

# ANTI-FUNGAL EFFICACY OF PROBIOTICS IN HEAT CURE DENTURES BASE RESIN: AN INVITRO STUDY

## Abstract

**Aim:** To assess if probiotics alter the adherence of candida species onto the denture base material made with Polymethylmethacrylate(PMMA).

**Materials and Method:** Sample number of 10 per denture base resin with Sample dimensions of length 10 mm × 10 mm and width 2 mm.

**Test group:** PMMA samples coated with probiotics at varying concentrations dipped in saliva containing candida species.

**Control group:** PMMA samples in saliva containing candidal species without probiotics.

**Results:** Significant difference of candida cell count on PMMA was evident before and after giving probiotic. On the heat cure denture base materials, there was a significant decrease in Candidal cell counts when the probiotic concentrations (2.5, 5, 10, 15 and 20 µg/ml) increased.

**Key word:** oral cavity, dentures, PMMA, Candida infection, Prophylaxis

## Introduction:

“Geriatric problems became more frequent with the increase in life expectancy. The oral cavity harbors a lot of microorganisms Either as a commensal and/or as a pathogen, Candida species has attracted the attention of many investigators”.[1]. “Owing to its ability to cause an infection when the host defence mechanism is either lowered or rendered inadequate, Candida albicans has been referred as a notorious opportunistic pathogen among the entire range of candida species. Candidiasis is a common disease among the elderly causing oral thrush in denture wearers. Apart from the medications available, the products that could help in treating oral candidiasis is vital for dentists”.[1,2] “As the denture wearing population increase around the globe, the incidence of denture biofilm-related problems, such as malodour, denture- stomatitis and aspiration pneumonia increases proportionately. It has been strongly suggested that, probiotic bacteria consumption may improve oral health”.[2,3]

However, the effect of probiotics on the microbial status of denture wearers remains unclear. The acrylic prostheses (i.e) dentures in edentulous individuals, possess a nonshedding, hard surface thereby facilitating candidal adhesion and subsequent fungal colonization.[4] The ability of the yeast to adhere to the epithelial cells superficially and to the fitting denture surface remains as the essential prerequisite for colonization of candida species and results in making the denture as a reservoir of infection. In fabrication of a denture bases, a lot of materials has been incorporated in the literature.[1,5] “Acrylic and metals among them are frequently used. Compare to acrylic denture bases (Polymethylmethacrylate), the advantage of metallic denture bases is its impermeable hydrophobic surfaces and are also considered superior in many respects”.[6] But, “Polymethylmethacrylate (PMMA) has been employed widely in denture fabrication owing to its ease and simplification in the fabrication technique. There may be a certain variation in the factors influencing the adherence of *C. albicans*, based on the type of material used in denture base fabrication”.[7,8] However, literature is scarce in reference to the *C. albicans* adherence in these materials. Hence, the aim of the following study is to assess whether probiotics alter Candidal adherence onto the denture base material, taking PMMA into consideration in this study.

### **Materials and Methods:**

To evaluate the adherence of *C. albicans* to PMMA denture base materials, an invitro study was conducted. Sample number of 10/denture base resin with Sample dimensions of length 10 mm × 10 mm with a width of 2 mm.

**Test group:** PMMA samples coated with probiotics at varying concentrations dipped in saliva containing candida species.

**Control group:** PMMA samples in saliva containing candidal species without probiotics.

### **Preparation of PMMA Specimens:**

Using a metal die, test samples were fabricated. A die was manufactured in such a way that it had 10 slots with dimension 10 mm×10 mm and 2 mm thick in each slot. The melted Modeling wax was poured into the die having these slots and was then allowed to cool to room temperature after which excess wax was removed using a wax knife. This process was repeated again until a total of 10 wax squares were made using the preformed die. Flasking of the samples were done after being taken out of the die. Polymethylmethacrylate

Heat cured denture resins (DPI, Mumbai, India) were used for packing the mould. According to the manufacturer's instruction, the samples were fabricated by mixing the liquid and powder. The flasks were secured in a dental clamp for 30 minutes followed by polymerization in an **acrylizer unit** (Confident company, India). A long curing cycle of 70° for 9 hours was carried out to reduce the residual monomer content which helped in obtaining good transparency of the material. Using standard techniques, the cured resin were then finished and polished according to the manufacturer's instructions.

### **Saliva Samples and Probiotic coating:**

After acquiring the PMMA discs of required dimensions, they were subjected to artificial aging of six months by immersion in the saliva collected from patients with clinically diagnosed Oral Candidiasis. A sample was then collected from the disks as a baseline data for culture of candidal species.

Then the Probiotic solution (Yakult probiotic) was prepared in different concentrations, namely 2.5, 5, 10, 15 and 20 mg/mL. Commercially available probiotic lozenges were diluted and used for this purpose. It contained *Lactobacillus casei* Shirota =  $6.5 \times 10^6$ , *L.sporogenes* =  $10 \times 10^6$  and *Bifidobacterium* species =  $2 \times 10^6$  according to the manufacturer. The formed disks were dipped in the prepared probiotic solution for a period of 48 hours at 37°C. A swab sample was collected from each disk after immersion in probiotic solution as **test group** for culture of candidial species.

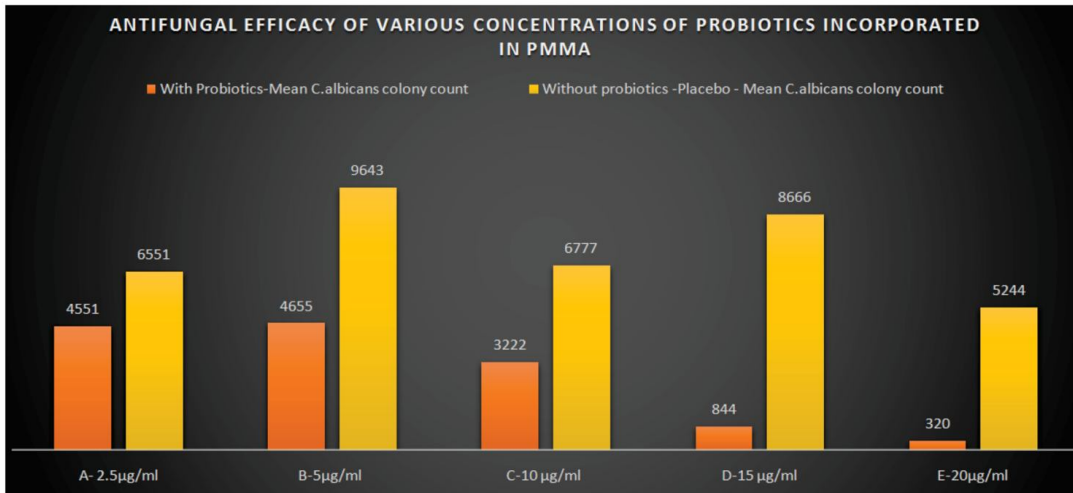
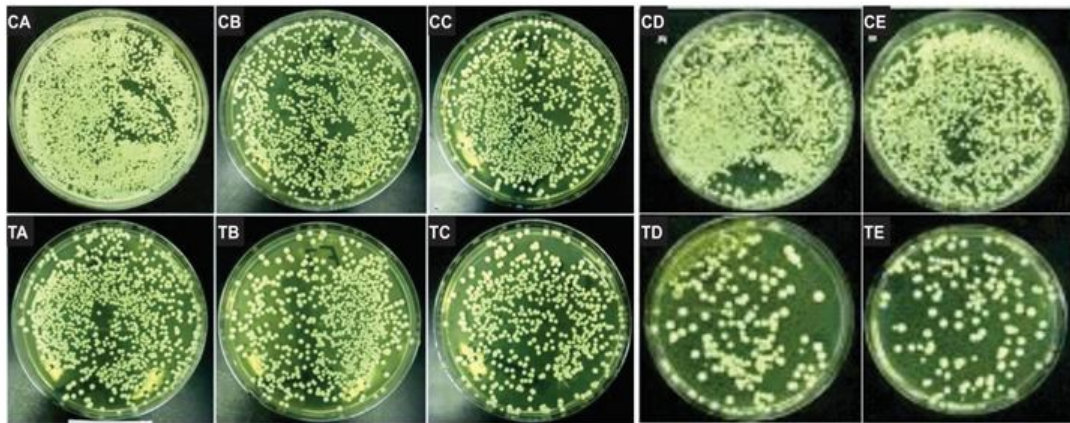
### **Culture preparation and Colony Counting:**

The preparation of culture and the growth of *Candida albicans* over the prepared specimens were assessed through microbial analysis. Swab samples collected from the disks before and after immersion in probiotic (Baseline and test samples) were smeared on the culture media in petri dishes and a incubation cycle of 24 hours at 37°C was carried out inside the ultraviolet chamber. After 24 hours, Candidal colonies were formed on the media. After the incubation period; using sterile forceps, the specimens were removed to avoid any contamination. The specimens were fixed on a glass slide using methyl alcohol for 1 minute followed by draining. Over the specimen that was placed on a glass slide, A drop of cedar-wood oil was applied and observed under oil immersion lens (1000×). For all the specimens, the same procedure was repeated as mentioned above and the cells were counted microscopically. The average candidal cells on the disks before and after introducing

probiotic were calculated using manual counting method and the data was tabulated and analysed using statistical analysis.

**RESULTS:**

10 samples of heat cure denture base materials were prepared and compared to assess the adherence of Candida (Candidal colony cell count) over them, It was done before and after coating with probiotic solution (Fig 1). Results suggest that the difference among average Candidal cell counts on heat cure denture base materials before and after giving probiotic is significant. Candidal cell counts decreased significantly with increase in probiotic concentrations on the heat cure denture base resin (Fig.2).



DISKS	MEAN VALUE OF C. ALBICANS COLONY COUNT - WITHOUT	PROBIOTIC CONCENTRATION (µg/ml)	MEAN VALUE OF C. ALBICANS COLONY COUNT -

	<b>PROBIOTICS</b>		<b>WITH PROBIOTICS</b>
DISK 1	6551	2.5	4551
DISK 2	9643	5	4655
DISK 3	6777	10	3222
DISK 4	8666	15	844
DISK 5	5244	20	320

<b>PROBIOTIC CONCENTRATION (µg/ml)</b>	<b>2.5</b>	<b>5</b>	<b>10</b>	<b>15</b>	<b>20</b>
<b>MEAN VALUE OF C.ALBICANS COLONY COUNT</b>	<b>4551</b>	<b>4655</b>	<b>3222</b>	<b>844</b>	<b>320</b>

#### **DISCUSSION:**

Among critically ill patients, prophylaxis of Candida infection is usually carried out with imidazoles or through oral use of nystatin. Recent reviews on both the treatments concluded that, they have a beneficial effect in reducing the invasive candidal infections. Although, these systemic and local antifungals have been effective in reducing the fungal colonization, their use comes along with adverse effects.[9] Furthermore, the number of yeasts resistant to antifungal drugs has increases thereby indicating the need for newer antifungal drugs.[10] Studies on probiotics against oral Candida have been performed in asymptomatic patients. Most of these studies focused on patients with oral candidiasis, and they proved that the probiotic was not the only treatment to reduce Candida, but rather can be used as an adjuvant along with the conventional antifungal therapy. A recent literature evidence suggest that the use of a probiotic product formed by Bifidobacterium longum, S. thermophilus and Lactobacillus bulgaris, in conjunction with oral local antifungal agents such as nystatin was proved to be more effective in the treatment of Candida-associated stomatitis compared with the conventional therapy.[11,12] The mechanisms of probiotics to act against oral Candid species involves a combination of factors, such as competition for adhesion, antimicrobial compound production, cytokine stimulation, induction of IgA secretion, maintenance of the epithelial defence barrier, and modulation of the adaptative and innate response of the immune system.[2,6,7]

Our particular study was done to assess the effect of probiotics on Candidal adherence on denture base resins. Candidal colony cell count was performed on heat cure denture base materials both before and after incorporation of probiotics at varying concentrations to the samples and the obtained results were compared and used for analysis.

In completely edentulous patients; Chhabra et al., conducted a study to evaluate the effect of denture adhesive with probiotics on Colony Forming Units (CFU) of Candida. The study results suggested a decrease in the number of CFU of Candida after using probiotics along with denture adhesives.[13] These results correlate with the results of our study. The present study results also correlate with the study conducted by Dos Santos et al., where individuals with Candidal species in the oral cavity used Yakult LB probiotic for 20 days and showed a significant Candidal reduction (46%) and the mean Candida CFU/ml counts (65%). This study concluded that this reduction can be attributed to competition between the yeasts rather than through specific immune stimulation response.[14]

Miazima et al., through their study concluded that., consumption of cheese supplemented along with probiotics daily, with either *L. rhamnosus* Lr-32 or *L. acidophilus* NCFM, was able to reduce the oral Candidal colonization in complete denture wearers. This strongly proves the potential of probiotics in reducing the risk of oral candidiasis in this highly susceptible population.[15] Another study done by Hatakka et al., found out the significance of probiotics on the prevalence of Candidal species in the elderly. The study results suggested that probiotic intervention decreased the risk of high yeast and also, the probiotic bacteria can be effective in controlling hyposalivation and oral Candidal count in the elderly.[6]

The data available with respect to application of probiotics in patients at varying concentration to prevent candidal adhesion to denture is still scarce and need further investigation and research.

## **CONCLUSION:**

The study was undertaken with the objective of evaluating the effect of probiotics on Candidal species adherence over the heat cure denture base material. Based on the results, following conclusion can be drawn:

1. Probiotic application on denture base resin (PMMA) did decrease the candida albicans count compared to the denture base without probiotic application.
2. As the concentration of probiotic over the denture base increase, the candida cell count decreased respectively.

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