Determination of antimicrobial potential of *Saccharomyces boulardii* and *Bacillus clausii* against some community acquired pathogens-*in vitro* study

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ABSTRACT

Probiotics are micro-organisms that have claimed to provide health benefits when consumed. This piece of work was designed to find out the possibilities to use probiotics as a therapeutic agent. For this, two common probotic strains *Sacchraomyces boulardii* and *Bacillus clausii* were isolated from the commercial probotic products of regular use and their antimicrobial activities were assessed against the standard strains of the common pathogens, *Pseudomonas, Escherichia* and *Staphylococcus* by using the disc diffusion method. Serial suspension of turbidity M.F.S. #1.0 showed the maximum antagonistic activity followed by the suspensions of the turbidity M.F.S. #1/10 and1/100. The positive outcomes of this study could be one more step to use these probiotic strains as an complementary and alternative medicines in the near future.

Key words: Probiotics, *Sacchraomyces boulardii*, *Bacillus clausii*, and Antagonistic effect.

INTRODUCTION.

Probiotics are used either as preventives (prophylactics) or as curatives (bio therapeutics) for particular diseases. Bacteria like *Lactobacillus, Enterococcus, Bacilli, Saccharomyces* and *Bifidobacterium* are extensively used as probiotics. Probiotics are commonly taken as a part of fermented foods such as yogurt, soy yogurt, yakult or as dietary supplements. Probiotics have been reported to have beneficial effects in several ailments like various types of Diarrhea (1) Urogenital infection (2) intestinal inflammation (3) allergies, lactose intolerance and exhibit anticarcinogenic and immunomodulatory effects. Probiotics helps to improve the Immune function. Some strains of Lactic acid bacilli may affect pathogen by means of competitive inhibition and there is evidence to suggest that they may improve immune function by increasing the number of IgA producing plasma cells, increasing or improving phagocytosis as well as increasing the proportion of T-lymphocytes and Natural killer cells (4,5).The efficacy of probiotic performance depends on their strong adherence and colonization of the human gut, which in turn improves the host immune system (6). Probiotic have been found to enhance the antimicrobial activity of antibiotics against the *E. coli* and *P. aeruginosa* (7,8). Also *Lactobacillus rhamnosus* has been shown to produce an antimicrobial substance that inhibits the growth of *Escherichig coli, Streptococci spp, Colstridium difficile, Bacteroides fragilis* and *Salmonella* spp., (9). There are many studies, which advocate the therapeutic utility of probiotics (10,11). In this study two probotic strains *Saccharomyces boulardii* and *Bacillus clausii* have been isolated from the commonly prescribed probotic products "G Norm" and " enterogermina" to see their antagonistic effects against the microbial pathogens *Pseudomonas aeruginosa* MTCC-103, *E. coli* MTCC-1652, and *Staphylococcus aureus* MTCC-740.

MATERIALS AND METHODS

Isolation and cultivation of probiotic strains

Both the strains *Sacchraomyces boulardii* and *Bacillus clausii* were isolated from the commercial probotic products "G Norm" and " enterogermina" respectively. Both the products were in powedered form and were containing the only strain. To isolate the *S.boulardii* a pinch of "G Norm", capsule powder was dissolved in 2 ml of water and the suspension was inoculated on the sabouraud dextrose agar medium and kept at 37^{o}C for 24 hr. After incubation the colonies were subculture to get the pure colonies. To isolate the *B. clausii* from "enterogermina", a loopful of enterogermina suspension was inoculated on Nutrient agar surface and kept at 37^{o}C for 24 hrs. After 24 hrs, the pale whitish colonies appeared on the plates which were sub cultured to get the pure culture. Pure colonies were stored at 4^{o}C in the butt slant tubes of nutrient agar.

Test pathogens

The standard strains of the pathogens, *Staphylococcus aureus* MTCC-740, *Pseudomonas aeruginosa* MTCC-103 and *Escherichia coli* MTCC-1652 were obtained from IMTECH, Chandigarh India and stored at 4^{o}C.

Antibiogram of probiotic strains

The antibiotic susceptibilities of *S.boulardii* and *B.clausii* were detected by swabbing their suspensions of the turbidity M.F.S.# 0.5 on the Muller-Hinton Media plates by placing the readymade antibiotic discs of Chloroamphenicol (C) Cefoxitin(CX), Azithromycin(AZM) Amoxyciilln(AMC) Ampicillin/Sulbactam(A/S) Meropenem(MRP) Ceftazidime(CAZ) Levofloxacin (LE) and incubated at 37ºC for 24 hrs.

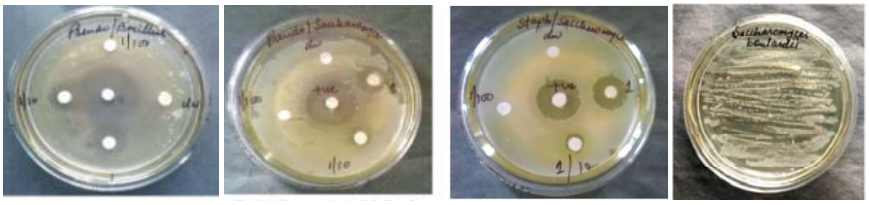
Antagonistic activity of probiotic strains

Antagonasitic activity of probiotic strains was studied by disc diffusion method according to National Committee for Clinical Lab Studies (NCCLS) guidelines (12). For this petriplates of diameter of 90 mm were poured 20 ml of Muller Hinton media, swabbed with *E. coli* MTCC-1652, *P. aeruginosa* MTCC-103 and *S.auresus* MTCC-740 suspensions of the turbidity M.F.S.# 0.5 and incubated at 37ºC for 15 minutes. Now the sterile blotting paper discs of 6mm diameter were loaded with the 20 ul of suspensions of *S.boulardii*& *B.clausii* of the turbidity M.F.S. ≠ 1.0 (3 x 10^8 cfu/ml) and the serial suspension of 1/10 (3x10^7 cfu/ml) and 1/100 (3x10^6 cfu/ml) each disc now contained 6 x 10^6 cfu/disc (for M.F.S. ≠ 1.0) , 6 x 10^5 cfu/disc (M.F.S. ≠ 1/10) and 6x10^4 cfu/disc (M.F.S. ≠ 1/100). Streptomycin disc was taken as a positive and sterile distilled water disc as negative control. Discs were placed on MHA surface and kept at 4ºC for 1 hr for proper diffusion. Plates were incubated for 24 hours at 37ºC.

RESULTS AND DISCUSSION

Table 1: Antagonastic activity of *Saccharomyces boulardii* and *Bacillus clausii* against *Pesudomonas aeruginosa* MTCC-103, *E. coli* MTCC-1652 and *Staphylococcus aureus* MTCC-740.

S. No.	Antibiotic/ Probiotic disc	Diameter of the zones of inhibition (mm)					
		P.aeruginosa		*E. coli*		*S.aureus*	
		S. boul.	*B. cla.*	*S. boul.*	*B. cla.*	*S. boul.*	*B. cla.*
1.	Antibiotic	20	20	21	23	16	18
2.	1	14	13	19	21	13	10
3.	1/10	8	11	18	17	6	9
4.	1/100	0	0	8	9	0	7
5.	Distilled water	0	0	0	0	0	0



(a) **(b)** **(c)** **(d)**

Fig. 1 : Showing the Zones of inhibition against *P. aeruginosa by* (a) *B. clausii* (b) *S.boulardii* (c) against *S. aureus by S. boulardii* (d) *S. boulardii* on sabouraud dextrose agar.

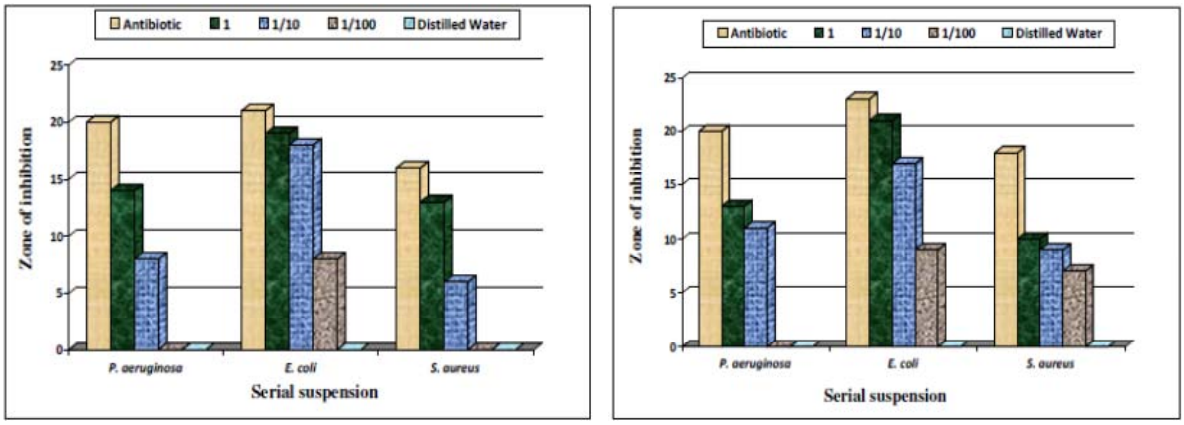


Fig.-2: Graphical representation of the Zones of inhibition by (a) *S.boulardii* (b) *B. clausii* against different pathogens.

Both the strains were confirmed by colony morphology, *S.boulardii* appeared as characteristic oval shaped cells under microscope and produced white colonies on sabraoud's agar. *B.Clausii* was viewed as rod-shaped, gram-positive, motile bacterium with spore.

Both the strains *S.boulardii* and *B.Clausii* showed maximum zones of inhibitions (13mm and 11mm respectively) for chloramphenicol and minimum for Cefoxitin and found resistance towards Ceftazidime (CAZ).)

Maximum inhibition of zone was produced by *S.boulardii* against *E. coli* MTCC-1652 (19 mm) followed by *P. aeruginosa* MTCC-103 (14 mm) and *S. aureus* MTCC-740 (13 mm) by serial suspension M.F.S. # 1.0, medium zones were observed in the serial suspension of M.F.S. # 1/10 (6 mm to 18mm). Similarly *B.clausii* has also produced maximum zone of inhibition against *E. coli* MTCC-1652 (21 mm) in the serial suspension of turbidity M.F.S. # 1.0 and minimum zone of inhibition against *S.aureus* MTCC-740 (10 mm). *B.clausii* showed intermediate senstivity(9-17mm) for the serial suspension of 1/10, also it produced zones against *S. aureus* MTCC-740 and *E. coli* MTCC-1652 (7&9mm) but gave no zone against *P. aeruginosa* MTCC-103 for serial suspension of M.F.S.# 1/100 turbidity (table-1&fig.-1&2).

CONCLUSION

It is clear from the above that *S. boulardii* produced better zone of inhibition than *B. clausii* against the *P. aeruginosa* MTCC-103 and *S.aureus* MTCC-740 but reverse was true against *E.coli* (M.F.S. # 1.0). It is clear from the above these strains may be used as the therapeutic agents for the various infections, specially against the resistant pathogens like *S. aureus* and *P. aeruginosa* etc. Apart from their antimicrobial potentials *S. boulardii* and *B. clausii* are well known to put their health benefits so there seems no harm to use them as a regular diet supplements. Finally it can be concluded that use of *S. boulardii* and *B. clausii* as drug or food supplement will not only protect us from harmful effect of pathogenic microorganisms but also provide uncountable health benefits on our physical and mental health.

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