



## Research Article

# Antimutagenic Potential of Probiotic *Lactobacillus Sporogenes* Using Ames Assay

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### Abstract

**Objective:** Probiotic are beneficial microbial nutrition supplements which have useful effects on human health by conserving of bowel microbial balance. There are many studies that have been recommended the use of probiotic products as cancer risk reducer. The aim of present study was to investigate antimutagenic potential of Probiotic *Lactobacillus sporogenes* against TA98 and TA100 strain of *Salmonella typhimurium*.

**Material and Methods:** Ames test was used in the present investigation to evaluate antimutagenic activity in TA98 and TA100 strains of *Salmonella typhimurium* using direct acting mutagens (Sodium azide) and different concentration of Probiotic *L.Sporogenes* (25, 50, 100 and 500 µg/0.1 ml/plate).

**Results:** Probiotic *Lactobacillus sporogenes* showed significant anti-mutagenicity against mutagen sodium azide in TA98 and TA100 tester strains whereas it showed anti-mutagenicity result in inhibition of 93-97% and 62-88% of his<sup>+</sup> revertants induced by sodium azide in TA98 and TA100 strains respectively.

**Conclusion:** The anti-mutagenicity of Probiotic *Lactobacillus sporogenes* the observed in the present study implies chemopreventive pharmacological importance of Probiotic *Lactobacillus sporogenes* and encourages its use as a biotherapeutic agent.

**Keywords:** Anti-mutagenicity; Sodium azide; *Salmonella typhimurium*; Ames test; *Lactobacillus sporogenes*

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## Introduction

Cancer is one of the most significant deaths causing in the world [1]. Cancer can take over 200 different forms, including lung, prostate, breast, ovarian, hematologic, skin, and colon cancer, and leukemia, and both environmental factors (tobacco smoke, alcohol, radiation, and chemicals) and genetic factors (inherited mutations and autoimmune dysfunction) are associated with an increased risk of developing cancer. Bacterial and viral infections are also strongly associated with some types of cancer (stomach cancers and cervical cancer, respectively). The enormous numbers and diversity of the human gut microflora is reflected in a large and varied metabolic capacity, particularly in relation to xenobiotic biotransformation, carcinogen synthesis and activation. The metabolic activities of the gut microflora can have wide ranging implications for the health of the host, resulting in both beneficial and detrimental effects [2].

Probiotics are defined as viable microorganisms that have beneficial health of a host [3]. They are considered to be “Generally Recognized as Safe” (GRAS) and used for different applications in food [4]. They have been used for therapeutic purposes like replenish the normal gut flora [5].

Probiotics have been of scientific and commercial interest due to a range of health promoting attributes, including suppression of growth of pathogens, control of cholesterol levels, immune system modulation, improvement of lactose digestion, vitamin synthesis, increased bioavailability of minerals and possible anticarcinogenic activity [6-9].

Anti-genotoxicity and anti-mutagenicity are now frequently included among the functional properties for characterizing probiotic microorganisms [10, 11].

The probiotics recommended for human applications are primarily two classes of lactic acid producing microorganisms: the bifidobacteria and lactic acid bacteria (LAB) including species of *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* [12, 13]. Some yeast strains such as *Saccharomyces cerevisiae* and *Saccharomyces boulardii* have also emerged as probiotics for their influence on the human intestinal flora [14-16].

During recent years, probiotics have received considerable attention as dietary agents to modify the gut microflora and hence potentially modulate cancer risk [17].

*Lactobacillus sporogenes* are stable under the extreme conditions of heat, acid and bile salt conditions. [1] Due to their stability, strains of *Lactobacillus sporogenes* survive through gastro intestinal condition of host and exhibit beneficial effects [18].

The aim of present study was to investigate anti-mutagenic potential of probiotic *Lactobacillus sporogenes* against mutagen sodium azide in TA98 and TA100 strain of *Salmonella typhimurium* by using Ames test.

## Materials and methods

### Probiotic strain

Probiotic strain of *Lactobacillus sporogenes* were procured from Unique Biotech Limited, Unit-II, Hyderabad, India

### Chemicals and Reagents

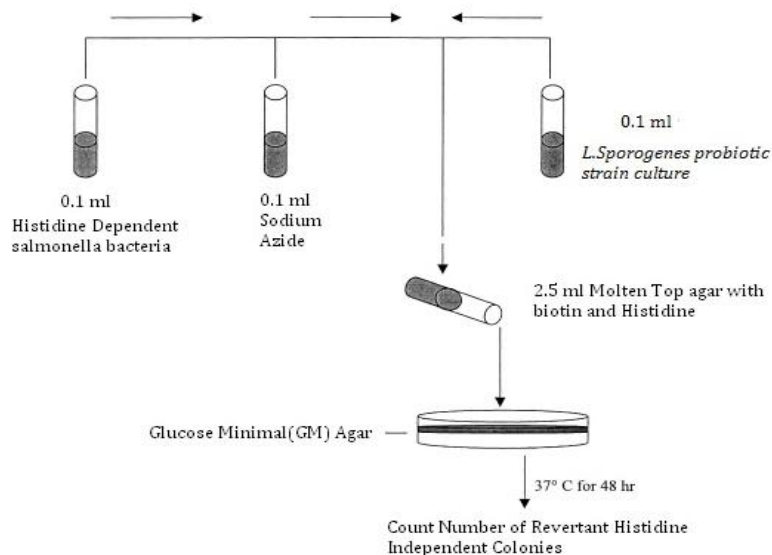
Sodium azide (SA), Dimethyl sulfoxide, Histidine, biotin, Magnesium sulfate, citric acid monohydrate, potassium phosphate dibasic anhydrous, sodium ammonium phosphate, dextrose, Agar, sodium chloride. All chemicals employed in the studies were of analytical reagent grade.

## Bacterial strains

Clinical strains of two human pathogenic bacteria of Gram-negative bacteria *Salmonella typhimurium* TA98 (MTCC 1251) and *Salmonella typhimurium* TA100 (MTCC 1252) were used for the Ames assay. All the microorganisms were obtained from the Institute of microbial technology (IMTECH), Chandigarh and maintained in the Department of Pharmaceutical Microbiology, SSR College of Pharmacy, Silvassa. A fresh nutrient broth culture was grown to a density of  $1-2 \times 10^9$  cells/ml for 12 hour at 37 °C before each experiment.

## Determination of antimutagenicity against direct acting mutagens

*Salmonella* mutagenicity assay was conducted as previously described by Mortelmans K and Zeiger E, 2000 [19]. Plate incorporation method was done for anti-mutagenicity assay without microsomal activation. Fresh bacterial cultures of *S. typhimurium* strains TA 100 and TA 98 ( $1-2 \times 10^9$  cells/ml) were mixed with 2ml of molten agar containing 0.5 mm histidine/biotin solution, different concentration of *L.Sporogenes* (25, 50, 100 and 500 µg/0.1 ml/plate) and direct acting mutagens such as sodium azide (2.5µg/plate). Further it was spread over minimal glucose agar plates. Plates were incubated for 48 hours at 37 °C and the revertant colonies were counted. Steps involved in the plate incorporation method depicted in the Figure 1.



**Figure 1** Schematic representation of Steps involved in the plate incorporation method

Percent inhibition of mutagenicity was determined by the following formula:

$$\text{Inhibition (\%)} \text{ of mutagenicity} = 1 - \frac{\text{His}^+ \text{ revertant induced / plate by mutagen in the presence of probiotic } L.Sporogenes}{\text{His}^+ \text{ revertant induced / plate by mutagen alone}} \times 100$$

## Statistical analysis

Results were expressed as Mean  $\pm$  S.E.M. Statistical significance was tested using one way ANOVA as appropriate using computer based fitting program (GraphpadPrism 6.0.). Differences were considered to be statistically significant when  $p < 0.05$ .

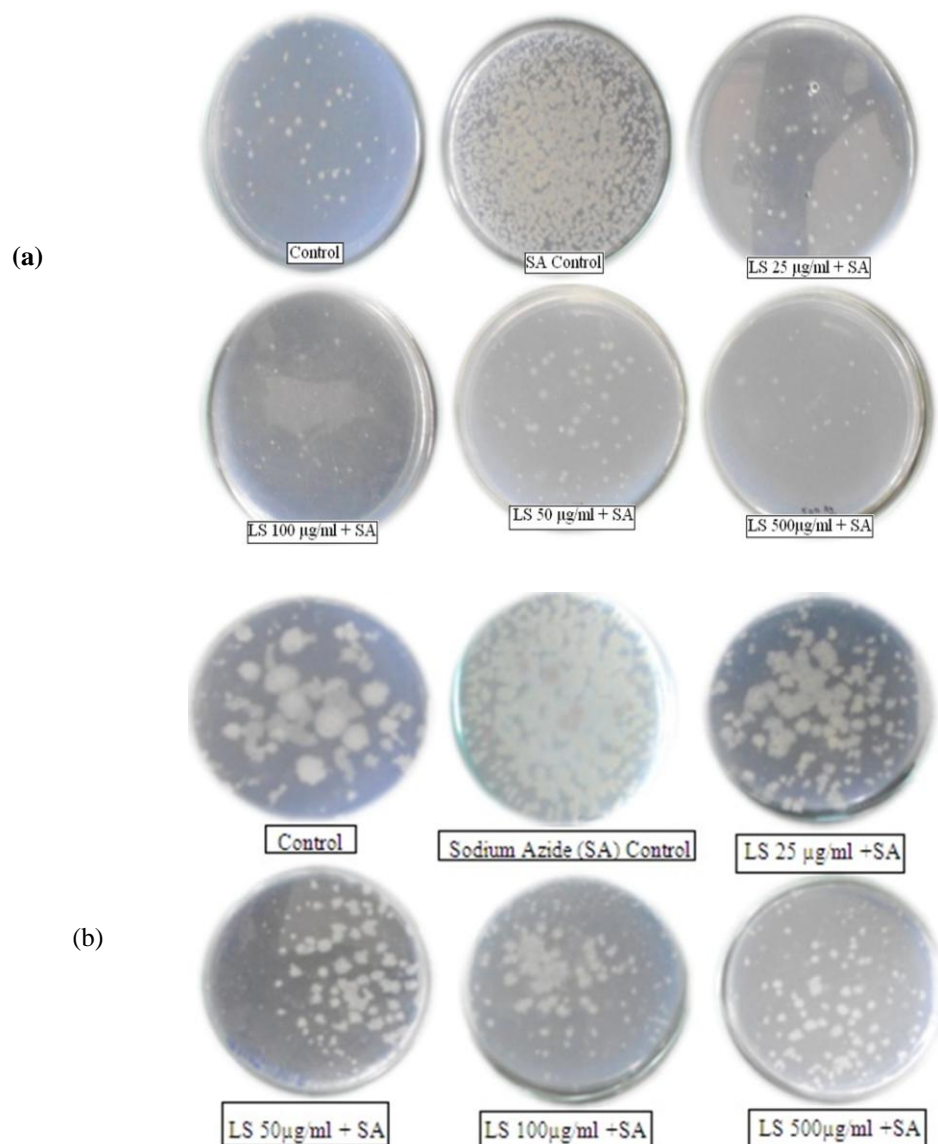
## Results and Discussions

### Antimutagenic activity of Probiotic *L.Sporogenes* against TA98 and TA100 strain of

***Salmonella typhimurium***

In the present investigation, different doses of *L.Sporogenes* (LS) was selected for evaluation purpose LS 25, LS 50, LS 100 and LS 500 groups were given 25 µg, 50 µg, 100 µg and 500 µg per plate respectively. Sodium azide (SA) serves as positive control.

Evaluation of anti-mutagenic potential of Probiotic strain *L.sporogenes* was demonstrated by colony characteristic using Ames test against *Salmonella typhimurium* TA98 (MTCC 1251) and *Salmonella typhimurium* TA100 (MTCC 1252) as shown in figure 2 a) and b), respectively.



**Figure 2** Evaluation of anti-mutagenic potential of Probiotic strain *L.sporogenes* using (a) *Salmonella typhimurium* TA98 (MTCC 1251) (b) *Salmonella typhimurium* TA100 (MTCC 1252)

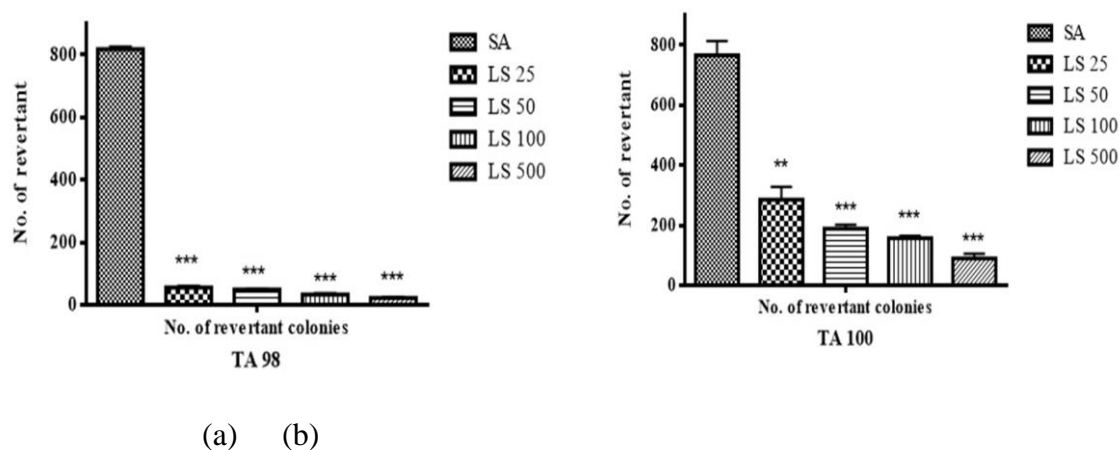
Experimental Results of anti-mutagenic studies (given in Table 1) revealed that culture of *L.Sporogenes* was effective in reducing the mutagenicity caused by the mutagen (sodium azide).

Table 1 In vitro Anti-mutagenic activity of *Lactobacillus sporogenes* against sodium azide on *Salmonella typhimurium* strain TA 98 and TA 100.

Treatment	Dose ( $\mu\text{g}/\text{plate}$ )	TA 98		TA 100	
		Number of his <sup>+</sup> revertant/plate	% Inhibition	Number of his <sup>+</sup> revertant/plate	% Inhibition
Positive Control	SA	817 $\pm$ 7.33	---	765 $\pm$ 47.4	---
	25	56 $\pm$ 4.27**	93.14 $\pm$ 1.58**	285.3 $\pm$ 42.74**	62.75 $\pm$ 2.57**
Co-incubation (SA + LS)	50	48 $\pm$ 2.07**	94.1 $\pm$ 1.05**	188 $\pm$ 13.3**	75.42 $\pm$ 0.93**
	100	33 $\pm$ 3.12**	95.97 $\pm$ 1.40**	156.3 $\pm$ 8.373**	79.61 $\pm$ 0.64**
	500	21 $\pm$ 4.41**	97.43 $\pm$ 0.06**	89.67 $\pm$ 15.59**	88.37 $\pm$ 0.80**

n=3 Trial, \*\*\*p<0.001 extremely significant, \*\*p<0.01 very significant, \*p<0.05 significant, <sup>ns</sup>p>0.05 nonsignificant, significant compared to positive control, values are expressed as Mean $\pm$ SEM.

The percent inhibition of sodium azide induced mutagenicity was recorded as 93.14 $\pm$ 1.58, 94.1 $\pm$ 1.05, 95.97 $\pm$ 1.40, and 97.43 $\pm$ 0.06 in TA 98 and 63.75 $\pm$ 2.57, 75.42 $\pm$ 0.93, 76.61 $\pm$ 0.64, 88.37 $\pm$ 0.80 in TA100 for dose 25, 50, 100, and 500  $\mu\text{g}/\text{plate}$  respectively (Table 1). Antimutagenic activity of *L.Sporogenes* in *Salmonella typhimurium* TA98 (MTCC 1251) and TA100 (MTCC 1252) against sodium azide was shown in figure 3 (a) and (b), respectively.



**Figure 3** Anti-mutagenic activity *L.Sporogenes* in (a) *Salmonella typhimurium* TA98 (MTCC 1251) (b) *Salmonella typhimurium* TA100 (MTCC 1252) against sodium azide. There was a statistically significant difference (p<0.001) at all dose level with respect to control and among *L.sporogenes* when analyzing the data by One Way ANOVA.

As is clear from figure 3 (a) and (b), a dose dependent response was observed with maximum percentage inhibition obtained at maximum dose of *L.Sporogenes* tested.

According to one way ANOVA the protective effect of *L.Sporogenes* against SA induced mutagenicity in

TA 98 and TA 100 was verified and found significant ( $p < 0.05$ ).

In the present study, the Ames test is a basic toxicological test that can be used to determine if a substance is potentially genotoxic. Ames test serves as a quickest way to analyze the carcinogenic potential of a compound. It is a very easy and cheap test that can be set up in most labs. In this test *Salmonella typhimurium* strains carrying mutations in the histidine operon is being used, hence are histidine dependent. Mutagens are agents (physical or environmental) that can induce a genetic mutation or the increase in rate of mutation whereas antimutagens are agents that inhibit the effects of mutagens. Addition of mutagens, bacteria reverse back to histidine independent and form colonies in histidine deficient medium. So addition of anti-mutagenic agents considerably reduces reverse mutation capability of mutagens.

Different bacterial strains used here detect different mutagens. TA 100 detect mutagens causing base pair substitutions, TA 98 detects frame shift mutagen.

Mutagens may be either direct acting or requiring microsomal activation. Direct acting mutagens interact directly with DNA to produce mutation. In this study direct acting mutagens like Sodium azide, were used and able to reverse the mutation of bacteria to form colonies in minimal glucose agar plates.

Sodium azide on positive plate shown maximum growth and the auxotrophic strain of *Salmonella typhimurium* showed back mutation and converted to its wild type average growth was found on negative control plate which was without addition of sodium azide.

A preventive effect on malignant development could be mediated by production of anti-mutagens and LAB binding of mutagens, and this has been reviewed [20]. One possible mechanism for the anti-mutagenic properties of LAB involves a physical binding of the mutagenic compounds by these bacteria. In a review of the therapeutic role of dietary LAB, Fernandes et al [21]. suggested that the cellular uptake of nitrites by LAB reduced the formation of nitrosamines from nitrites. In vitro assessment of possible antimutagenic actions of LAB, specifically *L. casei* and a blend of *B. longum* and *L. gasseri*, indicated a significant reduction in mutagen induced chromosome aberrations and micronuclei [22]. In a study with healthy adults, consumption of fermented milk containing *L. acidophilus* ( $\sim 10^{10}$ - $10^{11}$  cfu) contributed to a significant decline in mutagenic activity in the urine and feces following three days of supplementation [23].

In general, live cells of probiotic bacteria showed higher anti-mutagenic activity, and this was permanent, in contrast to killed cells. In this in vitro study, butyric acid, and to a lesser extent, acetic acid inhibited mutagens. Strains of *B. lactis* were shown to express anti-mutagenic properties, probably linked to cell wall constituents. The anti-mutagenic effect was active also after acid and bile treatment, mimicking the GI transport, and interestingly, enhanced in the presence of whole milk [24]. One mechanism for this effect can be binding of mutagens, and heterocyclic aromatic amines were shown to be bound to the cell wall of certain bacteria, such as *B. longum* and other LAB, and thereby be detoxified [25, 26]. In *L. plantarum* KLAB21, however, the anti-mutagenic effect was mediated by three glycoproteins which are secreted extracellularly [27].

## Conclusion

The results showed that most significant inhibition of mutagenicity induced to TA 98 by the direct acting mutagens such as sodium azide. The anti-mutagenicity of probiotic *Lactobacillus sporogenes* the observed in the present study implies chemopreventive pharmacological importance of probiotic *Lactobacillus sporogenes* and encourages its use as a Biotherapeutic agent.

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