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A Study showing antagonistic effect of *Streptococcus thermophilus* against *Candida albicans*

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Abstract: ***Live microorganisms, have beneficial effects on their host's health, are called as probiotics. Probiotics are live microorganisms which when administered in appropriate amount gives health benefit on the host. A study was carried out on inhibitory effects of Streptococcus thermophilus on growth of Candida albicans. Results of the antagonistic effect of Streptococcus thermophilus against Candida albicans was determined by using the Kirby Bauer disc diffusion method, Streptococcus thermophilus produced highest antimicrobial activity against Candida albicans Streptococcus thermophilus may be used as probiotics when preceded by further studies.***

Keywords: *Probiotcs, Norgut, Streptococcus thermophilus, Candida albicans.*

1. INTRODUCTION

Streptococcus thermophilus also known as *Streptococcus salivarius* subsp. *Streptococcus thermophilus* also known as *Streptococcus salivarius* subsp. *Thermopilus*[1]is a gram-positive bacterium, and a fermentative facultative anaerobe, of the *viridans* group. It tests negative for cytochrome, oxidase, and catalase, and positive for alpha-hemolytic activity. It is non-motile and does not form endospores. *S. thermophilus* is fimbriated. It has an optimal growth temperature range of 35 - 42 °C while *L. bulgaricus* has an optimal range of 43-46 ^{0}C; *Candida albicans* is opportunistic pathogenic yeast that is a common member of the human flora. It does not proliferate outside the human body [2]. It is detected in the gastrointestinal tract and mouth in 40-60% of healthy adults. It is usually a commensally organism, but can become pathogenic in immune compromised individuals under a variety of conditions. It is one of the few species of the *Candida* genus that causes the human infection candidiasis, which results from an overgrowth of the fungus. Candidiasis is for example often observed in HIV-infected patients. *C. albicans* is the most common fungal species isolated from biofilms either formed on (permanent) implanted medical devices or on human tissue[3]. *C. albicans*, *C. tropicalis*, *C. parapsilosis*, and *C. glabrata*, are together responsible for 50–90% of all cases of candidiasis in humans. A mortality rate of 40% has been reported for patients with systemic candidiasis due to *C. albicans*. Estimates range from 2800 to 11200 deaths caused annually in the USA due to *C. albicans* causes candidiasis. The human gut is populated by a wide array of bacterial species, bearing important metabolic as well as immune functions, all leading to marked effects on the nutritional and health status of the host[4] .Probiotics are defined as 'living micro- organisms, which upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition'[5,6].Alternatively, probiotics are also known as live microorganisms belonging to natural biota with low or no pathogenicity, but with functional importance to the health and well-being of the host and investigated with regard to their medicinal use[7,8] Probiotics were originally used to influence human health through alterations in intestinal microbiota. At present, probiotics and their effects on human health have been demonstrated by incorporating them in traditional foods as single or mixed microbial culture preparations i.e. production of functional foods/nutraceuticals or as pharmaceutical preparation to get more health benefits[7].The effects of probiotics on human health are affirmative and well defined in prevention and treatment of various ailments such as acute or chronic gastrointestinal infections (diarrhea, lactose intolerance, (Inflammatory Bowel disease) IBD, etc.)[9] Immunological or inflammatory disease i.e. alleviating symptoms of allergies [10, 11], cancer [12] and AIDS (Acquired Immuno Deficiency Syndrome) [13],respiratory and urinary tract infections. Appropriate diet, broadly including nutraceuticals/functional foods/food supplements fortified with probotics, prebiotics and synbioitcs, with novel nutritional and therapeutic benefits culminates in a properly functioning Gastro Intestinal (GI) tract, resulting in attainment of proper human physiology, hence a healthy living. The objective of this work was a Study showing antagonistic effect of *Streptococcus thermophilus* against *Candida albicans.*

MATERIALS AND METHODS

Bacterial isolation and cultivation:

A Probiotic strain *Streptococcus thermophilus* was isolated from commercially available capsule 'Norgut'. For this, half of capsule was suspended in 2 ml of MRS broth in anaerobic condition and kept at 37 ^{0}C for 24 hrs while another half was suspended in normal saline and inoculated on sabraoud's agar and kept at 37 ^{0}C for 24 hrs in aerobic condition. After incubation a loopful MRS broth was dispensed to MRS agar and kept in Mc intosch jar with an anaerobic gas packet for 48 hr at 37^{0}C. *Streptococcus thermophilus* was isolated from Norgut plate. Pure colonies were obtained by repeated plating. All the probiotic strains were confirmed by Gram's staining, cell and colony morphology.

Antibiotic resistance:

Antibiotic resistance of probiotic strains was assessed using antibiotic discs (Hi Media, India) by using disc diffusion method according to the national committee for clinical laboratory standards (NCCLS) guidelines. Probiotic suspension 0.5 McFarland standard was inoculated by swabbing the MHA surface 3 times by rotating at 60 ^{0} to ensure even distribution. After 10 min. readymade disc of antibiotic Vericonazol were placed on Mueller hinton agar (MHA) surface and kept at 37 ^{0}C for 24 hrs.

Antagonistic activity:

Antagonasitic activity of probiotic strains was studied by disc diffusion method according to National Committee for Clinical Lab Studies (NCCLS)[14].For this petriplates of diameter of 90 mm were poured 20 ml of Muller Hinton media, swabbed with *Candida albicans* suspensions of the turbidity M.F.S.# 0.5 and incubated at 37ºC for 15 minutes. Now the sterile blotting paper discs of 6 mm diameter were loaded with the 20ul of suspensions of *Streptococcus thermophilus* the turbidity M.F.S. ≠ 1.0 (3 x 10^8cfu/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 6x10^4cfu/disc (M.F.S. ≠ 1/100). Vericonazol 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.

MIC of probiotic strains:

For testing the Minimum Inhibitory Concentrations (MIC) of probiotic strains, 3 steril blank discs of 6 mm diameter were transferred with 20 ul of their respective serial suspensions i.e. the suspension of turbidity equal of ≠ 1.0, (3 × 10^8 cfu/ml), 1/10 (3 × 10^7 cfu/ml) and 1/100 (3 × 10^6 cfu/ml). These discs were kept at 37°C for 1 hr. so that to absorb in their full capacity. These impregnated discs now contained approximately 6×10 ^{6}cfu/disc (for Mac Farland standard ≠ 1.0), 6 × 10^5 cfu/disc (for 1/10 serial suspensions) and 6 × 10^4 cfu/disc (for 1/100 serial suspension). Now a petriplate of MHA was swabbed with *C. albicans* clinical isolate of E.coli and kept at 37°C for 3 hrs. After it the probiotic discs were placed gently on the surface of MHA plate, along with the sterile water disc (negative control). The plates were kept at 4°C for 1 hr diffusion and then incubated at 37°C for 24 hrs zones of inhibition were measured.

RESULTS AND DISCUSSION

The result of characteristics of the isolates of Streptococcus *thermophilus* is as shown in table 1. All the colonies of *Streptococcus thermophilus* isolated are gram positive growth pH 5.4, showed on MRS agar medium *Streptococcus thermophilus* isolates showed characteristic cocci shape, big creamy white colonies, circular and irregular shaped. Incubation temperature of 37°C. The result of measurement of antimicrobial activity(zone of inhibition) of *Streptococcus thermophilus* against the clinical isolates is as shown in table 2 Out of this clinical isolates *Streptococcus thermophilus* showed the highest zone of inhibition (3.50 mm) against *Streptococcus thermophilus* against *Candida albicanus*(4.20 mm), and no or least zone of inhibition was shown by 1/100 (0.00mm). In case of *Streptococcus thermophilus* the maximum zone of inhibition was measured by the serial suspension of M.F.S. ≠1.0 against the C*andida albicans* (sample no.-2092, 2018, 259, 263 & 2093,) (12, 12,12, 14, 12 mm) and the medium zone of inhibition were observed in the serial suspension of M.F.S. ≠1/10 (10, 8, 9, 12, 8 mm) and one zone was seen in the serial suspension of M.F.S. ≠ 1/100 (6mm). Growth inhibition is highest with *Streptococcus thermophilus* against *Candida albicans* (1.60 mm) and no or least zone of inhibition was shown by 1/100 (0.00mm). of *Streptococcus themophillus*. The result of measurement of antimicrobial activity (zone of inhibition) of *Streptococcus thermophilus* against the clinical isolates the highest zone of inhibition (3.50 mm) against Streptococcus thermophilus against Candida albicanus (4.20 mm), and no or least zone of inhibition was shown by 1/100 (0.00mm).

Table 1: Characteristics of the Isolates of *Streptococcus thermophilus.*

Characteristics of isolates	*Streptococcus thermophilus*
Growth on MRS agar at ph 6.3	+
Growth on MRS agar at pH 5.4	-
Incubation temperature	42 ^{0}C
Cell morphology	Cocci
Gram reaction	+
Colony size	Big
Colony shape	Circular
Colony colour	Irregular Creamy white

Key: + = positive, - = Negative

Table 2: Showing zone of inhibition of *Streptococcus thermphilus* against the *C. albicans.*

S.No.	Sample No.	Zone of diameter (mm)				
		+ve	1	1/10	1/100	-ve
1.	2092	18	12	10	6	nil
2.	2018	16	12	8	nil	nil
3.	259	18	12	9	nil	nil
4.	263	20	14	12	nil	nil
5.	2093	20	12	8	nil	nil
6.	2180	18	nil	nil	nil	nil
7.	215	20	nil	nil	nil	nil
8.	278	28	nil	nil	nil	nil

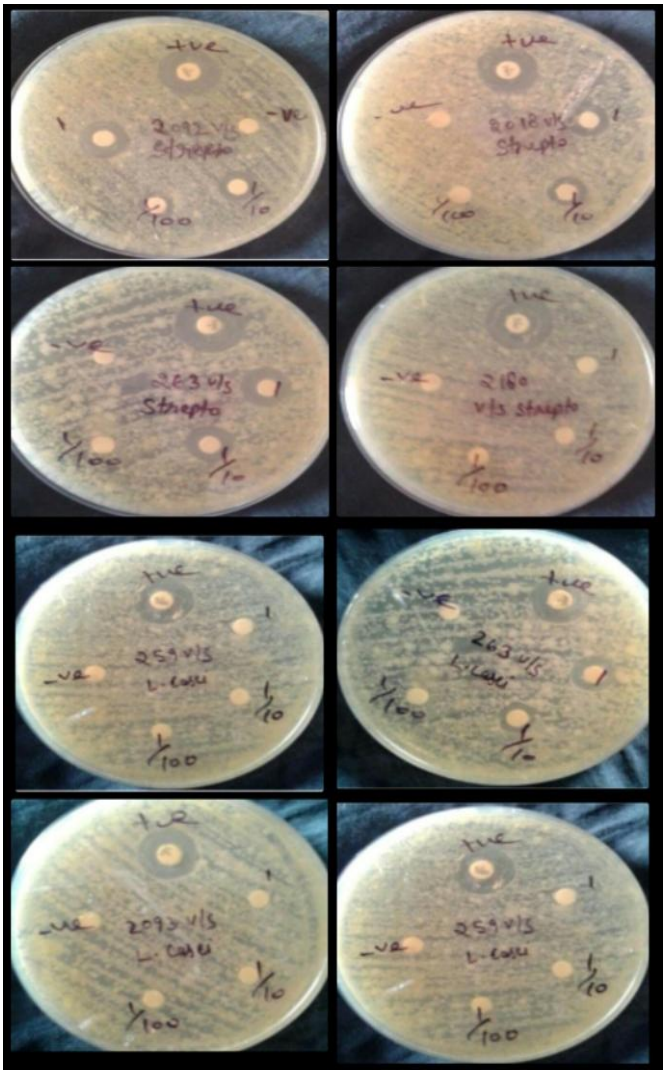

Figure 1: Showing antimicrobial effect of *Streptococcus thermphilus* against the *Candida albicans*.

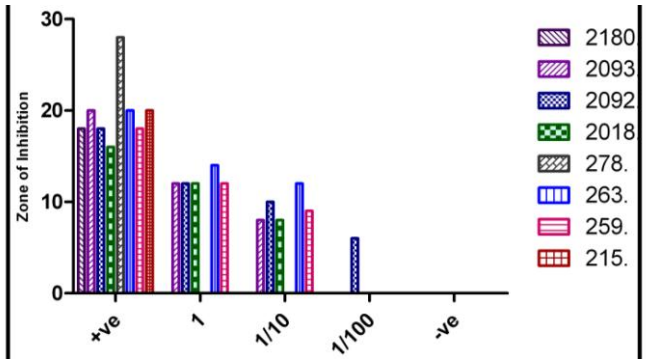

Figure. 2: Graph representing comparative zone of inhibition of the various clinical isolation of *Streptococcus thermophilus* against the *C. albicans*

CONCLUSION

The results of this study indicate that 'Norgut' exhibit a wide diversity of *Streptococcus thermophilus* occurring naturally in the norgut capsule and can be used as a potential natural source to isolate a variety of strains of *Streptococcus thermophilus*. It is clear from the above that *Streptococcus thermophilus* produced better zone against *C. albicans* It is clear from the above these strains may be used as the therapeutic agent for the various infections, specially against the resistant pathogen like *C. albicans* Since some strains of *Streptococcus thermophilus* possess potential probiotic and therapeutic properties including anti-inflammatory and anticancer activities, as well as other features of interest, these isolates can be further screened for their probiotic and related properties and exploited for health and economic benefits

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