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Exploratory randomized trial of Weissella cibaria CMU and oral microbiome changes in peri implant mucositis

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ABSCTRACT

The oral microbiome plays an important role in maintaining peri-implant health, and microbial imbalance is a key factor in the development of peri-implant mucositis (PIM). Probiotics have been proposed as a strategy to modulate the oral microbiome, but clinical evidence in peri-implant conditions remains limited. In this randomized exploratory trial, patients with PIM received the probiotic Weissella cibaria CMU (OraCMU) or placebo for four weeks following standard supragingival scaling and oral hygiene instruction. Subgingival microbiome profiles and clinical parameters were evaluated at baseline and follow-up. While overall microbial diversity showed no marked differences between groups, OraCMU administration was associated with favorable compositional changes within the subgingival microbiome. This included enrichment of bacterial taxa commonly associated with peri-implant health and suppression of taxa linked to inflammatory peri-implant conditions. Consistent trends toward reduced abundance of key periopathogenic species were observed in the probiotic group compared with placebo. A modest improvement in probing depth was also noted in patients with moderate PIM receiving OraCMU. Together, these findings indicate that short-term probiotic supplementation may support ecological stabilization of the peri-implant microbiome. Further longitudinal studies are needed to determine whether such microbiome modulation translates into sustained clinical benefit.

KEYWORDS: Microbiota, Mucositis, Dental Implants, *Weissella*, *Firmicutes*, *Bacteroidetes*.

INTRODUCTION

The oral microbiome is a complex ecosystem of microorganisms, their genetic material, metabolic byproducts, and the surrounding microenvironment. This network of host–microbe and microbe–microbe interactions is sensitive to subtle environmental changes, which can disrupt microbial equilibrium and compromise homeostasis. For example, *Streptococcus* species help maintain balance by forming biofilms and producing hydrogen peroxide to inhibit pathogens [1].

Peri-implant mucositis (PIM) is a reversible inflammatory condition triggered by microbial dysbiosis and biofilm accumulation. While standard treatment involves mechanical debridement and antibiotics [2], these may not fully restore microbial balance. If inadequately managed, PIM may progress to peri-implantitis, threatening implant success—especially in older adults, as oral health greatly affects their quality of life. Consequently, strategies that target microbial imbalances as soon as these appear are critical for preventing disease progression.

Recent research has suggested that probiotics may be able to improve oral health by modulating the microbiome, restoring microbial homeostasis, and inhibiting colonization by pathogenic species. Clinical studies have shown that oral administration of probiotics as an adjunct therapy in nonsurgical mechanical treatment of gingivitis or periodontitis improved clinical parameters such as the bleeding on probing (BOP), probing depth (PD), gingival index (GI), and plaque index (PI); it also reduced the levels of some periodontal pathogens and pro-inflammatory cytokines [3,4]. In particular, the Gram-positive rod-shaped lactic-acid bacterium *Weissella cibaria (W. cibaria)*, isolated from fermented foods, shows antimicrobial and anti-inflammatory activity. It inhibits *Fusobacterium nucleatum*, reduces malodor, and downregulates IL-6 and IL-8 [5,6]. It also interferes with *Staphylococcus aureus* biofilms and reduces biofilm formation on titanium implants [7,8] .

These findings suggest that *W. cibaria* CMU (OraCMU) may offer a noninvasive, microbiome-targeted approach to PIM. However, clinical evidence in peri-implant settings remains limited. To explore this potential, we conducted an exploratory randomized controlled trial to investigate the clinical and microbiological effects of OraCMU. Clinical parameters and microbiome profiles were assessed over a 4-week period to examine whether probiotic-driven modulation may support peri-implant health.

RESULTS

Baseline characteristics

Although 44 patients were initially enrolled, only 40 who completed both baseline and follow-up assessments were included in the final analysis. Twenty patients were assigned to each of the two groups (OraCMU and placebo). The two groups had similar baseline characteristics (Table 1).

The mode of prosthesis retention did not differ significantly between groups (OraCMU: screw-retained 75.0%, cement-retained 25.0%; placebo: screw-retained 90.0%, cement-retained 10.0%; $P = 0.405$).

One patient (5%) in the OraCMU group and none in the placebo group consumed alcohol ($P = 0.494$). Three patients (15%) in the OraCMU group and five patients (25%) in the placebo group were current smokers ($P = 0.402$). Systemic conditions were similarly distributed in both groups: three patients (15%) in the OraCMU group and one patient (5%) in the placebo group had diabetes ($P = 0.263$); six patients in the OraCMU group (30%) and seven patients (35%) in the placebo group had hypertension ($P = 0.811$); and five patients in each group (25%) had hyperlipidemia ($P = 1.000$). Implant locations (maxilla vs. mandible) were also similar in each group ($P = 1.000$). A total of 5 patients (25%) in the OraCMU group and three patients (15%) in the placebo group had severe periodontitis, whereas eight patients (40%) in the OraCMU group and 12 patients (60%) in the placebo group had moderate periodontitis ($P = 0.195$).

Clinical parameters show no significant differences between groups

Changes in clinical indicators (ΔV = V2 – V1) were compared between groups to evaluate the effect of OraCMU. As summarized in Table 2, no statistically significant difference was observed in the primary outcome (ΔBOP) between the OraCMU and placebo groups ($P > 0.05$).

Similarly, no significant between-group differences were found for the secondary outcomes—ΔPD, ΔGI, or ΔPI—when averaged across all six peri-implant sites (all $P > 0.05$). Although a numerical reduction in PD was noted in the OraCMU group among patients with moderate PIM (Fig. 2), this comparison was performed post hoc as an exploratory analysis. The severity categories were defined a priori based on the mean baseline PD (4-5 mm for mild, 5–6 mm for moderate, and ≥7 mm for severe).

Distinct taxonomic trends following probiotic administration

Following quality control of the raw sequencing data, a total of 78 high-quality samples from 20 patients in the placebo group and 19 patients in the OraCMU group were retained for taxonomic analysis. Microbial composition was assessed at both the phylum and genus levels before and after the 4-week intervention (Fig. 3). At baseline, both groups had a similar oral microbiome profile, dominated by the phyla *Firmicutes*, *Fusobacteria*, *Bacteroidetes*, and *Proteobacteria*, and by genera such as *Streptococcus*, *Fusobacterium*, *Veillonella*, *Neisseria*, and *Leptotrichia*. Other genera, including *Campylobacter*, *Rothia*, and *Parvimonas*, were present at lower abundance.

To address the heterogeneity inherent to genus-level analyses, additional exploratory analyses were conducted at the species level, focusing on taxa belonging to established periodontal microbial complexes. Species-level directional changes were summarized descriptively based on pre- and post-intervention group-level mean relative abundances (Table 3).

Phylum-level changes

- At the phylum level, *Fusobacteria* increased in both groups after treatment (placebo: 12.8% → 16.5%, +28.9%; OraCMU: 11.3% → 14.9%, +31.9%).
- *Bacteroidetes* decreased in both groups, with a greater reduction observed in the OraCMU group (placebo: 8.98% → 7.01%, –22.0%; OraCMU: 9.53% → 6.90%, –27.6%).

- *Firmicutes* showed a slight decrease in the placebo group (45.97% → 44.61%, –3.0%) but a slight increase in the OraCMU group (45.89% → 46.01%, +0.3%).
- *Actinobacteria* remained stable in the placebo group (7.12% → 7.11%, –0.1%) and increased in the OraCMU group (5.36% → 6.05%, +12.9%).

Genus-level changes

- At the genus level, *Veillonella* decreased in the placebo group (12.74% → 10.15%, –20.3%) but increased in the OraCMU group (6.33% → 8.66%, +36.7%).
- *Fusobacterium* increased in both groups (placebo: 7.40% → 10.15%, +37.2%; OraCMU: 6.74% → 9.75%, +44.6%).
- *Parvimonas* increased in both groups, with a greater increase observed in the OraCMU group (placebo: 5.36% → 6.30%, +17.5%; OraCMU: 5.48% → 7.86%, +43.4%).
- *Neisseria* decreased in both groups, with a greater decrease observed in the placebo group (placebo: 5.99% → 3.32%, –44.6%; OraCMU: 6.10% → 4.73%, –22.5%).
- *Dialister* decreased in the OraCMU group (4.05% → 2.76%, –31.9%) but remained stable in the placebo group (3.75% → 3.71%, –1.1%).
- *Campylobacter* showed a slight increase in the placebo group (2.56% → 4.07%, +58.9%) but remained stable in the OraCMU group.
- *Rothia* disappeared in the placebo group (2.55% → undetectable) but appeared at a low level in the OraCMU group (undetectable → 2.63%).
- *Filifactor* was detected at baseline in the OraCMU group (3.80%) but was undetectable after treatment; it was undetectable in the placebo group at both time points.

Species-level directional changes in key periodontal complexes

Species-level analysis demonstrated group-specific directional changes in relative abundance across selected periodontal microbial complexes (Table 3). Within the red complex, *Treponema denticola* showed a decreasing trend in the OraCMU group and an increasing trend in the placebo group. In the orange complex, *Fusobacterium nucleatum* and *Eubacterium nodatum* both exhibited decreasing trends in the OraCMU group. In the placebo group, *F. nucleatum* showed an increasing trend, whereas *E. nodatum* did not show a consistent directional change. In the yellow complex, *Streptococcus gordonii* showed an increasing trend in the OraCMU group, while a mixed directional pattern was observed in the placebo group.

Although *S. gordonii* demonstrated an increasing trend following OraCMU administration, this finding should be interpreted within an ecological framework rather than as evidence of enhanced pathogenicity. *S. gordonii* is widely regarded as an early colonizer and a commensal member of the oral microbiome, contributing to biofilm initiation and community organization rather than serving as a core peri-implantitis–associated pathogen[9,10]. Its role in peri-implant disease is context-dependent and fundamentally distinct from that of red- and orange-complex taxa traditionally implicated in disease progression.

Importantly, the contrasting species-level trajectories observed between groups are consistent with established models of oral biofilm maturation[11]. In the OraCMU group, enrichment of a yellow-complex species (*S. gordonii*) accompanied by decreasing trends in red- and orange-complex pathogens such as *T. denticola*, *F. nucleatum*, and *E. nodatum* suggests attenuation of progression toward a mature, pathogen-enriched plaque community. In contrast, the placebo group exhibited increasing trends in late colonizers, consistent with a natural ecological shift toward a more mature and potentially pathogenic biofilm structure. These findings suggest that OraCMU administration may modulate the ecological trajectory of peri-implant biofilms by delaying or attenuating plaque maturation rather than by directly suppressing individual pathogens.

Species-level findings are therefore presented as qualitative, exploratory trends derived from a limited set of well-characterized periodontal taxa, intended to complement genus-level analyses rather than to provide comprehensive species-level profiling.

Minor taxa changes

At baseline, the OraCMU group exhibited a higher relative abundance of low-frequency taxa, including elevated levels of *Filifactor*, suggesting that a dysbiotic microbial profile was associated with severe inflammation. Following probiotic administration, the microbial composition shifted: *Filifactor* was no longer detected, whereas *Rothia* appeared at a low relative abundance. *Campylobacter*, which remained stable in the OraCMU group, showed a slight increase in the placebo group (2.56% → 4.07%, +58.9%). *Rothia* was initially present at low levels in the placebo group but had disappeared after 4 weeks.

Alpha diversity preserved without significant alteration after OraCMU

The impact of OraCMU treatment on the alpha diversity of the oral microbiome in patients with PIM was assessed using a LMM, incorporating participant-specific random effects and fixed effects of treatment, time, and their interaction. Although baseline characteristics were well balanced between the placebo and OraCMU groups, we investigated the potentially confounding effects of clinical variables on alpha diversity to adjust for covariates (Supplementary Table 1). First, we conducted univariable analyses to examine potential associations between clinical variables and alpha diversity indices (Supplementary Table 1). These revealed that the Shannon and Simpson indices were significantly associated with implant position ($P = 0.018$ and 0.011, respectively), and the Chao1 index was weakly correlated with age ($P = 0.019$).

Although the OraCMU group showed a slight decrease in alpha diversity following treatment, LMM analysis indicated no significant changes in Shannon entropy, the Simpson index, or Chao1 richness, either before or after adjusting for covariates (Fig. 4). Specifically, the fixed effects of treatment, time, and their interaction were not statistically significant across all three alpha diversity metrics ($P > 0.05$). Furthermore, even after adjusting for implant position with the Shannon and Simpson indices and for age with the Chao1 index, the model outputs remained nonsignificant ($P > 0.05$), suggesting that OraCMU administration did not substantially affect within-sample microbial diversity.

Compositional shifts remain subtle despite time-dependent variation

To further evaluate whether OraCMU treatment influenced overall microbial community composition, beta diversity analyses were performed using Bray–Curtis and unweighted UniFrac distance metrics. The PCoA plots of these distances showed no apparent clustering by treatment group (placebo vs. OraCMU) or by time point (Pre vs. Post), and within-subject microbial shifts over the 4-week intervention period were not consistent in either group (Fig. 5).

To counteract potential confounding effects, PERMANOVA of the patients' baseline characteristics was performed using Bray–Curtis and UniFrac matrices (Supplementary Table 2). This analysis identified significant associations between Bray–Curtis distances and age ($R^2 = 0.022$, $P = 0.013$), implant position ($R^2 = 0.019$, $P = 0.046$), and hypertension ($R^2 = 0.020$, $P = 0.036$). According to their associations with UniFrac distances, age ($R^2 = 0.025$, $P = 0.029$) and the presence of diabetes ($R^2 = 0.027$, $P = 0.026$) contributed significantly to microbial variance.

The effect of OraCMU on beta diversity was then evaluated using a model incorporating treatment, time, and their interaction, with permutations constrained within subjects. The Bray–Curtis-based PERMANOVA showed no significant contribution of treatment, time, or their interaction to overall microbial dissimilarity ($P > 0.05$). These findings remained robust even after adjusting for age, implant position, and hypertension. For the UniFrac distances, the effects of treatment ($P = 0.017$) and time ($P = 0.008$) were initially significant; however, the interaction term was not ($P = 0.626$). After adjusting for age and the presence of diabetes, the effects of treatment ($P = 0.021$) and time ($P = 0.009$)

remained significant, whereas the interaction term remained nonsignificant (P = 0.667). These results indicate that although some global shifts may occur over time, or between groups, the specific effect of OraCMU on oral microbiome composition over time was not statistically different from the effects of placebo.

DISCUSSION

This exploratory randomized controlled trial evaluated the clinical and microbiological effects of OraCMU in patients with PIM over a 4-week period. Although the study was powered for the primary outcome (BOP), only four BOP-positive participants were enrolled per arm, limiting the ability to detect between-group differences. No statistically significant differences were observed in PD, GI, or PI when averaged across all peri-implant sites. While the OraCMU group exhibited a numerical reduction in PD, the difference was not statistically significant. This lack of measurable clinical change likely reflects the short follow-up period, the predominance of mild PIM cases, and the standardization of oral hygiene and scaling before the intervention, which minimized baseline inflammation in both groups. These observations were based on exploratory, non-prespecified analyses with a small sample size and should be interpreted cautiously.

The lack of statistically significant differences in microbiome profiles may be partly attributable to the inherent pathophysiology of PIM. This condition is particularly prevalent among older adults, and its pathogenesis is increasingly understood to arise not from a single keystone pathogen but from context-dependent shifts in microbial community structure and function. This ecological concept has been supported by prior studies demonstrating that peri-implant inflammation is driven by dysbiosis at the community level rather than by dominance of a specific pathogen [12-14]. Therefore, genus-level comparisons alone are insufficient to capture biologically meaningful changes relevant to peri-implant health. Instead, interpretation requires integration of species-level dynamics within known ecological frameworks.

OraCMU administration was associated with enrichment of commensal and functionally relevant species, suggesting a probiotic-associated ecological shift in the peri-implant microbiome. Whereas *Veillonella* decreased following placebo treatment, its relative abundance increased after OraCMU administration. Because *W. cibaria* CMU produces lactic acid and *Veillonella* utilizes lactate as a primary carbon source, this increase may reflect probiotic-induced metabolic reshaping of the oral environment that favors commensal proliferation through cross-feeding interactions. As a secondary colonizer, *Veillonella* metabolizes lactic acid produced by S*treptococci*, thereby influencing local acidification and biofilm homeostasis [15,16].

In parallel, the commensal genus *Rothia*, which was detected only after OraCMU administration, has been reported to metabolize carbohydrates and proteins while exerting anti-inflammatory effects within the oral cavity [17]. Notably, both *Veillonella* and *Rothia* are recognized as nitrate-reducing bacteria that participate in the nitrate–nitrite–nitric oxide metabolic pathway, a process implicated in pH regulation and modulation of local inflammatory responses [18,19]. Enrichment of these taxa has previously been observed in periodontal pockets following successful therapy and has been associated with a transition toward a more balanced and less inflammatory microbial environment [20].

Taken together, the coordinated increase in lactate-utilizing and nitrate-reducing commensal genera following OraCMU administration suggests a shift toward metabolic and ecological stabilization of the peri-implant biofilm. Such microbiome-level reorganization may precede measurable clinical improvement and reflects probiotic-driven modulation of community function rather than direct suppression of pathogenic species.

At the community level, we also observed changes in the *Firmicutes*-to-*Bacteroidetes* (F/B) ratio. Although interpretation at the phylum level should be made with caution because *Firmicutes* encompass both beneficial and pathogenic taxa, the F/B ratio can provide a broad ecological context when considered alongside genus-level data. Although the F/B ratio alone is not a definitive measure of oral microbial health, an increase in the F/B ratio combined with suppression of pathogenic genera and enrichment of commensals suggests a probiotic-mediated stabilization of the oral microbiome [21,22]. In this study, the F/B ratio increased by 38.62% in the OraCMU group compared with 24.23% in the placebo group. This phylum-level change, interpreted together with genus-level shifts such as increases in *Veillonella* and *Rothia* and reductions in *Dialister* and *Fusobacterium*, supports a pattern consistent with ecological stabilization rather than dysbiosis. Although the scaling procedures prior to the interventions could account for some of the increase in the F/B ratio in both groups, the greater increase in the OraCMU group (despite a slightly higher proportion of moderate-to-severe PIM cases) suggests an additional effect due to probiotic supplementation.

Furthermore, reductions in pathogenic genera such as *Dialister* and a relatively attenuated increase in *Fusobacterium* were observed exclusively in the OraCMU group. Although the relative abundance of *Fusobacterium* increased numerically in both groups, the proportional rise was smaller in the OraCMU group despite a higher baseline inflammatory burden. Given that *Fusobacterium* is a heterogeneous genus comprising both pathogenic (*F. nucleatum*) and less virulent or transitional species such as *F. periodonticum* and F*. polymorphum* [23,24], genus-level changes do not necessarily indicate pathogenic proliferation. Previous studies have shown that shifts in *Fusobacterium* abundance after periodontal or peri-implant therapy may reflect ecological reorganization and a transition toward less pathogenic species rather than persistent dysbiosis [25]. To address this limitation, species-level analyses were conducted to complement the genus-level findings.

Among Fusobacterium species, *F. nucleatum*, a key pathogenic member of the orange complex that plays a central role in bridging early and late colonizers and in amplifying inflammatory biofilm maturation, showed a decreasing trend in the OraCMU group, whereas an increasing trend was observed in the placebo group (Table 3). Notably, although genus-level Fusobacterium increased numerically in both groups, these species-level trajectories indicate qualitatively different patterns of microbial change between treatment arms. The observed reduction in *F. nucleatum* and the concomitant attenuation of red-complex taxa following OraCMU administration may also be interpreted in the context of bacterial coaggregation, an ecological mechanism that plays a critical role in oral biofilm maturation. *F. nucleatum* functions as a key bridging organism that physically and metabolically links early colonizers to late pathogenic species. Disruption of this bridging function has been shown to impair the establishment of mature, pathogen-enriched biofilms.

Recent studies have demonstrated that certain probiotic species, including *Limosilactobacillus reuteri*, can coaggregate with *F. nucleatum* and interfere with its ability to serve as a structural bridge within periodontal biofilms, thereby reshaping community composition and immunogenicity[26]. Although direct coaggregation was not assessed in the present study, our findings raise the possibility that OraCMU may exert similar ecological effects by modulating interspecies interactions within the peri-implant biofilm.

Similarly, *T. denticola* (red complex), a species repeatedly associated with peri-implant and periodontal inflammation, exhibited a decreasing trend following OraCMU administration but an increasing trend in the placebo group. *E. nodatum* (orange complex) also showed a decreasing trend in the OraCMU group, whereas no consistent directional change was observed in the placebo group. Taken together, these species-level directional patterns are consistent with the possibility of an early, favorable ecological shift in the peri-implant microbiome following OraCMU administration.

Thus, the observed trend may represent ecological stabilization with a shift toward less virulent *Fusobacterium* lineages, consistent with post-treatment microbiome recovery patterns [25]. Interestingly, the disappearance of *Filifactor—frequently associated with peri-implantitis—was no longer detected after OraCMU administration, which may be consistent with a probiotic-associated reduction of disease-related taxa and warrants further investigation in larger cohorts* [27]. These microbiological changes, although subtle, align with the clinical trends and collectively point toward a beneficial ecological shift following probiotic intervention.

From a clinical perspective, the modest magnitude of microbiological and clinical changes observed in this short-term trial does not necessarily imply the absence of probiotic efficacy. Instead, it may indicate that probiotic-driven microbial reorganization precedes measurable clinical improvement, a pattern consistent with prior studies of ecological restoration following nonsurgical periodontal therapy.

This study had several limitations. First, the follow-up duration was relatively short.

Reorganization of peri-implant microbial communities following mechanical biofilm removal generally requires at least 2 weeks [28], and clinical resolution of inflammation may take 3 weeks or more [29]. Although our follow-up period was 4 weeks, this may have been insufficient to capture statistically significant taxonomic shifts. Previous studies have suggested that subgingival microbiota associated with periodontal and peri-implant inflammation can exhibit dynamic changes up to 12 weeks after treatment [15]. Second, only 20% of participants were BOP-positive, limiting statistical power for the primary outcome. Third, the small sample size and few severe PIM cases hindered subgroup analyses. Fourth, although the mode of prosthesis retention was recorded and found to be similarly distributed between groups, detailed prosthetic contour characteristics (e.g., emergence profile morphology) were not systematically quantified. Prosthetic design may influence plaque accumulation and local inflammatory responses around implants, potentially affecting peri-implant microbial ecology [30]. Future studies incorporating standardized assessment of prosthetic contour parameters would help clarify their impact on peri-implant inflammation and microbiome dynamics. Finally, because this study relied on 16S rRNA sequencing, functional attributes such as bacterial protease activity, virulence factor expression, or host–microbe–virus interactions could not be evaluated. Future studies integrating metagenomics, metatranscriptomics, or host immune profiling will be required to elucidate these mechanisms.

Nevertheless, this study has strengths. The randomized controlled design minimized confounding, and the groups were well-balanced at baseline. Microbiome profiling via 16S rRNA sequencing provided high-resolution data, and concurrent clinical-microbiological assessment offered a rare multidimensional view of probiotic effects in peri-implant care.

Future studies should adopt larger sample sizes, extended follow-up, and multi-omics approaches—including metagenomics and metabolomics—to explore functional consequences of microbiome shifts. Stratification based on baseline microbiome profiles may optimize probiotic responses. Longitudinal sampling at multiple time points could better characterize microbial succession and resilience.

In conclusion, although global clinical and microbiome shifts were not statistically significant, OraCMU administration was associated with compositional trends suggesting partial suppression of potentially pathogenic genera, enrichment of commensals, and a modest increase in the F/B ratio. Such compositional changes may indicate probiotic-driven modulation of the peri-implant microbiome and an early phase of ecological stabilization preceding measurable clinical improvement. While short-term effects on clinical parameters were minimal, these findings suggest that *W. cibaria* CMU may help restore microbial balance and support peri-implant health over time. Larger-scale studies with extended follow-up are warranted to confirm these preliminary observations and to determine whether these microbiome-level changes translate into sustained clinical benefits.

METHODS

Subjects and sample collection

This study was conducted between December 2022 and November 2023 at Seoul National University Bundang Hospital. The study protocol was approved by the Institutional Review Board of Seoul National University Bundang Hospital (IRB No. B-2208-773-004). All methods were performed in accordance with the relevant guidelines and regulations, including the Declaration of Helsinki and institutional ethical standards. This randomized controlled trial was designed and reported in accordance with the CONSORT (Consolidated Standards of Reporting Trials) 2010 statement and the STORMS (Strengthening the Organization and Reporting of Microbiome Studies) guidelines for microbiome research [31,32]. The study employed a parallel-group randomized design with a 1:1 allocation ratio, comparing the probiotic intervention (OraCMU) with placebo. Adult patients with PIM visiting the outpatient clinic were enrolled. Written informed consent was obtained from all participants prior to enrollment in the study.

Eligibility criteria

Inclusion criteria were systemically healthy adults aged 20–70 years with PIM of varying severity (mild to severe), as defined by the American Academy of Periodontology and the European Federation of Periodontology [14,33]. Diagnostic criteria included bleeding and/or suppuration on gentle probing, in the absence of additional bone loss beyond initial remodeling. PD was measured circumferentially at six sites per implant (mesial, distal, buccal, and lingual aspects), and cases were included when at least one site exhibited a PD ≥ 6 mm in conjunction with clinical signs of inflammation. All implants were initially assessed using panoramic radiographs and a comprehensive intraoral examination. When multiple implants or multiple inflamed sites were present in a single participant, only one site per participant was selected as the index site. The index site was defined as the site demonstrating the most severe clinical presentation, determined by the deepest probing depth in combination with inflammatory signs. All clinical measurements (GI, BOP, PD, and

PI) and microbiological sampling were performed at this selected index site. PD values from all six aspects of the selected implant were recorded for analysis.

Exclusion criteria were as follow: the presence of uncontrolled systemic disease; antibiotic use during the month immediately prior to screening or anticipated use during the study period; currently pregnancy or breastfeeding; acute peri-implant symptoms; known hypersensitivity to probiotics; and intake of probiotics or supplements during the 7 days before enrollment.

Baseline data included demographic characteristics, oral hygiene status, comorbidities, and medication use.

Randomization and Intervention

Participants were anonymized and randomly assigned to either the experimental (OraCMU) or the placebo group. The randomization sequence was produced using a computer-generated permuted block algorithm (random block sizes of 4 and 6) to ensure balance between groups. Sequence generation and the preparation of sequentially numbered, opaque, sealed envelopes were performed by an independent coordinator not involved in participant recruitment or outcome assessment, ensuring allocation concealment; investigators enrolling participants and assessing outcomes remained unaware of upcoming assignments. Participants were enrolled and assigned to interventions by J.-S. Hong, who opened the next sequentially numbered envelope at the time of allocation. Both participants and the clinical examiner were blinded to group allocation. The probiotic and placebo powders were identical in appearance, texture, and packaging, maintaining participant blinding, and the examiner (H.-J. Lee) performing clinical assessments was not involved in allocation procedures and remained unaware of treatment assignments throughout the study.

Before the intervention, all participants received full-mouth supragingival scaling. Standardized toothbrushes and toothpaste were provided, and participants were instructed to use only these products throughout the study. The use of additional oral hygiene products or medications was prohibited.

The experimental group received OraCMU powder (2 g; 2.0 × 10^8 colony-forming units/sachet; Oraticx, Inc., Seoul, South Korea). The placebo group received 2 g isomalt-based placebo powder identical in taste, texture, and appearance but lacking *W. cibaria*. Participants applied the powder evenly across the oral cavity after morning brushing and before bedtime. Eating, drinking, or brushing was prohibited for 30 min after application. Compliance was assessed after 4 weeks by weighing the remaining sachets (Fig. 1).

Clinical assessment

In accordance with the trial registration, the primary outcome was BOP. Secondary outcomes included the PD, GI, PI, and oral microbiome profiles. All outcomes were pre-specified and assessed at baseline and at week 4.

A single experienced and trained examiner assessed BOP, PD, GI, and PI at baseline and week 4. This examiner was H.-J. Lee, who remained blinded to group allocation throughout the study. All measurements were performed under standardized clinical conditions using a consistent probing force and technique to minimize variability. PD was measured at six peri-implant sites per implant using a calibrated periodontal probe Although formal intra-examiner reliability testing was not performed, the use of a single examiner ensured methodological consistency throughout the study.

The severity of PIM was categorized a priori according to the mean baseline probing depth (PD) around the target implant: mild (4-5 mm), moderate (5–6 mm), and severe (≥7 mm). This stratification reflected the clinical extent of peri-implant inflammation and was based on the 2018 AAP/EFP case definitions [14,33]. While this subgrouping was predefined, comparative analyses of PD changes among severity groups were conducted post hoc as exploratory analyses.

Sample collection and microbiome profiling

Subgingival plaque was collected from the deepest pocket using a sterile curette, transferred to Periopaper strips (Oraflow, Smithtown, NY, USA), and stored at –80 °C.

Oral microbiome profiling

The PHiCS Institute (Seoul, South Korea) performed 16S rRNA-targeted next-generation sequencing. Microbial DNA was isolated from each sample using the QIAamp DNA Microbiome kit (QIAGEN, Venlo, the Netherlands). DNA quality was assessed using Qubit dsDNA HS Assay kits (Thermo Fisher Scientific Inc., Waltham, MA, USA). Polymerase chain reaction (PCR) targeting the V3 and V4 hypervariable regions of 16S rRNA genes was conducted using the KAPA HiFi HotStart ReadyMix PCR kit (Roche, Basel, Switzerland) in accordance with the manufacturer's instructions. Libraries were constructed using the NextEra XT DNA Library Preparation kit (Illumina Inc., San Diego, CA, USA) and were pooled to a final loading concentration of 8 pM. Paired-end (2 × 300 bp) sequencing was performed using the MiSeq platform (Illumina Inc.).

Demultiplexed FASTQ files were processed using a Divisive Amplicon Denoising Algorithm 2-based pipeline implemented within the QIIME2 platform [34,35]. Specifically, amplicon sequencing variants (ASVs) were taxonomically classified against the 99% SILVA rRNA taxonomy using a pretrained scikit-learn naïve Bayes machine learning classifier with the QIIME q2-feature-classifier plugin [36,37].

Given the inherent limitations of species-level resolution in V3–V4 16S rRNA amplicon data, species-level analyses were restricted to taxa with confident assignment and were interpreted descriptively.

Statistical analysis

All statistical analyses were performed using R software (ver. 4.1.2; R Development Core Team, Vienna, Austria). QIIME artifacts were imported into R using the qiime2R package and transformed into phyloseq objects via the phyloseq package [38,39]. To mitigate technical bias, feature tables were normalized via rarefaction prior to downstream analysis.

Alpha diversity was assessed using Shannon entropy, Simpson’s index, and the Chao1 index. Associations with patient characteristics were evaluated using Pearson's correlation for continuous variables and Wilcoxon rank-sum test for binary variables. Beta diversity and intersample dissimilarities were measured using Bray–Curtis and unweighted UniFrac distances. Principal coordinate analysis (PCoA) was used to visualize these trends. Permutational multivariate analysis of variance (PERMANOVA), implemented via the vegan package [40], was used to quantify variance explained by covariates.

Intervention effects on alpha diversity were analyzed using linear mixed model (LMM; lme4 R package) [41], with random intercepts for participants and fixed effects for treatment, time, and their interaction. Group effect sizes were expressed as estimated mean differences divided by their standard errors.

Normality was assessed using the Shapiro–Wilk test. Changes in clinical indicators ($\Delta V = V2 - V1$) were compared between groups using the Mann–Whitney U test. Raw feature counts were centered log-ratio transformed for compositional analysis. Categorical variables were analyzed using the chi-square test or Fisher's exact test, as appropriate. Within-subject changes in beta diversity were tested using the Wilcoxon signed-rank test. All P-values were two-sided, and $P < 0.05$ was considered statistically significant.

Sample size and power calculations

Although the trial registration specified a planned enrollment of 22 participants per arm, four screened individuals were excluded prior to randomization due to withdrawal of consent and ineligibility. Therefore, 20 participants per arm (40 total) were ultimately randomized and included in the final analysis.

Based on 20 subjects per arm, a two-sided α of 0.05, and 80% power, we estimated the minimum detectable differences for both primary and secondary outcomes. For BOP (a binary outcome), assuming a 40% event rate in the control group (based on [42]), the study was powered to detect a between-group difference of approximately 41.7 percentage points (i.e., from 40.0% to 81.7%). For change in probing depth (ΔPD, a continuous outcome), assuming a pooled standard deviation of $\sigma = 0.391$ mm (based on 0.389 mm and 0.393 mm in the control and treatment arms, respectively; [42]), the minimum detectable mean difference was 0.36 mm.

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AUTHOR CONTRIBUTIONS

Mi-Sun Kang, Hyo-Jung Lee, and Kyoung Un Park contributed to the conceptualization and methodology of the study. Hyunji Kim, Sujin Oh, and Jin-Sil Hong were responsible for data curation. Formal analysis was performed by Hyunji Kim and Sujin Oh. Investigation was conducted by Jin-Sil Hong. Resources were provided by Jin-Sil Hong and Hyo-Jung Lee. Hyo-Jung Lee supervised the study. Validation was carried out by Sujin Oh. Hyunji Kim contributed to data visualization and drafted the original manuscript. Hyo-Jung Lee and Kyoung Un Park reviewed and edited the manuscript. All authors read and approved the final manuscript.

Data availability statement

The sequence data described in this study have been deposited in the Sequence Read Archive (SRA) and are available under BioProject accession number PRJNA1263118 (https://www.ncbi.nlm.nih.gov/bioproject/ PRJNA1263118).

Conflict of interest disclosure

One author, Mi-Sun Kang, is employed by the R&D Center of Oraticx, Inc., the manufacturer of the probiotic product used in this study. All other authors declare no conflicts of interest.

Ethical approval statement

The study protocol was approved by the Institutional Review Board at Seoul National

University Bundang Hospital (No. B-2208-773-004).

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TABLES

Table 1. Clinical characteristics of patients in the experimental group.

Variables	Probiotics (N = 20)	Placebo (N = 20)	*P*-value
Sex, N (%)			
Male	10 (50.0)	14 (70.0)	
Female	10 (50.0)	6 (30.0)	0.110
Age, yrs (mean±SD)	61.55 ± 6.14	59.85 ± 9.46	0.504
Alcohol consumption, N (%)			
Yes	1 (5)	0 (0)	
No	19 (95)	20 (100)	0.494
Smoking, N (%)			
Yes	3 (15)	5 (25)	
No	17 (85)	15 (75)	0.402
Systemic disease, N (%)			
Diabetes	3 (15)	1 (5)	0.263
Hypertension	6 (30)	7 (35)	0.811
Hyperlipidemia	5 (25)	5 (25)	1.000
Implant position, N (%)			
Maxilla	9 (45)	8 (40)	
Mandible	11 (55)	12 (60)	1.000
Mode of prosthesis retention, N (%)			
Screw-retained	15 (75.0)	18 (90.0)	0.405
Cement-retained	5 (25.0)	2 (10.0)	
Periodontitis[†], N (%)			
Mild	7 (35)	5 (25)	
Moderate	8 (40)	12 (60)	
Severe	5 (25)	3 (15)	0.195
Gingival Index (GI, median [IQR])			
Buccal GI	1.00 [1.00–1.00]	1.00 [1.00–1.00]	0.655
Distal GI	1.00 [1.00–1.00]	1.00 [1.00–1.00]	0.407
Lingual GI	1.00 [1.00–1.00]	1.00 [1.00–1.00]	0.655
Mesial GI	1.00 [1.00–1.00]	1.00 [1.00–1.00]	0.179
Bleeding on Probing (median [IQR])	4.00 [4.00–4.00]	4.00 [4.00–4.00]	0.468
Pocket depth[*], mm (PD, median [IQR])			
Mesiolingual PD	6.00 [5.75–7.50]	7.00 [6.00–8.00]	0.399
Lingual PD	6.00 [5.00–8.00]	6.00 [5.00–7.50]	0.967
Distolingual PD	6.00 [5.00–8.00]	6.50 [5.00–8.50]	0.784
Mesiolbuccal PD	6.00 [6.00–7.25]	6.50 [6.00–8.00]	0.618

Buccal PD	6.00 [5.00–7.00]	7.00 [6.00–8.00]	0.093
Distobuccal PD	6.00 [5.00–7.00]	7.00 [5.79–9.00]	0.054
Plaque Index (median [IQR])	4.00 [4.00–4.00]	4.00 [4.00–4.00]	0.671

[†] Periodontitis severity was assessed by dental experts and classified according to the Centers for Disease Control and Prevention-American Academy of Periodontology (CDC-AAP) definition.

[*] Pocket depth values represent measurements obtained from six peri-implant sites of the index implant selected for each participant.

Table 2. Effect of OraCMU treatment on changes in clinical indicators

Variables	**Probiotics**	**Placebo**	***P*-value**
ΔGI			
ΔBuccal GI	- 0.55 ± 0.51	- 0.60 ± 0.68	0.988
ΔDistal GI	- 0.40 ± 0.60	- 0.45 ± 0.69	0.923
ΔLingual GI	- 0.75 ± 0.55	- 0.60 ± 0.68	0.354
ΔMesial GI	- 0.30 ± 0.57	- 0.45 ± 0.69	0.381
ΔBOP	- 1.05 ± 1.43	- 1.3 ± 1.30	0.521
ΔPD			
ΔMesiolingual PD	- 0.55 ± 1.15	- 0.90 ± 1.29	0.453
ΔLingual PD	- 1.00 ± 1.56	- 0.75 ± 1.62	0.729
ΔDistolingual PD	- 0.75 ± 1.45	- 0.70 ± 1.13	0.777
ΔMesiolbuccal PD	- 0.40 ± 1.04	- 0.05 ± 1.54	0.609
ΔBuccal PD	- 0.45 ± 0.89	- 1.05 ± 1.64	0.146
ΔDistobuccal PD	- 0.55 ± 1.43	- 1.05 ± 1.57	0.416
ΔPI	- 1.75 ± 1.21	- 1.35 ± 1.23	0.314

ΔV (change) = V2 - V1. The statistical significance of ΔV was determined by the Mann-Whitney U Test ($P < 0.05$). Values are presented as mean ± SD.

- Abbreviations: GI, Gingival Index; BOP, Bleeding on Probing; PD, Probing Depth; PI, Plaque Index.

Table 3. Directional Changes in Species Abundance Between Pre- and Post-Intervention in the OraCMU and Placebo Groups

		Δ Probiotics (Pre → Post)	**Δ Placebo (Pre → Post)**
Red complex	*Treponema denticola*	↓↓	↑/ →†
Orange complex	*Fusobacterium nucleatum*	↓	↑↑
	Eubacterium nodatum	↓↓	→
Yellow complex	*Streptococcus gordonii*	↑↑	↑/ →†

Δ = Post – Pre

Arrows indicate the direction of change in group-level mean relative abundance.

Symbols indicate descriptive directional trends. For borderline changes, the dominant direction is shown first (e.g., ↑/→), followed by stability to reflect uncertainty.

†showed a trend toward increase

Figure legends

Figure 1. Study Overview. Following dental scaling, participants were randomly assigned to two groups: the experimental group, which received 2 g sachets of OraCMU, and the control group, which received 2 g of isomalt. Both groups were instructed to consume the designated powder for 4 weeks. The powders were indistinguishable in appearance, flavor, and scent. Samples were collected afterward for comparative analysis. This figure was created by the authors using BioRender (BioRender.com; web-based platform, https://www.biorender.com/).

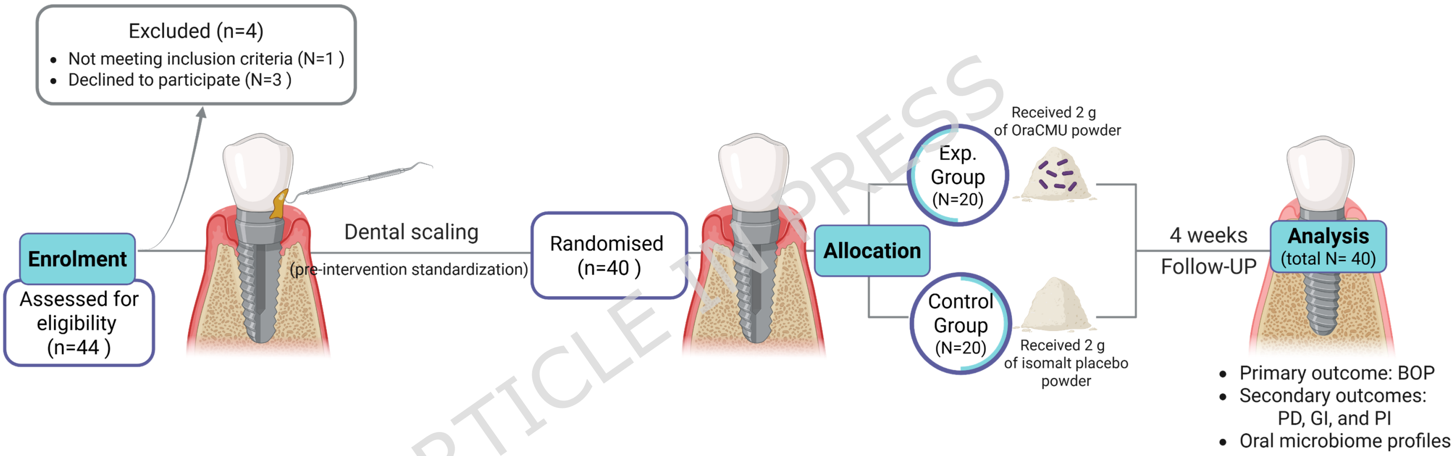
Figure 2. Changes in probing depth (ΔPD = post-treatment PD – pre-treatment PD) were compared between the probiotics and placebo groups, stratified by the severity of peri-implant mucositis (PIM). The probiotics group (N = 20) consisted of 7 patients with mild, 8 with moderate, and 5 with severe PIM, while the placebo group included 5, 12, and 3 patients in each respective category. (A) Probing depth was measured at multiple sites around each implant. This panel was created by the authors using BioRender (BioRender.com; web-based platform, https://www.biorender.com/). (B) Although consistent treatment effects were not observed across all severity levels, a statistically significant reduction in PD was observed in the probiotics group within the moderate PIM subgroup, suggesting a potential therapeutic benefit. However, in the severe group, the small sample size in the probiotics arm (N = 3) limits the reliability of statistical interpretation. This panel was created by the authors using GraphPad Prism version 8.4.2 (GraphPad Software, San Diego, CA, USA).

Figure 3. Probiotic treatment modulates the relative abundance of key microbial taxa. Relative abundances of dominant (A) phyla and (B) genera were profiled before and after treatment in the placebo and OraCMU groups. While both groups showed temporal shifts in microbial composition, OraCMU-treated patients exhibited increased *Veillonella* and relative preservation of *Firmicutes*. In contrast, *Fusobacteria* and *Fusobacterium* increased more notably in the placebo group. These trends suggest that OraCMU may contribute to microbiome stabilization by supporting beneficial taxa and attenuating overgrowth of disease-associated bacteria.

Figure 4. Alpha diversity indices remain stable following probiotic intervention. Alpha

diversity of the oral microbiome was assessed using Shannon entropy, Simpson index, and Chao1 index. Paired comparisons of pre- and post-treatment samples revealed no statistically significant changes in alpha diversity in either the placebo or OraCMU groups. Linear regression lines (blue for placebo, red for OraCMU) represent average trends over time. Although individual variation was observed, the overall within-sample diversity remained stable, suggesting that OraCMU does not perturb alpha diversity.

Figure 5. Subtle temporal shifts in microbial composition with no distinct treatment-specific clustering. (A) Principal coordinate analysis (PCoA) based on Bray-Curtis and unweighted UniFrac distances demonstrated no clear separation by treatment group (PBO vs. OraCMU) or timepoint (Pre vs. Post). Arrows connecting pre- and post-treatment samples for each subject reflect interindividual variability and direction of microbiome shifts. (B) Violin plots of within-subject distance changes (Post – Pre) revealed no significant differences between groups for either Bray-Curtis or UniFrac distances. While UniFrac metrics showed time- and treatment-related variance, the lack of interaction effect indicates that OraCMU does not result in a treatment-specific directional shift in beta diversity.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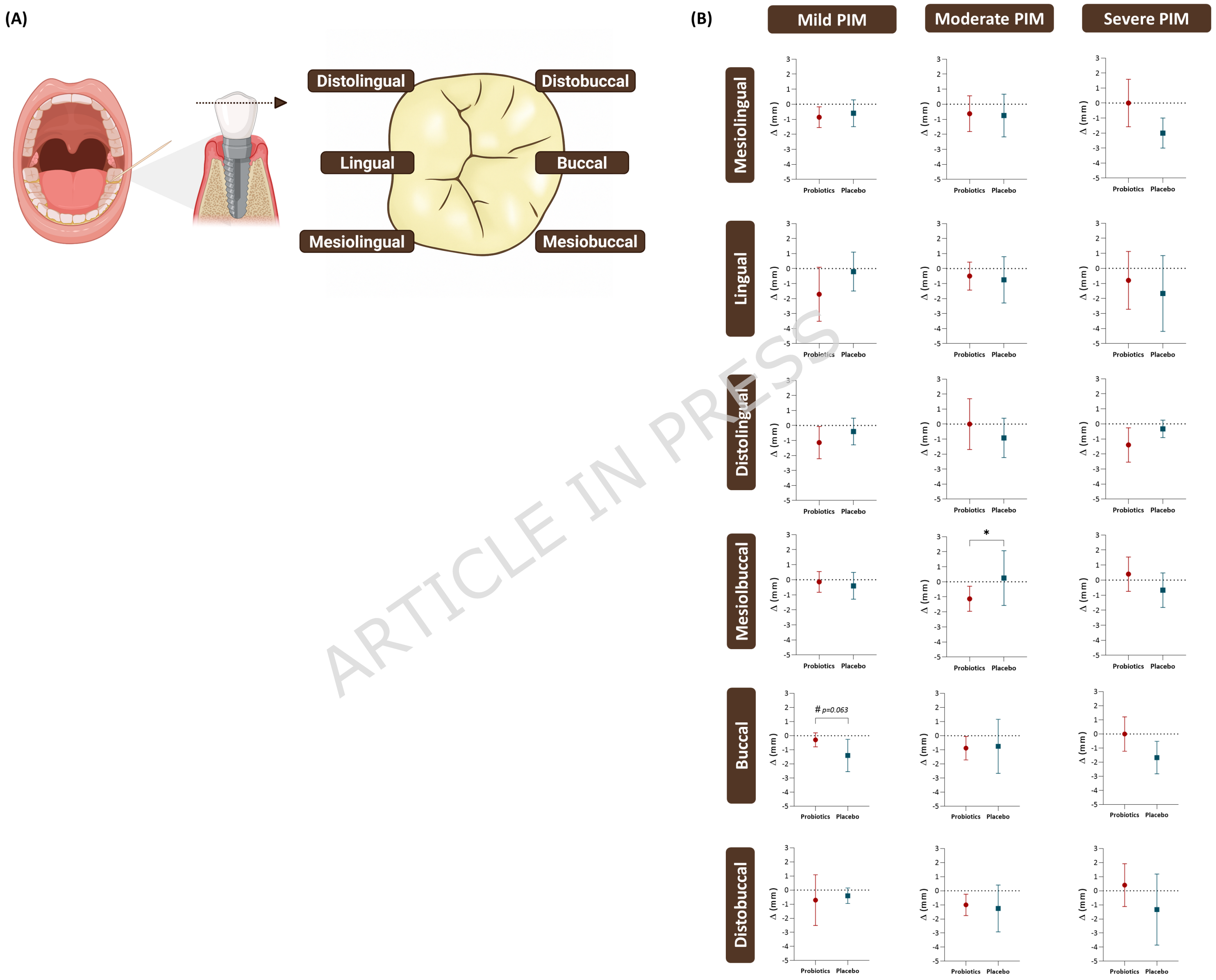
Enrolment
Assessed for eligibility (n=44)
Dental scaling
(pre-intervention standardization)
Randomised (n=40)
Allocation
Exp. Group (N=20)
Received 2 g of OraCMU powder
Control Group (N=20)
Received 2 g of isomalt placebo powder
4 weeks
Follow-UP
Analysis
(total N= 40)
Primary outcome: BOP
Secondary outcomes: PD, GI, and PI
Oral microbiome profiles


(A)
Distolingual
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Mesiolingual
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(B)
Mild PIM
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Lingual
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Mesiobuccal
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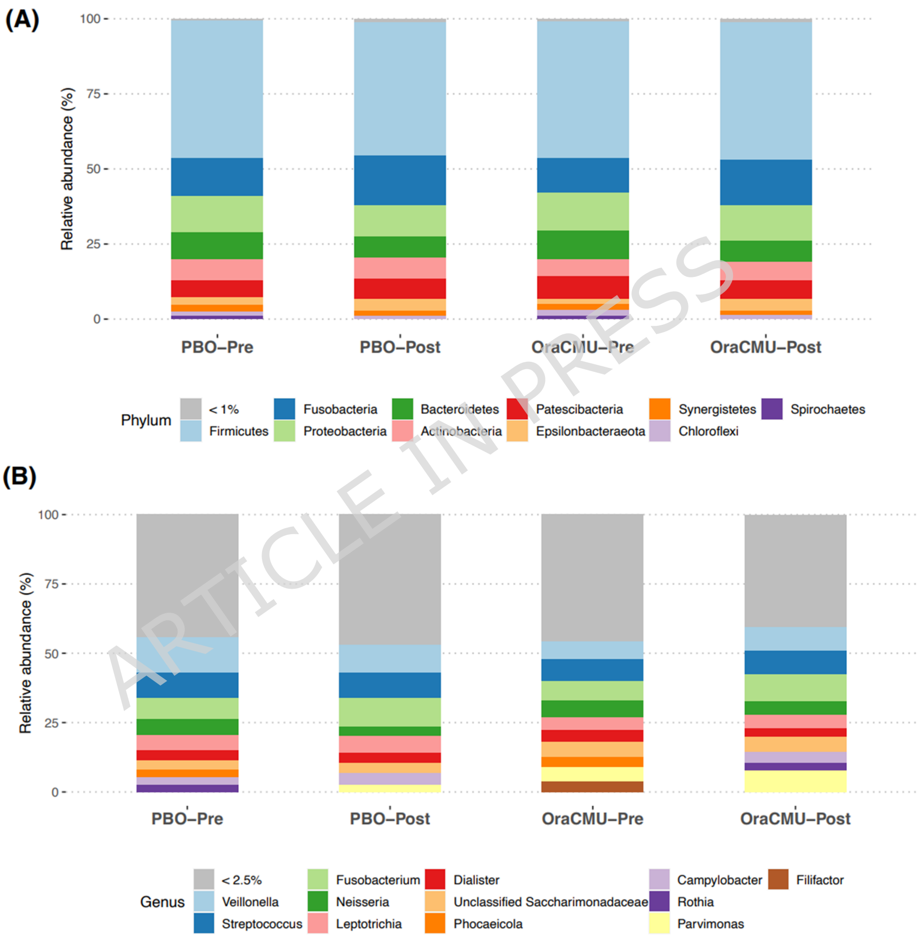
Probiotics
Placebo
*
p=0.063


(A)
Relative abundance (%)
100
75
50
25
0
PBO–Pre
PBO–Post
OraCMU–Pre
OraCMU–Post
Phylum
< 1%
Firmicutes
Fusobacteria
Proteobacteria
Bacteroidetes
Actinobacteria
Patescibacteria
Epsilonbacteraeota
Synergistetes
Chloroflexi
Spirochaetes
(B)
Relative abundance (%)
100
75
50
25
0
PBO–Pre
PBO–Post
OraCMU–Pre
OraCMU–Post
Genus
< 2.5%
Veillonella
Streptococcus
Fusobacterium
Neisseria
Leptotrichia
Dialister
Unclassified Saccharimonadaceae
Phocaeicola
Campylobacter
Rothia
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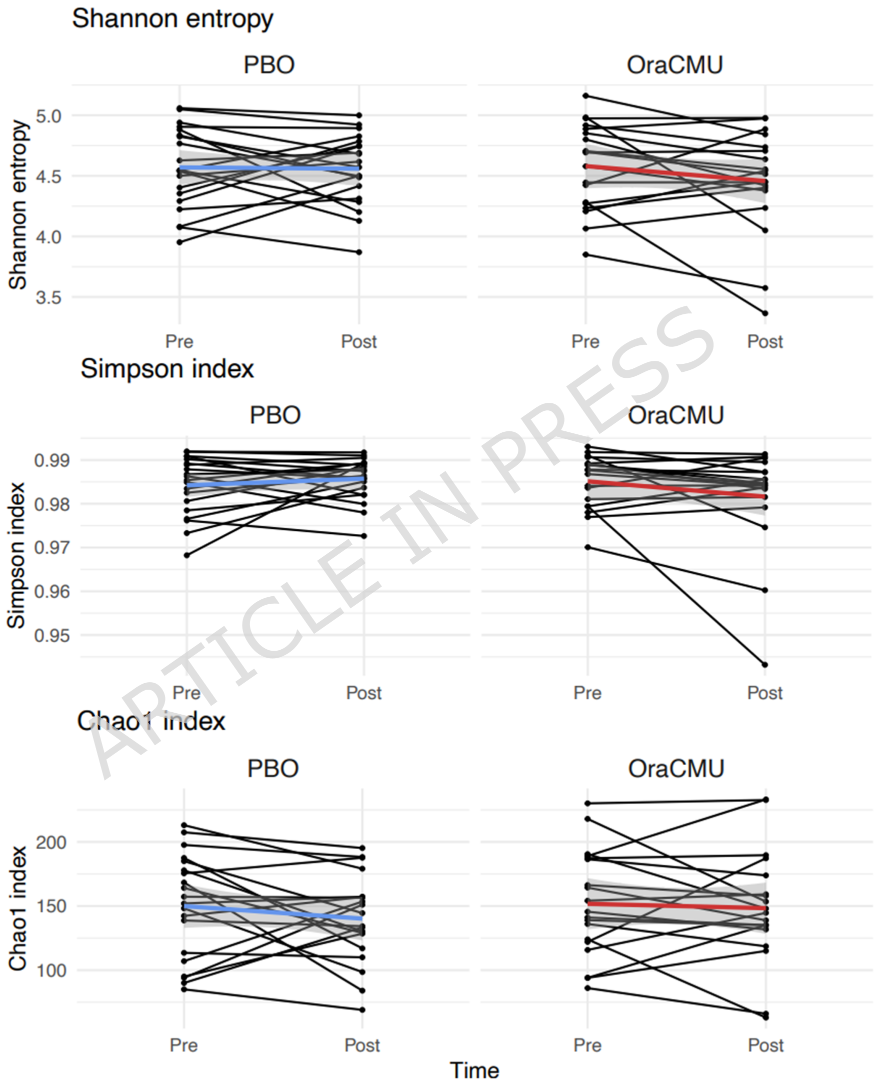
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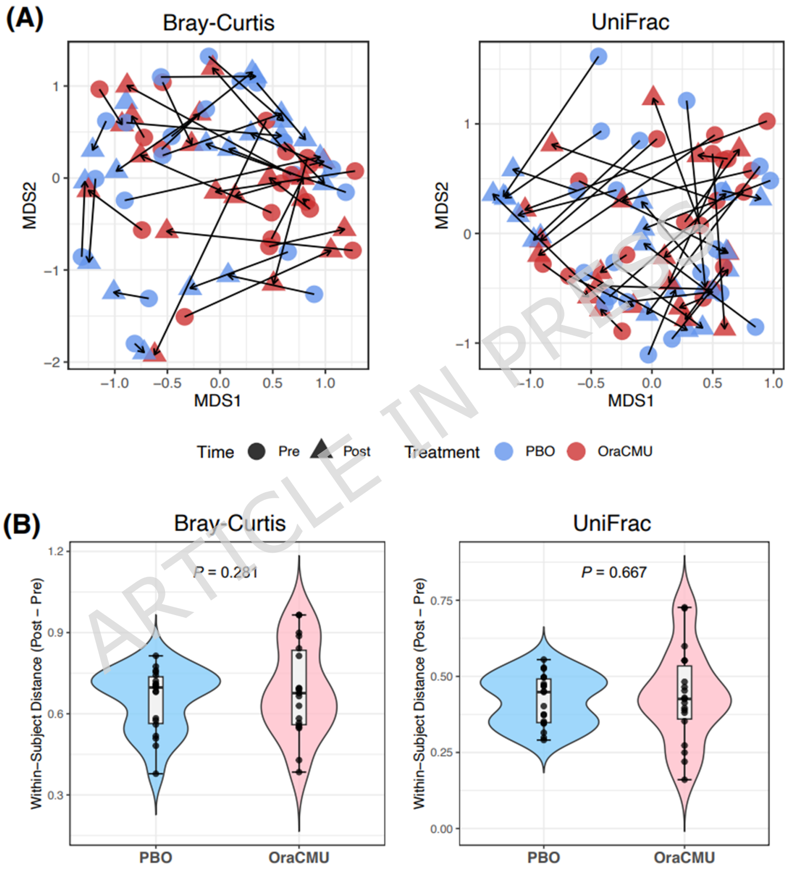
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Bray-Curtis
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Time ● Pre ▲ Post
Treatment ● PBO ● OraCMU
(B)
Bray-Curtis
UniFrac
P = 0.281
P = 0.667
Within−Subject Distance (Post − Pre)
1.2
0.9
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PBO
OraCMU