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An in vivo pilot study on permanence in saliva of two different probiotics strain administered through chewing gums

Submission date	Recruitment status	[_] Prospectively registered
23/01/2025	No longer recruiting	[_] Protocol
Registration date	**Overall study status**	[_] Statistical analysis plan
27/01/2025	Completed	[_] Results
Last Edited	**Condition category**	[_] Individual participant data
27/01/2025	Oral Health	[X] Record updated in last year

Plain English summary of protocol

Background and study aims
Probiotics are increasingly utilized to enhance oral health, with chewing gum recently proposed as a novel delivery method. This study aims to evaluate the persistence in saliva of Heyndrickxia coagulans SNZ1969 and Lacticaseibacillus rhamnosus GG—in both microencapsulated and free forms—administered via sugar-free chewing gums.

Who can participate?
Healthy adult volunteers aged 18 to 64 years

What does the study involve?
Participants were asked to chew a pellet of gum containing one of the probiotic strains. Following a washout period of one week, they were asked to chew a second pellet containing a different strain. After another one-week washout period, they chewed the third and final pellet. The three sugar-free chewing gums were administered in random order during the morning, at least two hours after breakfast and oral hygiene routines. Both participants and investigators were blinded to the specific probiotic strain contained in each gum. Volunteers were instructed to chew the gum for 10 min and to refrain from eating or drinking anything for the subsequent two hours. Saliva samples of at least 0.5 mL were collected from the floor of the mouth using sterile disposables at the following time points: before chewing gum use (T0), and at 1, 5, 10, and 20 min, as well as 1 and 2 h after the procedure began (T1–T6) to assess the amount of viable probiotic strain.

What are the possible benefits and risks of participants?
The probiotics tested in this study are effective on oral health, although there is no evidence of benefits after a single administration. There are no known risks associated with the use of the products administered during the study if applied following the manufacturer's instructions and all individual ingredients are already used in commercially used food products.

Where is the study run from?
The study is run by the Department of Biomedical, Surgical and Dental Sciences, University of Milan, Italy

When is the study starting and how is expected to run for?
October 2023 to May 2024.
Recruitment of participants, intervention, and microbiological measurements were carried out between March and May 2024.

Who is funding the study?
Perfetti Van Melle S.p.A., Italy

Who is the main contact?
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Contact information

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Additional identifiers

Clinical Trials Information System (CTIS)
Nil known

ClinicalTrials.gov (NCT)
Nil known

Protocol serial number
Nil known

Study information

Scientific Title
In vivo pilot randomized cross-over study on permanence in saliva of two different probiotics strain administered through chewing gums on healthy adults

Study objectives
The probiotic strains Heyndrickxia coagulans SNZ1969 and Lacticaseibacillus rhamnosus GG - the latter in both microencapsulated and free form - can remain in the oral cavity in viable form for up to two hours after chewing gum administration.

Ethics approval required
Ethics approval required

Ethics approval(s)
approved 13/02/2024, Ethical Committee of the University of Milan (Via Festa del Perdono 7, Milan, 20142, Italy; +39 02 503 12667; comitato.etico@unimi.it), ref: 24/24

Study design
Randomized cross-over pilot study

Primary study design
Interventional

Study type(s)
Safety, Efficacy

Health condition(s) or problem(s) studied
Prevention and reduction of gingivitis and halitosis in healthy adult

Interventions
This study is a randomized, cross-over pilot experiment on 10 subjects. Each subject tested one chewing gum and, after a washout period of one week, another chewing gum containing a different strain, in randomized order. The randomization was computer-generated. Both participants and experimenters were blinded to the specific probiotic strain contained in each gum.

All of the chewing gums used in the study were sugar-free, formulated with a mixture of polyols (excluding xylitol), and incorporated one of the specific probiotic strains under study. These included Lacticaseibacillus rhamnosus GG, supplied either in free form (biomass supplied by Chr. Hanse) or as microencapsulated cells (biomass supplied by AnaBio Technolo- ges LTD), and Heyndrickxia coagulans SNZ1969 (biomass supplied by Sanzyme Biologics Ltd), added as spores.

Participants were instructed to chew a gumball containing one of the probiotic strains. After a washout period of one week, they were asked to chew a second pellet containing a different strain. After another washout period of one week, they chewed the third and final pellet. The three sugar-free chewing gums were administered in random order (computer-generated randomization) during the morning, at least two hours after the breakfast and oral hygiene routine. Both participants and experimenters were blinded to the specific probiotic strain contained in each gum. Volunteers were instructed to chew the gum for 10 minutes and refrain from eating or drinking for the next two hours.

The primary outcome was viable bacterial cells in saliva, expressed as Colony Forming Units (CFU) /mL. Saliva samples of at least 0.5 mL were collected from the floor of the mouth using sterile disposables at the following time points: before chewing gum use (T0), and at 1, 5, 10, and 20 min, as well as 1 and 2 h after the procedure began (T1–T6). Samples were stored at 4 °C, transported to the laboratory, and processed within two hours. Aliquots of 300 µL of saliva were diluted in 600 µL of MDR buffer. Aliquots of 100 µL were plated onto selective media (GYEA-agar for H. coagulans SNZ1969 and RVB-MRS-agar for L. rhamnosus GG). For H. coagulans SNZ1969, part of the samples underwent viable count after pasteurization (incubation in a water bath at 90 °C for 10 min) to quantify bacterial spores. The fraction of vegetative forms of the SNZ1969 strain was determined by subtracting the spore count from the total count obtained without pasteurization, as previously described in detail. The plates were incubated at 37 °C for 72 h for the evaluation of L. rhamnosus GG and at 55 °C for 72 h in anaerobic conditions for the assessment of H. coagulans SNZ1969 CFUs and spores. The described procedures were repeated for each sample in triplicate. CFUs were identified by morphology, size and color and counted. If different morphologies were detected, three colonies per type were selected and analyzed by colony polymerase chain reaction (PCR), picking the colony into a PCR reaction with the strain-

specific primers PVM-Wc-1F 5’-TTGTCTTTGGATCAGTTACAG-3’ and PVM-Wc-1R 5’ GCATAGGAATACCTTGTGCA-3’ for H. coagulans SNZ1969 and the primers GG I 5'-CAATCTGAATGAACAGTTGTC-3' and GG II 5'-TATCTTGACCAAACTTGACG-3' for L. rhamnosus GG. Morphologies that revealed expected amplicons by agarose gel electrophoresis were confirmed as CFU and included in the final count. Some of the amplicons obtained were confirmed by Sanger sequencing.

Intervention Type
Supplement

Primary outcome(s)
Viable bacterial cells in saliva measured using dilution plating onto selective media to count colony-forming units per 1 mL of culture (CFUs/mL) before chewing gum use (T0), and at 1, 5, 10, and 20 min, as well as 1 and 2 h after the procedure began (T1–T6). For H. coagulans SNZ1969, part of the samples underwent viable count after pasteurization (incubation in a water bath at 90 °C for 10 min) to quantify bacterial spores.

Key secondary outcome(s))
Adverse effects reported by participants measured by an investigator who conducted brief telephone interviews with the participants on the evening of the intervention day and one week later to record any side effects associated with the chewing gum administered

Completion date
31/05/2024

Eligibility

Key inclusion criteria
1. Adult subjects aged 18 to 64 years
2. Presence of at least 24 natural teeth (excluding third molars)
3. Gingival index and plaque index scores ≤ 2
4. A stimulated salivary flow rate between 1.5 and 2.0 mL/min

Participant type(s)
Healthy volunteer, Employee

Healthy volunteers allowed
No

Age group
Adult

Lower age limit
18 years

Upper age limit
64 years

Sex
All

Total final enrolment
10

Key exclusion criteria
1. The presence of systemic diseases
2. Pregnancy or lactation
3. History of drug abuse
4. Smoking habits
5. Use of fixed orthodontic appliances
6. Allergies to any ingredients in the chewing gums used

Date of first enrolment
01/03/2024

Date of final enrolment
15/03/2024

Locations

Countries of recruitment
Italy

Study participating centre
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Sponsor information

Organisation
University of Milan

ROR
https://ror.org/00wjc7c48

Funder(s)

Funder type
Industry

Funder Name
Perfetti Van Melle SpA

Results and Publications

Individual participant data (IPD) sharing plan

The data generated during and/or analysed during the current study will be stored in a publicly available repository (https://dataverse.unimi.it/dataverse/scirio) and will be available at the end of the study. The participants received an information sheet before taking part in the study. They signed a consent to participate in the study and information relating to the processing of personal data according to Ar.13 / UE 2016/679.

IPD sharing plan summary

Stored in publicly available repository

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
Participant information sheet	Information on the processing of personal data in the research project		27/01/2025	No	Yes
Participant information sheet	Informed consent		27/01/2025	No	Yes
Participant information sheet	Participant information sheet	11/11/2025	11/11/2025	No	Yes