the overall *C. albicans* and *C. tropicalis* species and 100% of the *C. parapsilosis* species.

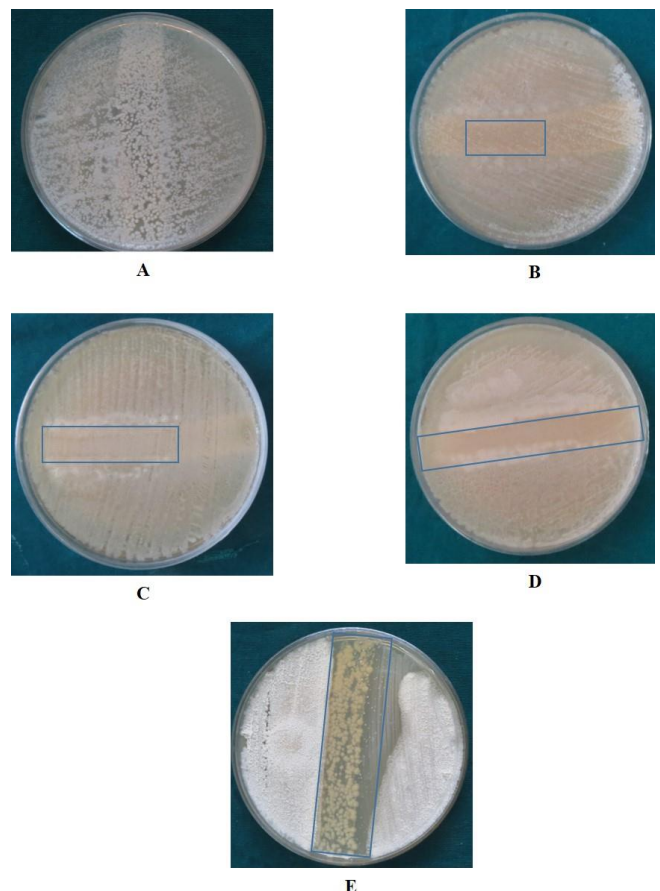
The rectangular box in figure 3 shows the clear zone (where there is no *Candida* growth).

- A) (-) = no *Candida* growth inhibition over the probiotic culture.
- B) (+/-) = minimal inhibition of *Candida* growth over the probiotic culture (clear zone observed over less than 50% of the total length of the streak).
- C) (+) = partial inhibition of *Candida* growth

over the probiotic culture (clear zone observed over more than 50% of the total length of the streak).

- D) (++) = total inhibition of *Candida* growth over the probiotic culture (clear zone observed over the entire length of the streak).

E) (+++) = total inhibition of *Candida* growth over and beyond the probiotic culture (clear zone observed over the entire length of the streak and extending beyond the probiotic streak).



**Figure 3.** Mueller-Hinton agar showing zones of inhibition.

### Discussion

Although the literature confirms the association of *Candida* with PMDs, the exact role of *Candida* organisms in epithelial dysplasia has not yet been established. However, studies performed by Krogh et al have suggested that these organisms might play an important role in oral carcinogenesis due to their ability to produce N-nitrosobenzylmethylamine.



N-nitrosobenzylmethylamine is a carcinogen that can act alone or in combination with other chemical compounds and can activate specific proto-oncogenes that could trigger the development of a cancerous lesion.<sup>6</sup> Additionally, in a study conducted by McCullough et al, the carriage rate of *Candida* was found to be correlated with epithelial dysplasia.<sup>9</sup> Hence, the treatment of *Candida* infection could possibly prevent malignant transformation and potentially reverse dysplasia.

Although antifungals (polyenes, azoles, and echinocandins) have been used to treat *Candida* infections, the existence of side effects and resistance patterns from these treatments are well established. Probiotics are live microorganisms that are widely used to treat diseases associated with the gastrointestinal tract. However, in recent studies, probiotics have been shown to play a beneficial role in the maintenance of oral health and oral cancer. Apart from having general health benefits, probiotics have also displayed the following specific benefits:

1. Antifungal activity by reducing filamentation and biofilm development.
2. Immunomodulation by inhibiting T cell activity, keratinocyte apoptosis, regulating levels of cytokines, and mast cell degranulation.
3. Inducing apoptosis in oral cancer KB cells through the upregulation of PTEN and the downregulation of MAPK signaling pathways.<sup>11, 17-22</sup>

Meurman et al. (2007), Hasslof et al. (2010), Mailander-Sanchez D et al. (2017), and Lijun Hu et al. (2019) all reported that probiotics (*Lactobacillus* species and *Bifidobacterium* species) have antifungal activity against *Candida* species in the oral cavity.<sup>17, 27-29</sup>

In this study, a commercially available probiotic (FLORA-SB) containing a combination of spores of *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium longum*, and *Bifidobacterium bifidum* was used to assess the anti-*Candida* activity.

Two samples in this study did not show any *Candida* growth and hence were not included in the analysis. Among the samples that showed *Candida* growth, the species isolated included *C. albicans*, *C. tropicalis*, and *C. parapsilosis*. The highest prevalence of *C. albicans* (72%) in the present study is comparable with that of earlier studies by

McCullough et al. in 2004 and Rindum et al. in 1994, who found that *C. albicans* comprised 78% of the isolated yeasts, making it the most frequent *Candida* species in precancerous and cancerous disorders.<sup>9, 30</sup> The species isolated from patients with oral leukoplakia included *C. albicans*, *C. tropicalis*, and *C. parapsilosis*, with the highest prevalence of *Candida albicans*. Conversely, in the oral lichen planus sample, only *C. albicans* was isolated. These results are similar to those in studies performed by F Gall et al. (2012) and Sarkar and Rathod (2014) in which *C. albicans* was found to be the predominant species in oral leukoplakia and oral lichen planus compared to non-*albicans*.<sup>4, 31</sup>

Overall, *C. albicans* and *C. tropicalis* each displayed 66% inhibition, and *C. parapsilosis* displayed 100% inhibition. Direct comparisons with earlier studies regarding *Candida* inhibition are not possible due to the lack of literature regarding the antifungal activity of probiotics in PMDs. In a randomized controlled trial, Hattaka et al. reported a reduction in the prevalence of *C. albicans* and *C. tropicalis* in the saliva of 90 elderly patients who used probiotics.<sup>19</sup> Mendonça FH et al. found that there was an approximately 8% reduction in *C. albicans* and *C. tropicalis* and complete inhibition of *C. parapsilosis*.<sup>32</sup> Furthermore, 1 of the 7 (14%) samples in the present study had epithelial dysplasia. Multiple *Candida* species (*C. albicans*, *C. tropicalis*, and *C. parapsilosis*) were observed in this sample. Earlier studies have also found an association between *Candida* and epithelial dysplasia.<sup>9, 33</sup>

It remains largely unknown whether the type of PMD influenced the results of this study because of the small sample size.

#### Limitations:

This study is limited by its small sample size, lack of a positive control (the methodology used in this study cannot be used for comparisons between a standard drug and probiotics), failure to establish the minimum inhibitory concentration, and lack of comparisons with *Candida* from healthy people without oral PMDs.

Hence, further *in vitro* studies with a robust sample size that evaluate the minimum inhibitory concentration of different species of probiotics against clinical isolates of *Candida* followed by clinical randomized controlled trials with long-term follow-up could confirm our results

and clarify the role of probiotics as a therapeutic agent or an adjunct in the management of PMDs.

## Conclusions

The probiotics used in this study showed varying grades of inhibition against the clinical isolates of *Candida* obtained from oral PMDs. Further in vitro and clinical trials are recommended to evaluate the role of probiotics in PMDs.

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## Declaration of Interest

The authors declare no conflicts of interest.

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