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ORIGINAL ARTICLE

Antifungal Effect of Probiotic *Pontibacter Sps.,* against Oral Candidiasis causing *Candida albicans*

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ABSTRACT
Candida albicans(C.albicans)is the pathogenic fungi that cause oral candidiasis. Probiotics are the live microorganism proven to possess antimicrobial properties encompassing action against pathogenic fungi and bacteria. The purpose of this work was to evaluate the in vitro antagonistic potential of Probiotic Pontibactersps against oral pathogenic fungi Candida albicans. The antimicrobial activity was evaluated using the Agar diffusion method using Amphotericin B as standard. The antagonistic activity was related to the area (mm²) of the inhibition zone observed (CLSI). The two-fold macro dilution method was used to determine Minimum Inhibitory concentration. The results of the antifungal activity of Pontibacter sps were analyzed thrice and expressed as mean ± standard deviation using Microsoft Excel 2007. The results of growth inhibition studies indicate that Pontibacter sps. inhibits both the strains of Candida albicans with minimum variation in zone of inhibition. Inhibition percentage as observed in MIC values indicates that Pontibactersps effectively inhibits oral pathogenic fungi even in lowest concentration. Furthermore, maximum inhibition percentage (97.8%) is attained with minimum concentration of 5000 µg probiotics in Candida albicans 183 in comparison to the same concentration of probiotics (96.3%)in Candida albicans 3958. The most promising results clearly indicate that Pontibacter sps has high fungal inhibiting potential to be used in the future for prevention and the therapy of Oral candidiasis.
Keywords: *Pontibacter sps, MIC, Antifungal, Oral Candidiasis, Probiotics*

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INTRODUCTION

The life- threatening infections caused by pathogenic microorganisms remains the cause of morbidity and mortality in most of the developing countries [1]. The use of antineoplastic and immunosuppressive agents, prosthetic devices and antibiotics have led to the development of complicated fungal infections [2].

Over 95% of all fungal infections are caused by *Candida albicans (C. albicans), Aspergillus fumigates, or Cryptococcus neoformans* [3-4]. Infection with the yeast-like fungal organism *Candida albicans* is termed as Candidiasis [5]. Candidiasis encompasses secondary or opportunistic infections that range from acute, sub-acute, and chronic to life-threatening mycoses [6]. Candidiasis can be localized to the throat, mouth, ears, nose, nails of toes and fingers, gastrointestinal tract, vagina, eyes, lungs, brain and kidneys or lead to candidemia, endocarditis, and meningitis systemically [7-9].

"Thrush", is the earliest oral disease documented. Hippocrates 460-377 BC and Gallen 130-200 AD described thrush and named in French as muguetas it causes whiteness of the infected tongue [10].

Despite the availability of effective antifungal drugs, the prevalence of antimicrobial resistance among key microbial pathogens is increasing at an alarming rate worldwide[2]. Moreover, most of the available antifungal drugs have undesirable side effects or very toxic (amphotericin B), exhibit drug-drug

interactions (azoles), leads to the resistance (fluconazole,5-flucytosine) during therapy [11] hence becomes ineffective for treatment. Consequently, it is obligatory to search for new antimicrobial agents that are more effective and less toxic is utmost importance to overcome these disadvantages.

The chemicals which are used to treat infectious diseases fall into two main categories, namely natural products and chemotherapeutic agents. Natural products include microorganisms and plants that usually produce secondary metabolites [12]. Production of antimicrobial compounds seems to be a general phenomenon for most bacteria to maintain their predominance [13]. The most promising compounds display low minimum inhibitory concentrations and are cost-effective in production [14].

Subsequently, the earlier reports confirmed that the predominant factor of preventing *Candida* infection is maintaining balanced microbial flora and a healthy immune system. This has led people to consider the use of probiotics as these beneficial microbes that are good at restoring disturbed microflora and boosting up immune functions [15-16].

Probiotics are usually administered an acceptable level to confer health benefits to the host [17-18]. The use of probiotics in order to improvise the oral health condition without any negative impact on the normal oralmicrobiota is a relatively new concept [19].

The efficacy of antifungal agents is primarily evaluated by invitro antifungal susceptibility tests. Hence, the focal theme of this study was *in vitro* evaluation of the impact produced by probiotic bacteria in the growth of oral pathogenic *Candida albicans.*

MATERIAL AND METHODS

Microbial Strains

Probiotic bacteria *Pontibacter sps* was isolated from *Labeorohita* fish gut [43].. The fungal pathogenic strains *Candida albicans* (MTCC 183 and 3958) were obtained from Microbial Type Culture Collection (MTCC, Chandigarh, India).

Chemicals and reagents

The chemicals and culture media which includes Sabouraud's Dextrose agar, Dextrose, Amphotericin were procured from Merck and Himedia, India

In vitro Agar diffusion assays

The antifungal activity of the probiotic bacteria was assessed using the standard method CLSI M38-A (formerly NCCLS). The fungal cultures were maintained in 0.2% dextrose medium. The inoculum of fungi was evenly spread on Sabouraud's Dextrose agar (HiMedia, India) using a sterile swab and its antimicrobial activity was determined using Amphotericin B as a standard. The Petri plates were incubated at 30°C for 2 days and subsequently examined for zones of inhibition. The inhibition was recorded as negative if the zone was absent around the agar well. The antagonistic activity was related to the area (mm^2) of the inhibition zone observed (CLSI).The experiments were duplicated and repeated three times.

Determination of minimum inhibitory concentration (*MIC*) *values*

MIC values determine the lowest concentration of the antifungal agents that effectively prevents visible growth of the pathogenic fungi.Briefly, the stock (5000 µg/mL) of the bacteria was prepared by dissolving 5 mg of the lyophilized culture in 1 ml of distilled water. Different concentrations (1000, 2000, 3000, 4000 and 5000 µg/ml) were prepared from the stock solution with distilled water. The tubes containing *Candida albicans* were incubated with the varying concentration of lyophilized bacteria, aerobically at 30°C for 24- 48 h. The fungi within the same culture media without the lyophilized bacteria served as a positive control. After incubation, the tube with the least concentration of bacterial cell solution showing prominent growth inhibition was taken as the MIC value and inhibitory percentage was calculated (CLSI method).Amphotericin B served as a negative control [20,21]. All experiments were carried out in duplicates and repeated thrice.

STATISTICAL ANALYSIS

The statistical analysis was carried out using SPSS software and expressed as mean ± SD.

RESULTS

Oral candidiasis is a fungal infection commonly occurring in the mucous membrane of the mouth Figure 1.

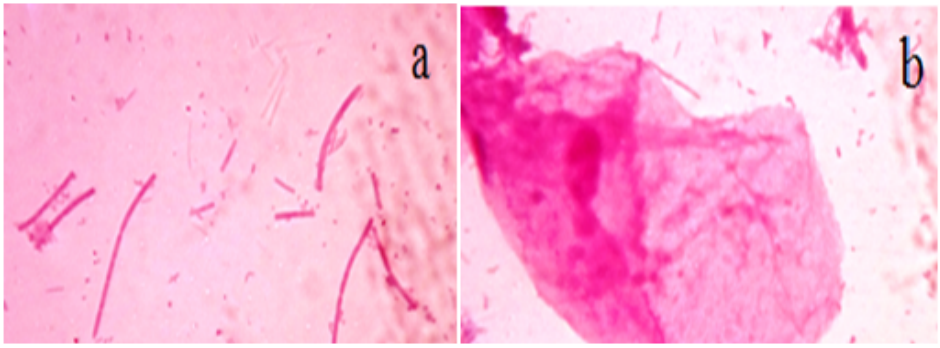

Fig. 1: Oral Candidiasis a) *Candida albicans*- hyphae form PAS (Periodic Acid Schiff stain) b) *Candida albicans* – adjacent to the oral squamous cell.

The results of growth inhibition and MIC studies are depicted in Figures 2 and 3, respectively. *Pontibacter sps.* inhibits both the strains of *Candida albicans* with a minimum variation in zone of inhibition(Fig. 1). Inhibition percentage as observed in MIC values indicates that *Pontibacter sps* effectively inhibits oral pathogenic fungi even in the lowest concentration. Furthermore, the maximum inhibition percentage is attained with a minimum concentration of probiotics in *Candida albicans183.*

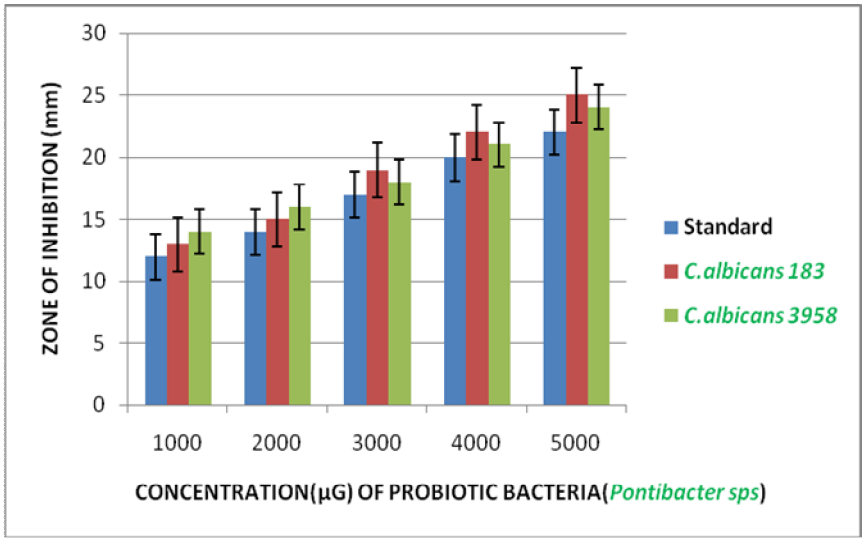

Fig.2: Zone of inhibition studies of probiotic bacteria *Pontibacter sps* against *C. albicans* 183 and *C. albicans* 3958.Data is expressed as mean±standard deviation (n=3)

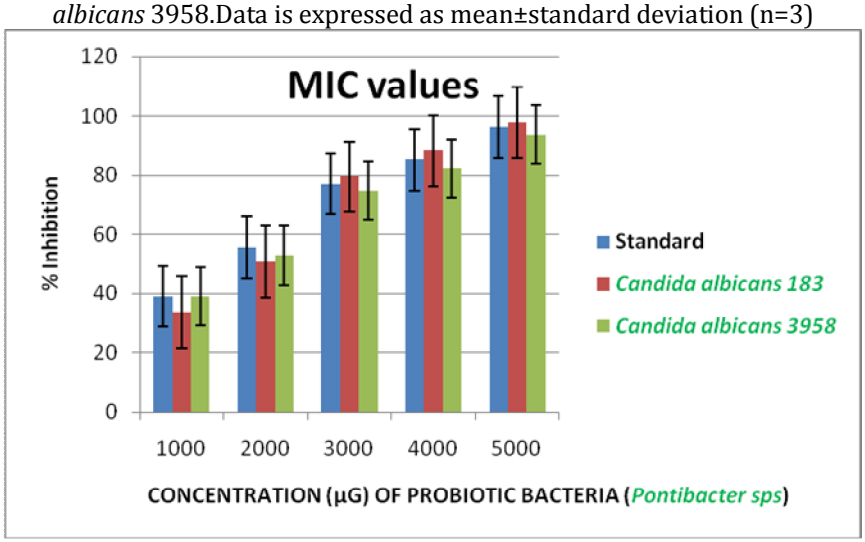

Fig. 3: Minimum inhibitory concentration of probiotic bacteria *Pontibacter sps* against oral pathogenic fungi *C. albicans* 183 and *C. albicans* 3958. Data is expressed as mean±standard deviation (n=3)

DISCUSSION

Previous researchers detected antifungal compounds production from different bacterial species [22] and the metabolitesof *Bacillus pumilus* grown on potato glucose broth, prevented the mycelial growth (spore germination) of *Aspergillus*, *Penicillium* and *Fusarium* species [23]. Similarly, Bapat and Shah have revealed the production of an extracellular antagonistic substance in *Bacillus brevis* which is fungicidal that acts by inhibiting the germination of conidia and has fungicidal to the vegetative mycelia of the pathogen [24].

Subsequently, the isolates of *Bacillus* spp. produced volatile metabolites that inhibited mycelial growth of *Fusarium oxysporum* with the highest effect in reducing *Fusarium* wilt of onion. Yiu-Kwok *et al.* emphasized that *Bacillus subtilis* filtrate was active at completely different dilutions against macroconidium germination and hyphal growth of *Fusarium graminearum* counting on the initial macroconidium density owing to their ability to advance with extracellular and soluble proteins and to complex with bacterial and fungal cell walls [25]. *Fusarium* sps.are soil and plant saprophytes that play an emerging role as pathogens in human and animal mycotoxicosis [26].

It has been known for years that commensal bacteria in our intestines play an important role metabolizing digesta in the lumen and in so doing provide an additional source of nutrients. *Bifido bacterium* species and LAB are natural commensal bacteria in the small and large intestines. These bacteria protect the host against potential pathogens by competitive exclusion and also by the production of antimicrobial agents [27].

Probiotic in diet boosts the human defense system against these fungal infections by synthesizing antagonizing substances on *C. albicans* [28-30]. Probiotics have been proven effective in treatment of oral diseases [31].

Consequently, *Lactobacilli* have received tremendous attention due to their health-promoting properties [32].However, most of the *Lactobacilli* do not maintain stable and numerically significant populations in the human intestinal tract, especially in the small intestine where they are presumed to form epithelial associations [33-34].*Lactobacillus species* have verified effective at inhibiting the expansion of microorganism and flora pathogens that normally cause vaginosis. The pathogens that are mostly inhibited by probiotic *Lactobacilli* are *Candida albicans*, *Escherichia coli*, *Streptococcus mutans*, and *Neisseria gonorrhea* [35-37].

The use of probiotics for the improvement of oral health without any negative impact on the normal oral microbiota is a relatively novel concept. Studies based on use of the intestinal probiotics *Lactobacillus rhamnosus*GG [38], *Lactobacillus reuteri* ATCC 55739 and *Bifidobacterium* DN- 173 010 [39] have been reported achieving reduced levels of *S. mutans* and, moreover, the children taking *Lactobacillus rhamnosus* GG developed fewer dental caries [32]. The prerequisites for the microorganism to be employed as a probiotic are antibiotic resistance, inhibitory activity against other commensal microorganisms, bile tolerance, intestinal etc. Strains of the genus *Bifidobacterium* has gained interest owing to its variety of resistance mechanisms to bile salts, a key effect of probiotic bacteria that must be generated in the presence of this biological fluid [40-42].Fish gut bacterium *Pontibactersps* (*Bifidobacterium)* bestows the probiotic potential due to its antimicrobial effect, antibiotic resistance and bile tolerance [43, 44, 45]. Hence, the present study confers the possible usage of probiotic *Pontibacter* sps the commensal fish gut bacteria as an alternative to antifungal therapy to reduce *C.albicans.*

CONCLUSION

The in-vitro study reveals the antifungal activity of probiotic *Pontibacter* sps the commensal fish gut bacteria and possibility as an alternative to antifungal therapy to reduce *C. albicans.* Detailed studies of the biochemistry of the antibacterial metabolites (antibiotics and extracellular hydrolytic enzymes) are in process, which we hope will be useful in our understanding of the mechanisms involved in antagonistic activity against the pathogenic fungi. The results of the present study paves way for further studies that deals with the process of isolation and identification of the active principles.

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