

EvoraPlus Oral Probiotic Tablet: New paradigm for Caries Prevention in Children

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ABSTRACT

Background: According to the World Health Organization, probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit to the host. The use of oral probiotics in children to improve oral health may lead to nonpathogenic bacteria replacing cariogenic bacteria. The Evora Plus oral probiotics tablet is the first such oral probiotics tablet containing a proprietary blend of three select species of naturally occurring oral bacteria, each with a specific function for maintaining a healthy oral environment. These strains include *Streptococcus oralis* KJ3sm, *Streptococcus uberis* KJ2sm, and *Streptococcus rattus* JH145.

Aim: To compare the levels of salivary total bacterial count, salivary pH levels, and salivary *S. mutans* count before and after consumption of the Evora Plus oral probiotic tablet.

Materials and methods: Around 40 caries-free schoolchildren from Daksh International School, Gadarwara, Madhya Pradesh, India, aged 12–14 years, were selected and divided equally into two groups—group A, in which total salivary bacterial count and pH were evaluated, and group B, in which salivary *S. mutans* count and pH were evaluated. These groups were further subdivided into group A1 and group B1, which served as the control groups not receiving the Evora Plus oral probiotic tablet, and group A2 and group B2, which were the test groups consuming the Evora Plus oral probiotic tablet for 30 days. Assessment of salivary samples for total bacterial count was done at baseline and after 30 days for groups A1 and B1. Salivary pH estimation was done at baseline and after 30 days for the entire group, and salivary *S. mutans* count was done at baseline and after 30 days for groups A2 and B2. Salivary pH level was checked using a digital pH meter; nutrient agar was used for salivary total bacterial count, while Mitis salivarius bacitracin agar was used for salivary *S. mutans* count. The number of colonies was counted and subjected to statistical analysis using unpaired and paired Student's *t*-test.

Results: The study showed a significant reduction in salivary total bacterial count and salivary *S. mutans* counts, as well as a significant increase in salivary pH after 30 days in the Evora Plus oral probiotic group compared with the group not receiving the Evora Plus oral probiotic tablet.

Conclusion: Cariogenic microorganisms could be reduced by an oral probiotic formulation. Thus, the use of the Evora Plus oral probiotic tablet could be recommended for the prevention of enamel demineralization.

Keywords: Evora Plus oral probiotic tablet, *Streptococcus mutans* count, Salivary pH, Salivary total bacterial count.

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INTRODUCTION

Science has the power to diagnose and treat infections before they cause any damage to a living organism. Preventive therapy in the form of probiotics is an alternative and potent way to combat infections by using harmless yet powerful strains of bacteria to displace and completely remove pathogenic microorganisms.^{1–3} Thus, Nobel laureate Elie Metchnikoff, known as the “father of probiotics,” introduced the concept of administering probiotics in the early 20th century. He proposed that ingesting such potent yet harmless strains of microorganisms could have substantial health benefits for humans. On the contrary, documentation of their influence in sites beyond the intestinal tract is now growing. Residential and transient commensals are competent enough to manipulate many colonized and noncolonized systems of human physiology through interrelated organ function and communication systems.^{4–8} Validation of probiotic-associated effects on sites other than the intestines spans the range from theoretical to evidence-based, supported by controlled human trials.^{9–12} To date, probiotics have been successfully used for treating diet-related and gastrointestinal disorders in the modern world of medicine. The modified use of probiotics in the resident human oral ecosystem still needs to be specifically adjusted because these microorganisms offer the advantage of being acclimated to it. Studies are still being carried out to determine the efficacy and

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efficiency of probiotics in the prevention of various oral diseases, including those affecting gums and hard tissues. Hence, such evidence from these studies has paved the way for a new concept of oral probiotics in dentistry.^{13,14}

Nowadays, the use of such harmless yet competent microorganisms has been introduced to treat a wide variety of clinical conditions linked to specific bacterial microorganisms, including oral health issues like dental decay and periodontal/

gingival problems. A combination of harmless microorganisms as probiotic strains is often used to enhance these beneficial effects.^{15,16}

Thus, the Evora Plus oral probiotic tablet (Fig. 1) was created by Dr Jeffrey Hillman, DMD, PhD, working at Oragenics, Inc., Alachua, Florida, United States of America, in early August 2010. It contains a proprietary blend of three selected species of naturally occurring wild strains of oral bacteria, each with a specific function for maintaining a healthy human oral ecosystem. These strains include *S. oralis* (KJ3sm), *S. uberis* (KJ2sm), and *S. rattus* (JH145). Keeping this in mind, the present *in vivo* study was undertaken to evaluate the efficacy and efficiency of the oral probiotic chewing mint tablet, Evora Plus, in caries prevention.^{17–19}

MATERIALS AND METHODS

In the present study, 40 caries-free healthy children aged 12–14 years were selected out of 200 schoolchildren for the study. The duration of the study was 30 days. All the selected children were provided with fluoridated toothpaste and soft-bristle toothbrushes. They were instructed to perform the modified Bass technique for standardization purposes (Fig. 2). Around 40 children were then randomly divided into two groups—group A and group B, with 20 children each. Group A, the experimental group, received the Evora Plus oral probiotic tablet (Fig. 3) and control group not receiving Evora Plus oral probiotics tablet.

These groups were further subdivided into group A1 and group B1, which served as control groups not receiving the Evora Plus oral probiotic tablet, and group A2 and group B2, which were test groups consuming the Evora Plus oral probiotic tablet for 30 days. Assessment of salivary samples for total bacterial count was done at baseline and after 30 days for groups A1 and B1. Salivary pH was measured at baseline and after 30 days of tablet consumption for the entire group, and salivary *S. mutans* count was assessed at baseline and after 30 days for groups A2 and B2. Unstimulated salivary samples were collected using the spitting method (Fig. 4) into two 10 mL sterile vials—the first vial for pH level estimation and the second vial for microbiological evaluation. The pH levels were measured with a digital pH meter immediately after spitting, while the other set of vials containing saliva samples were sealed and stored in a thermocol icebox for transportation to the microbiological lab within 2 hours for processing on the same day. Total salivary bacterial count was determined by streaking saliva, following serial dilution with saline, onto agar plates and culturing for 48 hours at 37°C (Fig. 5). Similarly, the salivary *S. mutans* count was assessed using the streaking method with salivary samples, following serial dilutions onto a selective agar medium known as Mitis salivarius bacitracin agar, and cultured in anaerobic conditions for 72 hours at 37°C. The colonies formed were counted using a digital colony counter (Fig. 6). The values thus obtained were tabulated and statistically analyzed.



Fig. 1: Evora Plus oral probiotic tablets



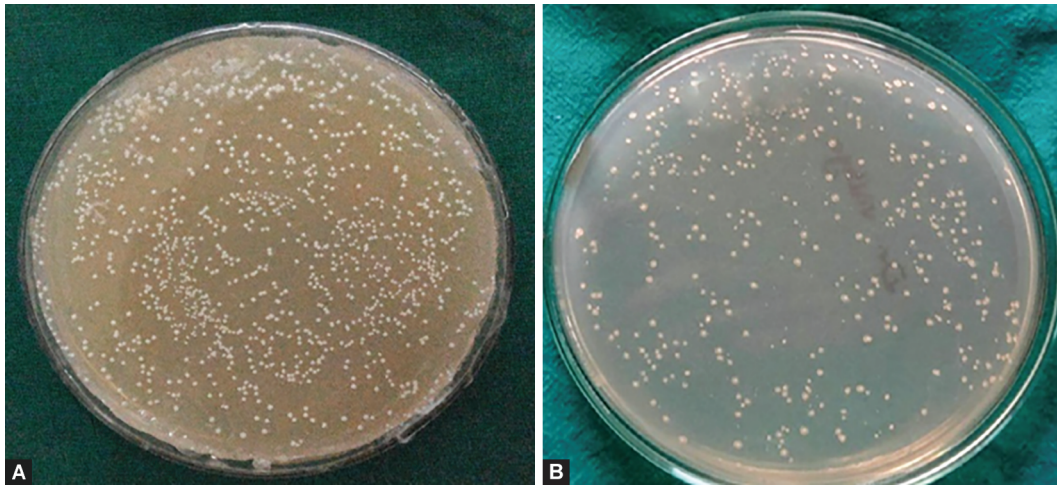
Fig. 2: Tooth brushing done under supervision for entire study group children



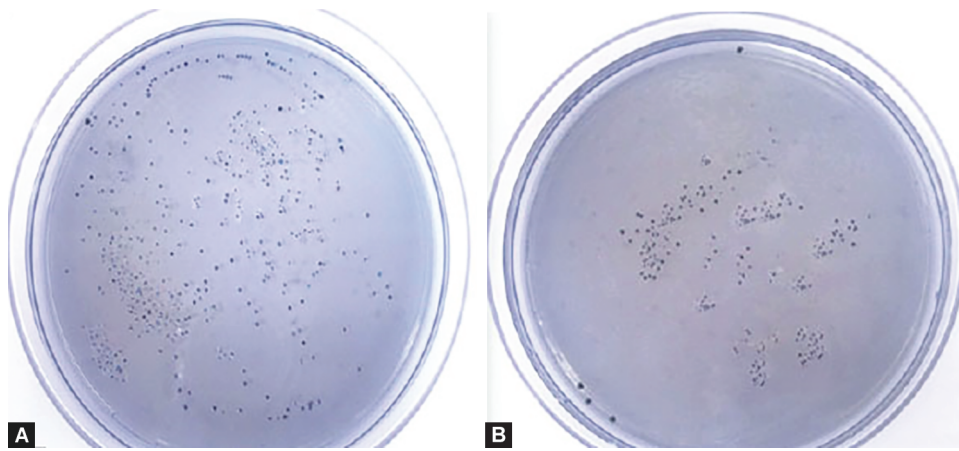
Fig. 3: Chewing Evora Plus oral probiotics tablet



Fig. 4: Spitting method for saliva collection



Figs 5A and B: Salivary total bacterial count. (A) Baseline salivary total bacterial count; (B) Salivary total bacterial count after 30 days administration of Evora Plus oral probiotic tablet



Figs 6A and B: Salivary *S. mutans* count. (A) Baseline salivary *S. mutans* count; (B) Salivary *S. mutans* count after 30 days administration of Evora Plus oral probiotic tablet

RESULTS AND OBSERVATION

Table 1 and Figure 7 reveal the comparison of mean salivary total bacterial colony count (CFU) between group A1 (control group) and group A2 (EvoraPlus oral probiotic tablet) at baseline and after 30 days. After 30 days, the mean salivary total bacterial colony count was reduced from 417.70 ± 26.32 to 378.84 ± 21.65 (group A1) and from 424.70 ± 28.06 to 167.80 ± 24.83 (group A2), respectively.

Table 2 and Figure 8 reveal the comparison of mean salivary pH at baseline and after 30 days between group A1 and B1 (control group) and group A2 and B2 (Evora Plus group). After 30 days, the mean salivary pH increased from 6.60 ± 0.433 to 6.68 ± 0.481 in group A1 and B1 and from 6.70 ± 0.519 to 6.98 ± 0.289 in group A2 and B2.

Table 3 and Figure 9 reveal the comparison of mean salivary *S. mutans* colony count between group B1 and group B2 at baseline and after 30 days. After 30 days, the mean salivary *S. mutans* colony count was slightly reduced from 514.90 ± 18.78 to 483.60 ± 17.82 in group B1 and from 350.00 ± 12.99 to 153.90 ± 10.00 in group B2.

DISCUSSION

The values drawn from the study concluded a significant increase in salivary pH after 30 days of administration of the Evora Plus

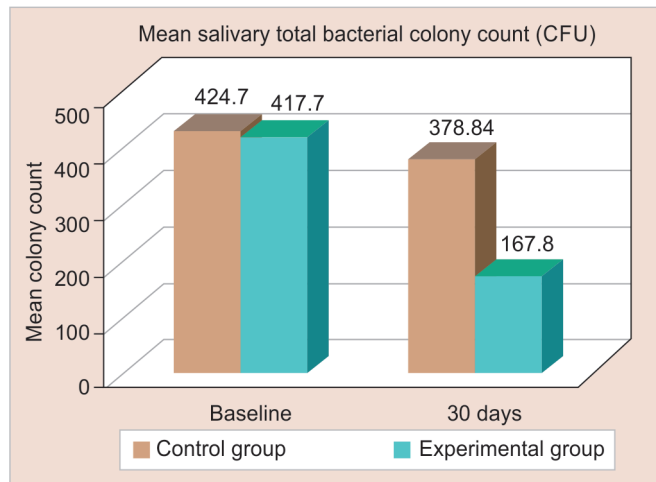
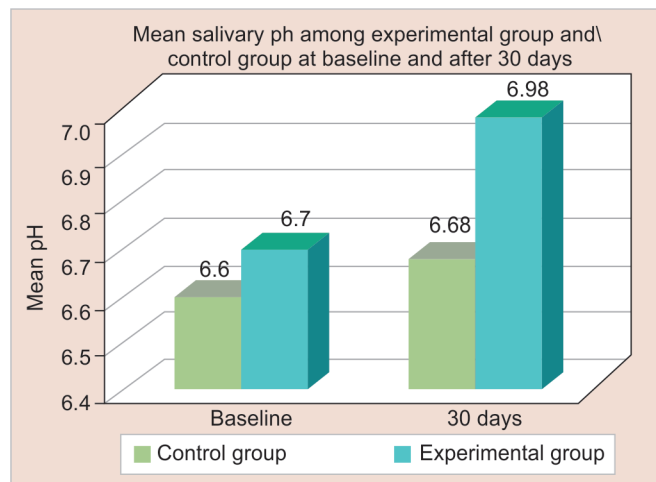
oral probiotic tablet in the group consuming the Evora Plus oral probiotic tablet, compared to the control group that was not consuming the Evora Plus oral probiotic tablet. As the number of harmful oral microorganisms decreases, the acidic oral environment becomes neutral, and the buffering capacity of saliva increases in the oral cavity. Thus, the salivary pH level increased. Similar results were observed by Hillman and Socransky, who reported an increase in salivary pH in the study group swishing with ProBiora3 mouthwash twice daily for 2 weeks. Similar results were also obtained from the studies by Chinnappa et al. and Bhalla et al.^{17,20}

The values drawn from the study concluded a significant reduction in total salivary bacterial count in the test group after 30 days of consumption of the oral probiotic tablet, compared to the control group. The selected microorganisms in the Evora Plus oral probiotic tablet, such as ProBiora3 organisms, might have reduced harmful microorganisms while leaving a healthy microbial ecosystem intact in the oral cavity. Similar results were obtained in the studies of Hillman et al., who conducted *in vivo* studies and concluded similar findings with the use of oral probiotics in reducing the number of disease-causing microorganisms in the oral cavity after 15 days of administration. Similar studies were done by Hillman and Shivers (1988), Hillman et al. (1989), Taubman et al., Walker and Buckley, and Hillman. Hillman also conducted an animal

Table 1: Mean salivary total bacterial colony count values of group A1 and group A2

Groups	Mean salivary total bacterial colony count			
	Baseline		30 days	
	Mean	SD	Mean	SD
Group A1	424.70	28.06	378.84	21.65
Group A2	417.70	26.32	167.80	24.83
Unpaired Student "t" test value	0.146		3.917	
Significance "p" value	0.885 (NS)		0.001 (HS)	

Bold values represent highly significant *p* values

**Fig. 7:** Mean salivary total bacterial colony count (CFU) among group A1 and group A2 at baseline and after 30 days**Fig. 8:** Mean salivary pH values of group A1 and B1 and group A2 and B2

study in rats and found a significant reduction in pathogenic oral microflora after administering 106 colonies of oral probiotic bacteria daily for 7 days.²⁰⁻³⁰

It was also observed in the present study that the salivary *S. mutans* count decreased significantly in the test group receiving the Evora Plus oral probiotic tablet compared to the control group not receiving the Evora Plus oral probiotic tablet after 30 days of intervention. *S. rattus*, one of the strains present in the Evora Plus oral probiotic tablet, is a wild variant of *S. mutans*. It produces

Table 2: Mean salivary pH values of group A1 and B1 and group A2 and B

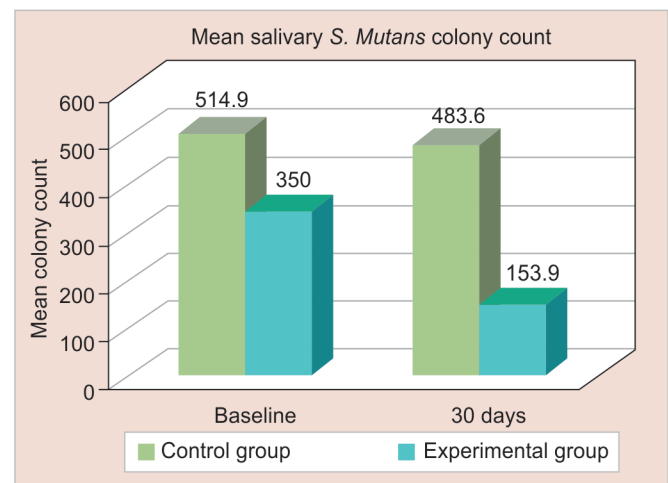
Groups	Mean salivary pH			
	Baseline		30 days	
	Mean	SD	Mean	SD
Group A1 and B1	6.60	0.433	6.68	0.481
Group A2 and B2	6.70	0.519	6.98	0.289
Unpaired Student "t" test value	1.042		3.877	
Significance "p" value	0.312		0.001 (HS)	

Bold values represent highly significant *p* values

Table 3: Mean salivary *S. mutans* colony count between group B1 and group B2

Groups	Mean salivary <i>S. mutans</i> colony count			
	Baseline		30 days	
	Mean	SD	Mean	SD
Group B1	514.90	18.78	483.60	17.824
Group B2	350.00	12.99	153.90	10.001
Unpaired Student "t" test value	2.782		8.041	
Significance "p" value	0.012 (S)		0.001 (HS)	

Bold values represent highly significant *p* values

**Fig. 9:** Mean salivary *S. mutans* colony count among group B1 and group B2 at baseline and after 30 days

less or no lactic acid from the metabolism of sugar compared to the parent *S. mutans*. This genetic defect in this strain has been identified and deleted, resulting in a mutation in the middle of the structural gene for the enzyme lactate dehydrogenase. As the strain does not produce lactic acid, it is virtually incapable of causing dental caries and can successfully compete as a primary invader for binding at adhesion sites in the oral cavity against *S. mutans*. Similar results were obtained by Hillman and Johnson et al., in their *in vitro* studies. The conclusions were consistent with those of Chinnappa et al. and Bhalla et al., who also showed a statistically significant reduction in *S. mutans* levels in saliva before and after the consumption of probiotic ice cream and curds, respectively, over a period of 7 days.^{21,24,25} Similar studies were conducted in orthodontic patients by Cildir et al., Zhu et al., and Hoorizad et al., showing a significant reduction in salivary *S. mutans* count 2 weeks after the administration of oral probiotic liquid.²⁶⁻²⁹

CONCLUSION

Hence, we can conclude that the addition of Evora Plus oral probiotics to an oral care regimen can restore the natural balance of beneficial bacteria, which may be depleted or compromised by modern diets, lifestyle stress, routine medication, illness, or other issues. Oral probiotics in any form support the health of hard and soft tissues in the oral cavity, whiten teeth, and freshen breath. These oral probiotics come in various forms such as tablets, lozenges, mouthwashes, and chewing gums, and are used to achieve caries reduction, changes in *S. mutans* and lactobacilli counts, plaque pH control, and reversal of root caries lesions.

Thus, we can envision that in the years to come, there will be stores selling oral probiotic food items with comprehensive oral health care and anticariogenic properties. Simply consuming these products may address issues related to dental caries and gum health for the next generation.

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