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SURVIVAL AND GERMINATION OF *LACTIC ACID BACILLUS* SPORES IN PRESENCE OF AMOXICILLIN/CLAVULANATE ANTIBIOTIC

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ABSTRACT

Background: *Lactic acid bacillus* spores (*Bacillus coagulans*) has excellent stability and shows therapeutic effects on intestinal diseases, such as acute diarrhoea, irritable bowel syndrome, antibiotic-related diarrhoea, constipation and colitis. Spores of *Lactic acid bacillus* resist gastric acid to reach the small intestine, where they can germinate and propagate. There is an existing misconception that antibiotics degrade the probiotics when given in conjunction. **Aim**: To study the survival, germination and proliferation of *Lactic acid bacillus* spores in presence of Amoxicillin/Clavulanate antibiotic. **Method:** 'Augpen LB®-625' tablet (FDC tablet of Amoxicillin 500 mg, Potassium Clavulanate 125 mg with *Lactic acid bacillus* 60 million spores) is dissolved in 0.1N HCl and pH 6.8 phosphate buffer. It was diluted and incubated in MRS broth and mixed with PNY agar media, followed by solidification and repeated incubation. The resulted number of colonies in each plate was counted. **Results:** It was observed that 60 million spores of *lactic acid bacillus* present in the 'Augpen LB®-625' tablet produced 7.3 billion CFU at 24 hours and 51 billion CFU at 72 hours under suitable conditions mimicking human intestinal pH, which helps in intestinal colonization with the probiotics. **Conclusion:** This study provides the evidence for survivability of the probiotic *Lactic acid bacillus* in acidic pH and its germination in presence of Amoxicillin and Clavulanate.

KEYWORDS: Germination, *Lactic acid bacillus*, Spores, Probiotic, Amoxicillin/Clavulanate.

INTRODUCTION

Amoxicillin/Clavulanate is a broad-spectrum antibacterial that has been in clinical use for many decades and is indicated for the treatment of bacterial sinusitis, acute otitis media, community-acquired pneumonia and acute exacerbations of chronic bronchitis. Amoxicillin/Clavulanate continues to be an important antimicrobial agent both now and in the future.[1] The widespread use of this combination has led to increase in the incidence of Antibiotic-Associated Diarrhoea (AAD), due to the disruption of colonic microflora by the antibiotics.[2,3] AAD complicates 2% to 25% of antibiotic treatment cases and its incidence during therapy with Amoxicillin/Clavulanate is 10% to 25%.[4]

Probiotics given along with antibiotic treatment is an effective and safe intervention for the prevention of AAD, as they reinforce the human intestinal barrier and help to maintain the gut flora and/or by competitively inhibiting the growth of pathogens.[3,5] Spores of *Bacillus spp*. commercially used as probiotics, unlike *Lactobacillus spp.*, are dormant. Spore-forming probiotics, as compared to non-spore formers, have an advantage since they are resistant to gastric acid and can remain stable at room temperature.[6] Bacterial spores contain a high concentration of dipicolinic acid which is responsible for its high tolerance to the acidic conditions in the stomach,[7] and they are able to pass through the stomach. On other hand, live probiotics are susceptible to gastric acid and bile salts, and their numbers decrease in the gastrointestinal tract. Therefore, spore-forming species are of great importance due to their high vitality.[6]

'Augpen LB®-625' tablet containing Amoxicillin 500 mg and Potassium Clavulanate 125 mg with *lactic acid bacillus* 60 million spores, as fixed-dose combination (FDC) formulation manufactured by Zuventus Healthcare Limited, Mumbai, India. There is always a concern in the medical fraternity that Amoxicillin/Clavulanate can degrade the lactic acid bacillus when given in conjunction with it. Spores after passing through the stomach, begin to germinate in the duodenum and proliferate in the upper part of the small intestine.[8] The suggested probiotic amount for intestinal colonization is $\geq 5 \times 10^9$ CFU/day.[9] We conducted an *in-vitro* study to provide evidence for the survivability of

lactic acid bacillus (*Bacillus coagulans*) in the required numbers when given along with the antibiotic.

MATERIALS AND METHOD

One tablet of 'Augpen LB®-625' containing Amoxicillin/Clavulanate 625 mg and Lactic acid bacillus 60 million spores was dissolved in 5L of 0.1N HCl and 5L of pH 6.8 phosphate buffer. A fraction of the solution was mixed with 0.9% saline solution (10^{-2} dilution), and heated on water bath at 75°C for 30 minutes followed by immediate cooling to about 45-50°C (solution A). One mL of its aliquot and PNY agar medium was added in each of the two sterile petri plates. After mixing and solidifying, petri plates incubated at 37°C for 72 hours and the number of colonies in each plate were counted for the initial count. One mL aliquot of solution A was added to three MRS broth test tubes and tubes were incubated at 37°C for 24, 48 and 72 hours for germination. The incubated and diluted solution (10^{-10} in saline) from each time point was again incubated and cooled PNY agar medium was added to the two sterile petri plates, each followed by solidification and incubation of media at 37°C for 72 hours. The number of colonies in each plate was counted and calculations were done after applying the dilution factor.

RESULTS

The growth of *Lactic acid bacilli* in two different mediums i.e. 0.1 N HCl and pH 6.8 phosphate buffer at different incubation period is shown in Table 1.

Table 1: Lactic Acid Bacillus count at different incubation periods.

	0.1N HCl medium		**pH 6.8 phosphate buffer medium**	
Factors	**'Augpen LB®-625' Tablet**	**'Augpen®-625' Tablet and *lactic acid bacillus* powder separately**	**'Augpen LB®-625' Tablet**	**'Augpen®-625' Tablet and *lactic acid bacillus* powder separately**
pH of 10^{-2} dilution	Observed pH 2.76	Observed pH 2.18	Observed pH 6.78	Observed pH 6.79
Initial count in 10^{-2} dilution	No Growth observed	No Growth observed	4.0 million spores/tablet	3.8 million spores/tablet
Incubation after 24 hours	No Growth observed	No Growth observed	7.3 billion CFU/tablet	9.3 billion CFU/tablet
Incubation after 48 hours	No Growth observed	No Growth observed	25.3 billion CFU/tablet	22.2 billion CFU/tablet
Incubation after 72 hours	No Growth observed	No Growth observed	51.0 billion CFU/tablet	34.8 billion CFU/tablet
Negative Control	No Growth observed	No Growth observed	No Growth observed	No Growth observed
Positive Control	Growth observed	Growth observed	Growth observed	Growth observed
Augpen LB®-625: FDC of Amoxicillin 500 mg and Potassium Clavulanate 125 mg with *lactic acid bacillus* 60 million spores (Zuventus Healthcare Limited) Augpen®-625: FDC of Amoxicillin 500 mg and Potassium Clavulanate 125 mg (Zuventus Healthcare Limited)				

The study results show no growth of lactic acid bacillus spores in acidic media whether it is used separately or in conjunction with antibiotic whereas there is a significant increase in lactic acid bacillus count in phosphate buffer (pH 6.8) after 24, 48 and 72 hours of incubation. The count at 72 hours was 51 billion CFU in the 'Augpen LB®-625' tablet group as compared to 34.8 billion CFU when the antibiotics and spores were added separately.

DISCUSSION

Manipulating the gut bacterial community by using probiotic bacteria is a therapeutic choice for treating intestinal microbial imbalance.[10] Amongst a large number of probiotics in use today, bacterial spore formers, mostly of the genus *Bacillus* are most effective to colonise human intestines.[11] Survival and germination are essential factors for spore probiotics for their beneficial effects in the gastrointestinal tract.[12] This study was conducted with the aim to show, how 60 million *lactic acid bacillus* spores can produce a count of ≥5 billion CFU after germination and the survival of *lactic acid bacilli* in presence of the antibiotic.

Earlier, Hyronimus *et al.* evaluated thirteen spore-forming *lactic acid bacilli* for their resistance to acid. This study indicated high acid tolerance of *Bacillus* spp.[13] Casula *et al* investigated whether spores can germinate in the gastrointestinal tract by using a murine model. The study stated that the spores germinated in significant numbers in the jejunum and ileum, suggesting their colonization into the small intestine.[14] Ghelardi *et al* documented that the orally administered *Bacillus* spp. follows transient colonization into the intestine.[15]

In the present investigation, *Lactic acid bacillus* spores showed no growth at acidic pH when incubated at 37°C for 72 hours, which indicated the ability of the spore to survive in the dormant environment under harsh gastrointestinal conditions (acidic media). An increase in the number of *Lactic acid bacillus* spores from 60 million to 7.3 billion at 24 hours explains the potential of survival, growth and proliferation at biological pH of 6.8. The proliferation to 7.3 billion CFU fulfils the recommended criteria of probiotic amount for intestinal colonization i.e. ≥5 billion CFU/day and confirms the survival of lactic acid bacillus in the presence of antibiotics.

CONCLUSION

This study provides an evidence of the survivability and proliferation of the probiotic *Lactic acid bacillus* in the presence of antibiotics. It is also shown that 60 million spores of lactic acid bacillus present in 'Augpen LB®-625' tablet can produce sufficient viable counts to match

the criteria of the required probiotic amount for intestinal colonization i.e. ≥5 billion CFU/day.

CONFLICT OF INTEREST

There are no conflicts of interest.

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