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ENHANCED ANTIMICROBIAL ACTIVITY OF PROBIOTICS THROUGH SELENIUM NANOPARTICLES ENRICHMENT AGAINST GASTROINTESTINAL PATHOGENS

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ABSTRACT: The decrease in susceptibility towards commonly administered antibacterial agents is due to excessive use of antibiotics every now and then, due to which the resistance toward these antibiotics is developed in pathogens. To overcome the problem of resistance in pathogenic microbes, the study of incorporation of Selenium metal to probiotics supplement was carried out. Antimicrobial activity of individual broth obtained from five different lactobacillus spp. viz. *Lactobacillus helveticas*, *Lactobacillus fermentum*, *Lactobacillus acidophilus*, *Lactobacillus gasseri* strains with varying concentration of Selenium (20mg/mL, 40mg/mL, 60mg/mL and 80mg/mL) were studied against pathogens like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi* and *Candida albicans*. The results showed that the antimicrobial property of the probiotic broth was significantly increased and showed good bacteriostatic activity against on *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. The report and such kind of further studies may pave the way for further investigations on the use of pure metals as effective antibacterial agents to overcome the problem of resistance in pathogens.

INTRODUCTION: Developing resistance of pathogenic bacteria towards antibiotic is an alarming situation and a matter of concern in the biomedical research and food organization¹. The decrease in susceptibility towards commonly administered antibacterial agents is due to excessive use of antibiotics every now and then, due to which the resistance toward these antibiotics is developed in pathogens^{2,3,4}.

Pathogens like *S. aureus*, *P. aeruginosa*, *E. coli*, *salmonella typhi* and *Candida albicans* are pathogens present in oral cavity, gastrointestinal track and urogenital track, can cause a variety of mild to serious infections and have been reported lately for their antibiotic resistance development. Probiotics is a group of microorganism which, when consumed in adequate amounts, can improve intestinal microbial balance and provide benefits for human health^{5,6}.

These bacteria produce lactic acid, acetic acid, hydrogen peroxide, and other antimicrobial substances, which allow them to prevent the colonization of pathogens⁷. They are benign employed for synthesis of Ag, Au, FeO₃, Se, and other nanoparticles^{8,9}.

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Selenium is required for maintenance of health and growth and hence widely used as a dietary supplement. Patients suffering from diseases like requiring kidney dialysis, HIV, Cardiovascular diseases, Thyroid diseases, Crohn diseases, are recommended for Se supplements⁷. However Selenium Nanoparticles (SeNPs) are being employed potentially in wide range for diet supplements and therapies¹⁰⁻¹².

Hence this present work aims to study and evaluate the antimicrobial effect of selenium-enriched metabolites of five different *Lactobacillus* spp. viz. *L. Helveticas*, *L. fermentum*, *L. acidophilus*, *L. gasseristrains* against pathogens like *S. aureus*, *E. coli*, *P. aeruginosa*, *S. typhi*, *C. albicans*.

MATERIALS AND METHODS:

Microbial strains: Probiotic strains of *Lactobacillus* spp. *L. Helveticas* NCDC 292, *L. fermentum* NCDC 141, *L. acidophilus* NCDC 11, *L. gasseristrains* were obtained from ICAR and the pathogenic bacteria *Staphylococcus aureus* NCTC6571, *S. typhi* NCTC8394, *P. aeruginosa* NCTC50082, *Escherichia coli* ZCIB9482, and *C. albicans* ATCC 14053 were obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Sector 39-A, Chandigarh - 160036, India and used in the present study.

Effect of Lactobacillus Species on Sodium Bisellinate Salt: DeMan-Rogosa-Sharpe (MRS) broth was used for inoculation of *L. helveticas*, *L. fermentum*, *L. acidophilus* and *L. gasseristrains* strains. The culture in each flask was allowed to grow for 24 h in 100 ml of broth at 37 °C inside an orbital shaker. Various concentrations of sodium bisellinate salt (20 mg, 40 mg, 60 mg and 80 mg) was dissolved in distal water and volume was made up to 100 ml.

Further this solution of sodium bisellinate salt was sterilized by a Millipore filter apparatus and was added aseptically to the lactobacillus cultures^{13, 14}. These lactobacillus cultures subjected to sodium bisellinate salt solution were allowed incubated at 37 °C for 96 h. The bacterial cells were eliminated from the culture by centrifugation at 5000 × g for 10 min. The supernatant from each concentration were collected and stored for further studies.

Preparation of Lactobacillus Cultures for Antimicrobial Activity Assay: *L. Helveticas*, *L. fermentum*, *L. acidophilus* and *L. gasseristrains* were grown aerobically in 100ml of MRS broth in different flask for 96 h. and the supernatants were collected after centrifugation at 5 000 × g for 10 min for antimicrobial assay against the selected pathogenic microbes.

Antibacterial Activity Screening:

Preparation of Lactobacillus Cultures for Antimicrobial Activity Assay: The samples were collected from sodium bisellinate treated culture flask as described earlier and were centrifuged at 10000 rpm for 10 minutes¹⁵. The supernatants were collected and assayed for anti-microbial activity against *S. aureus*, *P. aeruginosa*, *E. coli*, *S. typhi* and *C. albicans*.

Zone of Inhibition Assay: Bacterial cultures of *S. aureus*, *P. aeruginosa*, *E. coli*, *S. typhi* and *C. albicans* were obtained from pure stocks of department of molecular biology and biotechnology. Each was inoculated into 5 ml of brain heart infusion (BHI), and grown overnight for 18 h at 37 °C with shaking at 120 rpm. 500 µl of the overnight grown cultures were inoculated into 100ml BHI and incubated 4 h at 37 °C with shaking at 120 rpm. 1ml each of three cultures was added to lukewarm broth and poured and carefully distributed over the surface of solidified nutrient agar (BHI) plate. Cylindrical wells of 1cm diameter were then bored into the agar using a sterilized cork borer.

For all five species varying amount of selenium concentration (20 mg, 40 mg, 60 mg, and 80 mg/ml) were added to the wells were allowed to diffuse for 12 - 16 h at 37 °C^{16, 17}. The circular and clear inhibition zones around each hole were observed

RESULT AND DISCUSSION:

Examining the Primary Antimicrobial Activity of Pure Metal Using Zone of Inhibition Assay: The antimicrobial activity of selenium was tested against the five pathogenic bacteria species with the implementation of well diffusion assay. Diameter of zone of inhibition is directly proportional to the antagonistic effect of synergism of selenium and lactobacillus species.

The primary antimicrobial activity of selenium gradually increases in varying the concentration. Amongst five species selenium showed most

effective on *S. aureus*, *E. coli*, *Candida albicans* and shown less effective against *P. aeruginosa* in all the varying concentrations. (Table 1)

Zone of Inhibition Assay:

TABLE 1: ANTIBACTERIAL EFFECTS OF FERMENTED BROTH OF AND IN SYNERGISM WITH VARYING CONCENTRATION Selenium AGAINST STAPHYLOCOCCUS AUREUS, PSEUDOMONAS AERUGINOSA, ESCHERICHIA COLI, SALMONELLA TYPHI AND CANDIDA ALBICANS

Selected <i>Lactobacillus</i> Strains	Selected pathogenic microbes																			
	<i>Staphylococcus aureus</i>				<i>Pseudomonas aeruginosa</i>				<i>Escherichia coli</i>				<i>Salmonella typhi</i>				<i>Candida albicans</i>			
	Concentration of Selenium salt (mg)																			
	20	40	60	80	20	40	60	80	20	40	60	80	20	40	60	80	20	40	60	80
	Diameter of zone of inhibition in (mm)																			
<i>L. Helveticas</i>	30	40	42	45	0	20	21	30	18	20	35	40	30	30	30	32	20	30	45	48
<i>L. acidophilus</i>	30	40	42	41	20	20	20	22	30	30	30	40	35	30	30	31	19	30	46	49
<i>L. gasseristrains</i>	30	40	42	42	0	20	23	23	25	30	30	38	30	25	30	31	19	30	45	47
<i>L. fermentum</i>	0	40	42	42	0	20	22	22	19	35	40	40	0	0	0	20	30	30	43	45

Metal accumulation is known for disrupting bacterial cell wall and other cell components¹⁸. Growing bacteria in different metal concentrations resulted in cell clumping, cell damage, formation of bacterial aggregates and dehydration, indicating damage in cell membrane and leakage of cytoplasmic constituents. In addition to the reports stating that the metal tolerance depends on the interaction of the metal and the bacterial strain, it

has also been shown that extending the exposure time to metals does not allow development of metal resistance, and eventually kills bacteria. Antibacterial and Synergistic Effects were also determined by OD readings taken from spectrophotometer at 640 nm of Fermented Broth with varying concentration (20 mg, 40 mg, 60 mg and 80 mg) of Selenium against Extended Bacterial Fig. 1, 2, 3, 4.

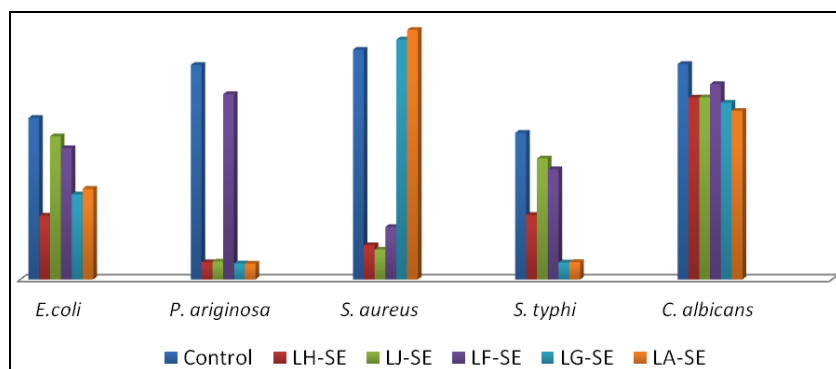


FIG. 1: INHIBITON EFFECT OF Selenium (20mg) CONCENTRATION WITH LACTOBACILLUS CULTURE IN TERMS OF OD @ 640nm (Note: All the results are the average of three different set of experiments)

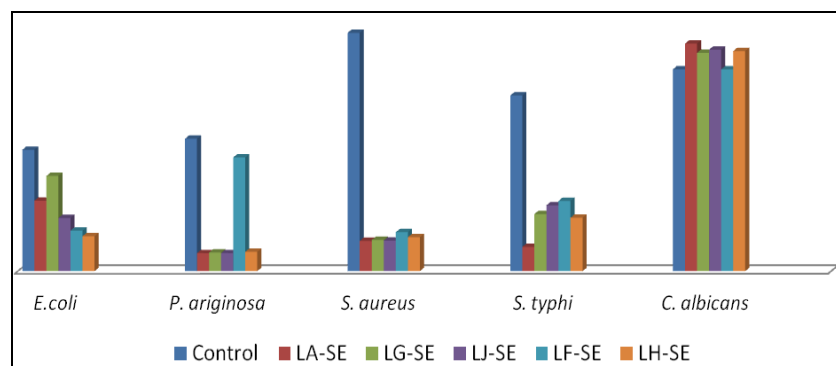


FIG. 2: INHIBITON EFFECT OF Selenium (40mg) CONCENTRATION WITH LACTOBACILLUS CULTURE IN TERMS OF OD @ 640nm (Note: All the results are the average of three different set of experiments)

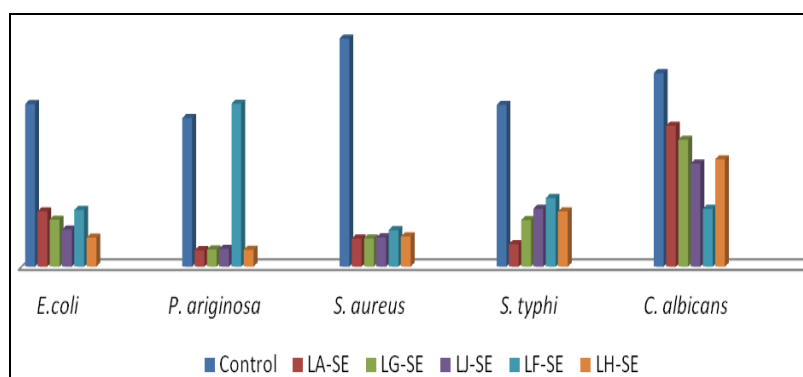


FIG. 3: INHIBITON EFFECT OF SELENIUM (60mg) CONCENTRATION WITH *LACTOBACILLUS* CULTURE IN TERMS OF OD @ 640nm (Note: All the results are the average of three different set of experiments)

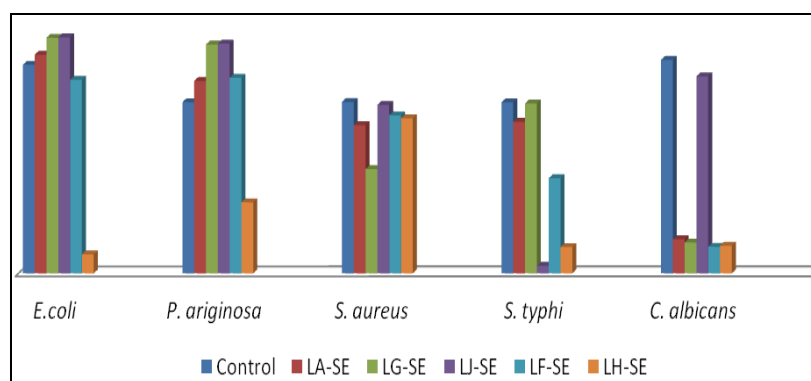


FIG. 4: INHIBITON EFFECT OF SELENIUM (80mg) CONCENTRATION WITH *LACTOBACILLUS* CULTURE IN TERMS OF OD @ 640nm (Note: All the results are the average of three different set of experiments)

The differential response of bacterial strains to metals could also be attributed to the difference in their cell wall structure. Gram positive bacteria such as *S. aureus* have a thick peptidoglycan layer that may reduce the entry of toxic metals. The lower susceptibility of *S. aureus* to metals compared to *P. aeruginosa*, as observed in graphs, also correlates with this assumption. This is well in agreement with a previous study demonstrating higher vulnerability of *E. coli* to heavy metals compared to *S. aureus*.

CONCLUSION: The enhanced antimicrobial activity of the selenium nanoparticles were illustrated in these studies. Further cellular mechanisms involved in the antibacterial activities of metals and their possible applications in health and medicine can be studied. The report and such kind of further studies may pave the way for further investigations on the use of pure metals as effective antibacterial agents to overcome the problem of resistance in pathogens.

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