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Salivary microbial profiles associate with responsiveness to warning oral sensations and dietary intakes

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ABSTRACT

Oral microbiota-host interactions are gaining recognition as potential factors contributing to interindividual variations in taste perception. However, whether such possible links imply specific bacterial co-occurrence networks remains unknown. To address this issue, we used 16 s rRNA gene sequencing to profile the salivary microbiota of 100 healthy individuals (52 % women; 18–30 y/o), who provided hedonic and psychophysical responses to 5 liquid and 5 solid commercially-available foods, each chosen to elicit a target sensation (sweet, sour, bitter, salty, pungent). The same cohort also completed several psychometric measures and a 4-day food diary. Unsupervised data-driven clustering of genus-level Aitchison distances supported the existence of two salivary microbial profiles (CL-1, CL-2). While CL-1 (n = 57; 49.1 % women) exhibited higher α-diversity metrics and was enriched in microbial genera assigned to the class *Clostridia* (e.g., *Lachnospiraceae_[G-3]*), CL-2 (n = 43; 55.8 % women) harbored greater amounts of taxa with potential cariogenic effects (e.g., genus *Lactobacillus*) and significantly lower abundances of inferred MetaCyc pathways related to the metabolic fate of acetate. Intriguingly, CL-2 showed enhanced responsiveness to warning oral sensations (bitter, sour, astringent) and a higher propensity to crave sweet foods or engage in prosocial behaviors. Further, the same cluster reported habitually consuming more simple carbohydrates and fewer beneficial nutrients (vegetable proteins, monounsaturated fatty acids). In summary, while the mediating role of participants' baseline diet on findings can not be definitively excluded, this work provides evidence suggesting that microbe-microbe and microbe-taste interactions may exert an influence on dietary habits and motivates further research to uncover a potential "core" taste-related salivary microbiota.

1. Introduction

The human oral cavity constitutes a flourishing habitat for a plethora of microbial taxa. Bacterial colonization targets both mucosal and dental surfaces, resulting in the construction of unique ecological niches that harbor distinct groups of microorganisms (Mark Welch et al., 2020). Nevertheless, this large site-dependent microbial heterogeneity is largely represented by the ensemble of microbes suspended in saliva. Indeed, saliva bathes the entire oral cavity and is enriched with numerous bacterial residents shed from all oral surfaces (Takeshita et al., 2016), which are closely associated with health status and dietary patterns (Belstrøm, 2020; Lu et al., 2019).

In addition to the salivary microbiota, differences in eating habits have also been linked to a myriad of host biological and attitudinal factors (Köster, 2009), of which the sense of taste is among the most influential (Kourouniotis et al., 2016). Taste perception varies widely between individuals, and several anatomical, demographic, psychological, or genetic sources of variation have been extensively discussed (e.g., Fischer et al., 2013; Monteleone et al., 2017). In particular, humans differ significantly in their genetically mediated responsiveness to bitter-tasting compounds, such as phenylthiocarbamide or 6-n-propylthiouracil (PROP; Bartoshuk et al., 2004).

Phenotypic responses to PROP bitterness range from null (Non Tasters: NTs) to moderate (Medium Tasters: MTs) or extreme (Super Tasters: STs) and are mainly ascribed to variations in the haplotypes of the TAS2R38 gene (Risso et al., 2016; Robino et al., 2022), with

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potential contributions from other bitter receptors (Hayes et al., 2008). Increasingly, ample evidence suggests that PROP perception is associated with acuity for a variety of oral stimuli (e.g., Dinnella et al., 2018; Nolden et al., 2020; Piochi et al., 2021), which has motivated its frequent use as a marker of generalized hypergeusia. However, recent large-scale studies noted that weak PROP tasting does not necessarily correspond to weak perceived intensities of other taste qualities (Fischer et al., 2014), and that the relationship between PROP and taste perception follows a linear pattern only in individuals with low density of fungiform papillae (Dinnella et al., 2018). Hence, while PROP acuity remains a valid indicator of interindividual differences in chemoperception, its actual predictive value needs to be reconsidered (Dinnella et al., 2018; Fischer et al., 2014; Nolden et al., 2020).

In attempting to further elucidate the sources of individual taste variation, it is worth noting that taste perception is intimately related to saliva. The salivary milieu (e.g., flow rate, protein content, ionic composition) plays a key role in taste and flavor perception, as it serves as a solubilizer of tastants and flavor-active stimuli that facilitates their transport near chemosensory receptors (Canon et al., 2018). Interestingly, chemoreceptors can also be triggered by a variety of salivary metabolites produced by microbial enzymes from endogenous and exogenous sources (nutrients), whose activity would increase the *peri*-receptor concentration of such molecules and ultimately induce sensory adaptation (Feng et al., 2018; Gardner et al., 2020; Leung & Covasa, 2021; Schwartz et al., 2021). Alternatively, salivary microbial communities may convert taste-active compounds into tasteless molecules, thereby promoting a reduced stimulation of chemosensory systems (Schwartz et al., 2021). Moreover, two recent reviews (Leung & Covasa, 2021; Schwartz et al., 2021) have examined a possible manipulative effect of oral microbes on taste receptor expression, although the direct consequences on taste are still debated.

Against this backdrop, the oral microbiota is emerging as an additional candidate to explain interindividual differences in taste perception (Besnard et al., 2018; Cattaneo, Gargari, et al., 2019; Cattaneo, Riso, et al., 2019; Feng et al., 2018; Gardner et al., 2020; Mameli et al., 2019; Solemdal et al., 2012; Valentino et al., 2022; Yousaf et al., 2022). For instance, acutely hospitalized elderly with poor sour sensitivity were found to harbor greater amounts of salivary *Lactobacilli* (Solemdal et al., 2012), whilst the abundances of the phylum *Actinobacteria* were inversely related to salt detection abilities in a small cohort of 21 adults (Feng et al., 2018). Furthermore, children and adolescents with higher proportions of salivary members of the phylum *Bacteroidetes* exhibited a generalized lower sensitivity to tastes (especially bitter), regardless of BMI (Mameli et al., 2019). Lastly, differences in salivary microbial composition have also been discussed in relation to PROP taster status, with STs housing more bacterial taxa of the genera *Prevotella, Veillonella*, *Alloprevotella*, and *Actinomyces* compared to NTs at the baseline of an 11-day oral rinsing intervention (Yousaf et al., 2022). However, the mechanistic links underlying such cross-sectional associations remain to be demonstrated empirically.

It is noteworthy that the polymicrobial salivary ecosystem is governed by ecological relationships among its residents (Marsh & Zaura, 2017). Indeed, microbes benefit from salivary environmental features (e.g., pH, nutrient availability, oxygen) and favorable inter-species interactions to establish opportunistic patterns of co-existence (Marsh & Zaura, 2017). These have recently referred to as "*stomatotypes*" (Willis et al., 2018), and have been linked to a variety of lifestyle and diet-related factors, including oral health (Takeshita et al., 2016), drinking water composition (Willis et al., 2018), and sugar intakes (Esberg et al., 2020). Given how oral health and diet may relate to the sense of taste (Diószegi et al., 2019; Kaur et al., 2021), this raises the question on whether specific co-occurring guilds of salivary bacteria can efficiently distinguish individuals with varying taste acuity and dietary habits. Importantly, unlike canonical bioinformatic pipelines aimed at detecting single bacterial markers related to taste phenotypes, identifying groups of co-abundant microbes offers the key advantage of capturing ecological relationships between taxa that are likely to share the same nutritional needs and exert similar functionalities to interact with the host (Wu et al., 2021; Yousaf et al., 2022).

However, this would not be very informative without filling in a few additional gaps in the newly-born taste-oriented microbiome research field. First is the widespread use of detection thresholds as exclusive measure of taste functioning in response to artificial stimuli such as single taste aqueous solutions (Besnard et al., 2018; Cattaneo, Riso, et al., 2019; Gardner et al., 2020; Mameli et al., 2019) or paper strips (Feng et al., 2018; Solemdal et al., 2012). Although common in the taste literature, detection thresholds suffer from poor predictive power with respect to everyday food perceptions, which are mostly allocated at suprathreshold level (Puputti et al., 2018; Webb et al., 2015). Hence, favoring complex food stimuli to water or paper-based single tastant delivers would tremendously increase the real-life (ecological) power of the results.

Second, conscious taste perception never arises as a standalone phenomenon. Indeed, hedonics, attitudes or psychological traits (among others) act as important confounders of how food tastes to different individuals (e.g., Laureati et al., 2018; Spinelli et al., 2018), and can promote dissimilarities in food choices (Köster, 2009) ultimately shaping the salivary microbiota (Valentino et al., 2022). Despite this, such factors have only been sparsely operationalized in previous reports (Mameli et al., 2019; Valentino et al., 2022). Lastly, research examining the interactions between taste, oral microbiota and dietary outcomes is still surprisingly little (Cattaneo, Riso, et al., 2019), thus raising the need to expand current knowledge. Taken collectively, these limitations highlight the importance of: a) investigating the associations between taste and the salivary microbiota in light of the ecological links among its inhabitants; b) assessing taste function using more ecologically valid stimuli and psychophysical tools; c) considering key mediators of taste perception; d) collecting measures of dietary behavior.

To address these gaps, this double-blind cross-sectional study represents the first attempt to examine whether distinct salivary microbial networks co-occur with variations in taste and flavor perception of real foods in healthy individuals, and to explore how these associations may reflect self-reported habitual dietary patterns. This work builds on a previous investigation (Menghi et al., 2023), in which the same individuals ($n = 100$) were assessed for associations between taste perception, diet, and distal gut microbiota, controlling for a variety of hedonic, attitudinal and psychometric covariates.

2. Methods

2.1. Participants

Data were collected from a cohort of 100 healthy young Italian adults (52 % women; 18–30 y/o; mean age = 23.7 ± 3.9; mean BMI = 22.5 ± 2.6) as part of a project focusing on the complex crosstalk between taste and the oral or gut microbiota (Menghi et al., 2023). Attendance was contingent upon meeting a long list of inclusion criteria designed to limit the influence of factors altering taste and/or oral microbial homeostasis on outcomes. Eligibility criteria included, but were not limited to: no evidence of a historical or current diagnosis of COVID-19; oral (e.g., periodontitis, chronic xerostomia) and gastrointestinal (e.g., coeliac or Crohn's disease) diseases; taste disorders (e.g., dysgeusia, anosmia); habitual smoking; pregnancy or breastfeeding; BMI ≥ 30 or ≤ 18.5 Kg/m^2; use of medications that may affect taste function (e.g., proton pump inhibitors) and use of (pre-) probiotics and antibiotics within the last 6 months prior to study entry. Full details on recruitment strategy, exclusion criteria and demographics can be found in our previous work (Menghi et al., 2023).

Informed consent was obtained electronically from each participant. Additionally, the study was conducted in accordance with the Declaration of Helsinki, and approved by the Research Ethics Committee of the University of Trento (n° prot. 2020-040, approved on 08/02/2021).

2.2. General procedure

In brief, eligible participants attended remotely a double-blind, 7-day (D-) lasting design. Data collection took place during four separate daily sessions (D1, D2, D3, D4), which volunteers were asked to complete within one week, along with a not-consecutive 4-day food diary (D1-D7). Remote attendance was subject to the autonomous collection of a bag containing all the materials needed to complete the study from various pick-up points located in the Autonomous Province of Trento (Italy). Also, bag collection was preparatory to accessing the online platforms used for data collection.

On D1, participants rated the bitterness evoked by two PROP impregnated strips using the generalized Labeled Magnitude Scale (gLMS; Bartoshuk et al., 2004), whilst D2 was designed to collect hedonics (Labeled Affective Magnitude scale; LAM; Schutz & Cardello, 2001) in response to 5 liquid and 5 solid commercially-available foods (Table 1), each evocative of a target taste (sweet, sour, bitter, salty) or sensation (pungent). Immediately after, familiarity (5-point Likert scale; 1 = Not at all familiar, 5 = Extremely familiar), and weekly frequency of consumption (5-point Likert scale; 1 = Never, 5 = Five or more times/week) of the evaluated food product categories were tested.

D3 was instead planned to gather a detailed demographic, attitudinal and psychosocial profile of our cohort, while the final working session (D4) was devoted to the collection of an unstimulated saliva sample just before asking volunteers to rate the intensity (gLMS) of oral sensations elicited by the ten products previously evaluated on D2. Completion of D4 marked the end of the protocol, and participants were allowed to return their salivary sample (D4-D7) upon confirming to have fulfilled all the expected tasks.

Both sensory (D1, D4) and hedonic (D2) work sessions were preceded by extensive text and video training to avoid idiosyncratic use of the gLMS (Bartoshuk et al., 2004) and LAM (Schutz & Cardello, 2001) scales, respectively. In addition, access to the online data collection platforms was only granted if volunteers confirmed to have refrained from eating, drinking (except water) and brushing their teeth for at least 3 h prior to the test, as well as to have respected common practices in sensory testing (Menghi et al., 2023). Lastly, data were collected using Eye Question (Elst, The Netherlands) and Alchemer (Louisville, CO, USA) software, with the exception of food diaries, which were collected and processed via Dietosystem® (DS Medica, Milan, Italy). The reader is referred to Menghi et al. (2023) for a detailed overview of the data collection.

2.3. Salivary microbial samples

2.3.1. Sampling and processing

Salivary sampling was performed as the initial task planned by the last work session (D4) using OMNIgene®•ORAL (OM-501, DNA Genotek Inc., Ottawa, Canada), a self-administered commercial kit that allows long-term (up to 1 year) storage of microbial DNA at room temperature. Volunteers were given text and video instructions to self-collect an unstimulated salivary sample by dropping it into the funnel attached to the OM-501 tube until it reached a marked fill line (~1 mL). Participants then mixed the DNA stabilization buffer (~1 mL; stored in the funnel lid) with their salivary specimen before closing and shaking the tube for 30 s to ensure homogenization. Overall, samples were delivered within 1 day (mean = 1.1 ± 2.3 days) upon collection and were immediately incubated in a water bath at 50° C for 1 h, mixed by inversion for 30 s, and aliquoted into 1 mL vials before storage at ambient temperature until ready to use.

Genomic DNA was extracted from 1 mL of stabilized saliva using the QIAamp DNA Microbiome Kit (Qiagen, Hilden, Germany) in line with manufacturer's recommendations. Total bacterial DNA was then quantified using a NanoDrop™ Lite spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and stored at −80 °C prior to amplification. Upon ice thawing, the V3-V4 hypervariable regions of the 16 s rRNA gene were PCR-amplified using the bacterial primers 341F (5′ CCTACGGGNGGCWGCAG 3′) and 806 R (5′ GACTACNVGGGTWTCTAATCC 3′) complemented by Illumina overhang adapters (Apprill et al., 2015; Klindworth et al., 2013). Lastly, amplicon libraries were prepared and purified according to Gaudioso et al. (2021), and subsequently sequenced using 300 bp paired-end reads on an Illumina® MiSeq platform (Control Software 2.6.2.1 and Real-Time Analysis software 1.18.54; San Diego, CA, USA).

2.3.2. Bioinformatics

Demultiplexed and primer free paired-end sequences were analyzed following the standard DADA2 microbiome pipeline (Callahan et al., 2016). In brief, amplicon products were first filtered and truncated (F: 283 and R: 213 bp) to retain sequences with a median PHRED > 30. Later, filtered reads were dereplicated and denoised before being inferred as amplicon sequence variants (ASVs). Complete denoised sequences were then derived by merging forward and reverse ASVs before removing chimeras as well as *Cyanobacteria* and mitochondrial reads. Lastly, ASVs were blasted against the expanded Human Oral Microbiome Database (version 15.22; Chen et al., 2010) for taxonomic assignment up to the genus level.

Table 1
Sets and evaluation order of food stimuli (Product) presented to participants within the liking (D2) and intensity (D4) tasks. Food-related information (Brand, Amount, Consistency) plus the full list of sensory descriptors (Target sensation, Other sensations, Flavor) used here are also tabulated. * In PR-08, participants rated bitterness before sweetness, astringency, and cocoa flavor. Adapted from "*Variations in oral responsiveness associate with specific signatures in the gut microbiota and modulate dietary habits*" by Menghi et al., 2023, Food Quality and Preference, 106, 104790, p. 4.

Acronym	Set	Order	Product (Brand)	Amount	Consistency	Target sensation	Other sensations	Flavor
PR-01	1	1	Pear juice (Yoga, Italy)	10 mL	Liquid	Sweet	Sour	Pear
PR-02	1	2	Grapefruit juice (Derby Blue, Italy)	10 mL	Liquid	Sour	Bitter	Grapefruit
PR-03	1	3	Ready to drink coffee (Pocket Bar, Italy)	10 mL	Liquid	Bitter	/	Coffee
PR-04	1	4	Olive pate (Madama Oliva S.r.l, Italy)	10 mL	Liquid	Salty	/	Olive
PR-05	1	5	Spicy tomato sauce "Arrabbiata" (Industrie Montali S.r.l, Italy)	10 mL	Liquid	Pungent	/	Tomato
PR-06	2	6	Biscuit (Lotus Bakeries NV, Belgium)	1 unit	Solid	Sweet	/	Caramel
PR-07	2	7	Lemon candy (Perfetti Van Melle S.p.A, Italy)	1 unit	Solid	Sour	Sweet	Lemon
PR-08	2	8	Dark chocolate (Venchi S.p.A, Italy)	1 unit	Solid	Bitter	* Sweet, Astringent	Cocoa
PR-09	2	9	Fries (Saiwa S.r.l, Italy)	4 units	Solid	Salty	/	Potato
PR-10	2	10	Ginger candy (Euro Company S.r.l, Italy)	2 units	Solid	Pungent	Sweet	Ginger

2.4. Sensory assessments

2.4.1. Food products

Five liquid and five solid commercially-available, ready-to-use, easily portionable, and widely distributed food products in the Italian market were selected for our scopes (Table 1). Most importantly, each matrix was expected to clearly evoke a recognizable target taste (sweet, sour, bitter, salty) or sensation (pungent) falling within an expected moderate/very strong range of intensity within the gLMS (Menghi et al., 2023). Adherence to the above criteria was confirmed by the results of three pilot tests. For details, please refer to our previous work (Menghi et al., 2023).

2.4.2. Scaling and sensory testing

Scale training and sensory assessments were conducted as previously reported (Menghi et al., 2023). Briefly, to avoid artifacts in the use of the gLMS (0 = no sensation, 100 = the strongest imaginable sensation of any kind; D1 and D4) and LAM (0 = greatest imaginable dislike, 100 = greatest imaginable like; D2) scales, volunteers were given extensive text and video instructions following standard guidelines (Bartoshuk et al., 2004; Schutz & Cardello, 2001; Webb et al., 2015). Particular emphasis was placed on the gLMS training. In this vein, volunteers were oriented to the scale by being asked to rate the intensity of five recalled extraoral stimuli that were assumed to be representative of the full length of the scale (Bartoshuk et al., 2004; Hayes et al., 2010; Menghi et al., 2023; Webb et al., 2015). Phenotypic responses to PROP bitterness were operationalized using two commercial paper strips (3–5 μg, MediSens, Groningen, The Netherlands). To this end, participants were instructed to place each strip on the tongue and spread the stimulus over the mucosal surfaces of the mouth for 10 s (Smutzer et al., 2013) before expectorating and waiting a further 5 s to rate the intensity of bitterness (gLMS). Ratings were then averaged, and volunteers classed as Non Tasters (NTs) or Super Tasters (STs) when their scores fell below the 25th (gLMS < 9.5) and above the 75th (gLMS > 31.3) percentiles of the distribution, respectively. All others were assigned to the Medium Tasters (MTs) group ($9.5 \geq$ gLMS ≤ 31.3).

Instead, two separate sets of five foods each were presented in the liking (D2) and intensity (D4) tasks (Table 1). Liquid products (Set 1) were always evaluated first, followed by the five solid foods (Set 2) after a 5 min break. Both series of stimuli (Set 1; Set 2) were presented in a fixed order and rated for relevant sensory attributes (Table 1). Particularly, pungent foods (spicy tomato sauce and ginger candy) were always assessed as last to control for potential carry-over effects driven by irritants, while psychophysical responses to target sensations were constantly collected before other product-related taste qualities and flavors (Table 1). Additionally, prior to beginning the evaluation of each sample, volunteers were provided with video instructions to facilitate sample preparation and portioning.

All food products were stripped of brand identifiers and stored in paper-based packaging with a 3-digit code and a label, the color of which had to be indicated by the participants (at the end of each evaluation) to ascertain the correctness of the tasting protocol (Menghi et al., 2023). Lastly, mineral water and unsalted crackers were used as palate cleaners during a 90 s break between all tastings (D1, D2, D4).

2.5. Psychometrics and demographics

On D3, volunteers were administered a series of psychometric and demographic measures to capture salient background information that could potentially act as confounders within the links examined here (Menghi et al., 2023). In detail, our cohort was first screened for food neophobia (i.e., unwillingness to try novel foods) and trait-anxiety levels using the common Food Neophobia Scale (Laureati et al., 2018; Pliner & Hobden, 1992) and the State-Trait Anxiety Inventory Questionnaire (trait anxiety subscale; Pedrabrissi & Santinello, 1989; Spielberger, 1983). Next, attitudes toward health- or taste-guided food choices and eating behaviors were operationalized using the Health and Taste Attitude Scale (Roininen & Tuorila, 1999; Saba et al., 2019) and the Dutch Eating Behaviour Questionnaire (Monteleone et al., 2017; van Strien et al., 1986), respectively. Lastly, volunteers were tested for facets of personality by the Big Five Inventory (Corr, 1998; Fossati et al., 2011). All measures employed were back-translated and validated into Italian, and were found to be internally consistent (Menghi et al., 2023). Full details on the psychometric tools used by the current study can be found in Menghi et al. (2023).

Participants then completed the D3 tasks by providing demographic information, i.e., age, gender, weight and height (later used to calculate the BMI as Kg/m^2), education level, occupation and annual income, as well as self-reported habitual diet type (Menghi et al., 2023). For this latter purpose, we classified participants as omnivores, flexitarians, vegetarians or vegans as described in De Backer & Hudders (2015).

2.6. Food diaries

Each participant was also invited to complete a food diary, listing all foods and beverages consumed on four (3 weekdays and 1 weekend day) of the seven days foreseen by the protocol. The food record was preceded by a practical video tutorial, which was designed to train the volunteers: a) to be meticulous in recording recipes and grammages; b) to use a photographic food atlas (Istituto Scotti Bassani, Milan, Italy), based on the Italian Food Composition Database (https://www.ieo.it/bda), as a landmark to detail portion sizes (Menghi et al., 2023).

Dietary intakes were tracked using a smartphone app (Dietosystem®, DS Medica, Milan, Italy), while Terapia Alimentare Dietosystem® (version 295 17.00.02, DS Medica, Milan, Italy) was employed to extract energy intakes (as Kcal) and exact quantities of a lengthy list (n = 93) of macro- and micronutrients. Lastly, the data were energy-adjusted by residual method (Poslusna et al., 2009) to unpack nutrient density from variations in total energy intake attributable to known covariates (gender, BMI, physical activity) and averaged.

2.7. Data analysis

Homogeneous groups of individuals with patterns of similarly co-occurring microbial consortia were derived using a compositionally coherent data analysis approach (Gloor et al., 2017), designed to capture the co-dependent nature of high-throughput sequencing products. First, the unfiltered ASV table was collapsed at the genus level before treating zero counts with geometric Bayesian-multiplicative replacement (Palarea-Albaladejo & Martín-Fernández, 2015), and centering log-ratio transforming the data (Gloor et al., 2017). Second, we calculated the Euclidean (or Aitchison) distances between samples (Gloor et al., 2017), which served as input for the subsequent derivation of salivary microbial patterns. For the latter, we replicated a previously reported (Menghi et al., 2023) unsupervised data-driven clustering method to objectively determine the algorithm and the number of clusters that best fit the data. According to previous tutorials (Kassambara, 2017), six different algorithms (HCA, K-means, PAM, SOTA, CLARA, and DIANA clustering) were tested within various clustering solutions (from 2 to 10), and evidence of optimal partitioning was certified by the lowest cluster connectivity and the highest silhouette width and Dunn index obtained (Brock et al., 2008). Salivary microbial profiles were then checked for differences in α-diversity metrics (Chao-1, Fischer, Shannon, Inverse Simpson indices) by Wilcoxon Rank Sum Test, whilst multivariate analysis of variance (PERMANOVA, n = 10000) was used to test for dissimilarities in Aitchison distances (inter-sample β-diversity) between groups.

Next, to deal with rarely occurring bacteria and reduce the likelihood of potential false positives, the differential abundance analysis was preceded by an unsupervised permutation (n = 10000) filtering of taxa with a null contribution to the total covariance of the data according to Smirnova et al. (2019). To this end, the functions *PERFect_sim* and the

PERFect_perm from the R package *PERFect* were subsequently applied to the original ASV table with default parameters (Smirnova et al., 2019). Clusters were thus checked for differentially abundant microbial taxa at different taxonomic levels (phylum, class, order, family, genus) using the Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC) with default parameters (Lin & Peddada, 2020). The filtered ASV table was also used as input to infer the abundances of functional microbial pathways via Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2; Douglas et al., 2020), later mapped against the MetaCyc database for subsequent annotation. As recommended by the original authors (Douglas et al., 2020), PICRUSt2 data should be treated as compositional. Therefore, we computed the Aitchison distances (as for bacterial counts) on the imputed bacterial functionalities and tested omnibus differences between clusters by PERMANOVA (n = 10000). Variations in MetaCyc pathway proportions between salivary microbial profiles were then tested using ANCOM-BC (Lin & Peddada, 2020) as described above.

Lastly, differences between salivary microbial profiles as a function of hedonics, psychophysics in response to the relevant sensory ballot of the ten food stimuli, attitudes and psychological traits, and dietary habits were calculated using the Wilcoxon rank sum test. All data are summarized as median ± interquartile range (IQR) unless otherwise stated. A p value < 0.05 was considered as statistically significant, and multiple inferences were adjusted with the Holm method (Holm, 1979).

2.8. Software

Bioinformatics and statistics were run in R 4.2.2 (R Core Team, 2022), with the exception of the PICRUSt2 analysis, which was launched on a Python 3.8.0 machine. Among others, α-diversity metrics were calculated via *phyloseq* (McMurdie & Holmes, 2013), while imputation of zeros and subsequent β-diversity analyses were performed using the *zCompositions* (Palarea-Albaladejo & Martín-Fernández, 2015) and *vegan* (Dixon, 2003) packages, respectively. The package *clValid* (Brock et al., 2008) was instead used for cluster derivation and validation, while the package *PERFect* (Smirnova et al., 2019) was used to filter the ASV table before differential abundance analyses. Bacterial metabolic activities were predicted using the full PICRUSt2 pipeline (Douglas et al., 2020) with default parameters (https://github.com/picrust/picrust2/wiki/Full-pipeline-script), and the *ANCOMBC* R package (Lin & Peddada, 2020) was used to test differentially abundant taxa and inferred MetaCyc pathways between salivary microbial profiles.

3. Results

3.1. Overall salivary microbial ecology

16 s rRNA gene amplicon sequencing of salivary specimens conclusively recovered 1717 unique ASVs from a total of 7,898,164 (mean = 78,981.6 ± 14,591.5 per sample) reads, which were later assigned to 10 phyla, 23 classes, 35 orders, 64 families, and 124 genera. Overall, the salivary microbiota of our cohort was governed by the phylum *Firmicutes* (74.1 ± 6.5 %), which has been reported to be the most abundant consortium inhabiting the healthy salivary microbial environment (Ruan et al., 2022). The phylum-level salivary bacterial composition of our cohort then included bacteria from the phyla *Actinobacteria* (24.3 ± 6.6 %), *Saccharibacteria_TM7* (1.2 ± 1.2 %), *Fusobacteria* (0.2 ± 0.8 %), *Proteobacteria* (0.1 ± 0.2 %), and *Bacteroidetes* (0.1 ± 0.1 %), which together accounted for over 99 % of the total amount of sequences generated.

Large overlaps were also observed at the genus-level with the 68 core residents of the healthy human salivary microbiota (Ruan et al., 2022), with members of the genera *Streptococcus* (48.1 ± 6.5 %), *Rothia* (15.2 ± 7.1 %), *Veillonella* (7.2 ± 3.6 %), *Gemella* (6.6 ± 3.5 %), *Granulicatella* (5.1 ± 2.2 %), *Scaalia* (4.1 ± 2.4 %), *Actinomyces* (2.4 ± 2.4 %), *Atopobium* (1.5 ± 2.4 %), *Saccharibacteria_(TM7)_[G1]* (0.9 ± 1.0 %), *Oribacterium* (0.4 ± 0.4 %), *Lachnoanaerobaculum* (0.4 ± 0.4 %) and *Solobacterium* (0.4 ± 0.3 %) detected in at least 99 % of the samples.

3.2. Derivation and description of salivary microbial profiles

To derive homogeneous salivary microbial profiles capturing ecological relationships among microbial taxa, a compositional data paradigm was employed (Gloor et al., 2017). To this end, the unfiltered ASV table (n = 1717) was collapsed to the genus level, zeros imputed and centered-log transformed prior to computing the Aitchison metric as a compositionally-aware pairwise distance between samples (Gloor et al., 2017). Such input was then employed to objectively determine the best clustering algorithm and grouping solution underlying the data. Results indicated that n = 2 clusters derived via Hierarchical Agglomerative Clustering (Fig. 1) represented the optimal partition (Supplemental Figure S1), as it provided the lowest connectivity and the highest silhouette and Dunn indices among all different combinations of algorithms (n = 6) and groupings (from 2 to 10) probed here (Brock et al., 2008). As expected, the salivary microbial consortia of the two clusters largely mirrored those previously reported (section 3.1), with *Firmicutes* and *Streptococcus* dominating the phylum- and genus-level bacterial composition of both groups, respectively. Relative abundances of phyla (n = 10) and genera (top 20) as a function of salivary microbial profiles are shown in Supplemental Figure S2.

In line with the strict selection criteria of our protocol (section 2.1; Menghi et al., 2023), both groups were homogeneous ($p > 0.05$) with respect to gender, age, BMI, habitual type of diet, food neophobia, trait anxiety, eating behaviors, and health-related attitudes toward foods (Table 2). In addition, both groups showed similar distributions of PROP taste phenotypes, though CL-2 tended to be populated by a higher number of PROP MTs (and fewer PROP NTs) than CL-1 (Table 2; $p = 0.084$). Nevertheless, some cluster-dependent differences in food-related attitudes and personality traits were observed (Table 2), with CL-2 exhibiting higher tendencies to crave sweet foods (Health and Taste Attitude Scale; Roininen & Tuorila, 1999) and to endorse prosocial

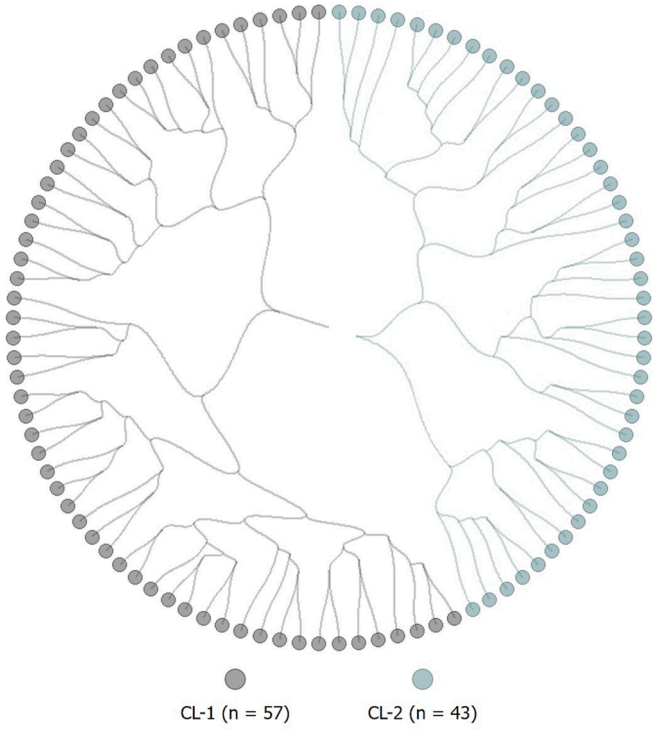

Fig. 1. Circular dendrogram depicting the Hierarchical Agglomerative clustering (Ward D2) of Aitchison distances between members (transparent circles) of CL-1 (n = 57; light gray) and CL-2 (n = 43; cadet blue).

Table 2
Demographic, psychological, and food-related attitudinal background of salivary microbial profiles (CL-1 = 57; CL-2 = 43). Data are tabulated as raw observations (n), mean ± SD or median ± IQR whenever stated. Statistically significant (p.value; $p < 0.05$) differences between clusters are depicted in bold and computed via chi-squared test (†), unpaired *t*-test (††) or Wilcoxon rank sum test.

	CL-1 (n = 57)	CL-2 (n = 43)	p. value
Gender (n)			
Women	28	24	0.507†
Men	29	19	
Age (mean ± SD)	23.8 ± 3.8	23.6 ± 4.2	0.876††
BMI (mean ± SD)	22.4 ± 2.6	22.5 ± 2.7	0.859††
Diet (n)			0.501†
Omnivores	38	24	
Flexitarians	14	14	
Vegetarians	4	5	
Vegans	1	0	
Food Neophobia Scale (median ± SD)	22 ± 10	24 ± 12.5	0.587
Trait Anxiety Inventory	44 ± 14	46 ± 12	0.524
Health and Taste Attitude Scale			
General health interest	4.5 ± 1.0	4.4 ± 1.4	0.302
Light product interest	3.8 ± 1.3	4.2 ± 1.3	0.113
Natural product interest	4.0 ± 1.8	3.8 ± 1.2	0.823
Craving for sweet foods	4.8 ± 1.8	5.7 ± 1.6	**0.005**
Using food as reward	4.7 ± 1.5	4.8 ± 1.8	0.900
Pleasure	4.8 ± 1.0	4.7 ± 1.2	0.613
Dutch Eating Behaviour Questionnaire			
Restrained Eating	2.6 ± 1.0	2.7 ± 1.3	0.805
Emotional Eating	2.5 ± 0.8	2.5 ± 0.9	0.269
External Eating	3.3 ± 0.4	3.2 ± 0.9	0.933
Big Five Inventory			
Extraversion	3.3 ± 1.1	3.3 ± 1.4	0.569
Agreeableness	3.6 ± 0.7	3.9 ± 0.7	**0.029**
Conscientiousness	3.7 ± 0.9	3.4 ± 0.8	0.329
Neuroticism	3.0 ± 1.1	3.1 ± 1.1	0.839
Openness	3.7 ± 0.9	3.9 ± 1.0	0.246
PROP Taster Status (n)			
Non Tasters (NTs)	19	6	0.084†
Medium Tasters (MTs)	25	25	
Super Tasters (STs)	13	12	

behaviors (Agreeableness; Big Five Inventory; Corr, 1998).

3.3. Salivary microbial profiles showed differences in α- and β-diversities

First, we examined the differences in bacterial richness and evenness between salivary microbial profiles. While both groups did not differ ($p > 0.05$) in both Chao-1 and Inverse Simpson indices, CL-1 showed higher Shannon (CL-1: 3.7 ± 0.8; CL-2: 3.2 ± 0.7; $p = 0.006$) and Fisher (CL-1: 3.7 ± 0.8; CL-2: 3.2 ± 0.7; $p = 0.006$) metrics than CL-2 (Supplemental Figure S3). Clusters were later tested for β-dissimilarities, and PERMANOVA highlighted statistically significant differences ($R^2 = 0.081$; $p < 0.001$) between the groups (Supplemental Fig. S4a). Additionally, we tested whether such differences translated into variation in the degree of compositional homogeneity within clusters (Supplemental Fig. S4b) and found that Aitchison distances among members of CL-1 (13.8 ± 3.6) were significantly shorter ($p < 0.001$) than those observed in CL-2 (15.4 ± 3.5). Overall, the results of the ecological analysis indicated that CL-1 harbored a more complex and homogeneous salivary microbiota.

3.4. Salivary microbial profiles differed in relative abundances of a large panel of taxa

Second, we uncovered the differentially abundant bacteria housed by the two clusters. Prior to performing the analysis, contaminant taxa were removed in accordance with the standard two-step procedure outlined by Smirnova et al. (2019). This enabled us to objectively retain all those ASVs with informative power in regard to the total covariance of the dataset, thus preventing inflated results driven by rarely occurring microbial communities and controlling the occurrence of false discovery rates (Smirnova et al., 2019). After permutation filtering (n = 10000), 585 out of 1717 ASVs (34.1 %) were considered important for estimating the underlying covariance of the data and were retained for downstream applications. Despite the massive filtering loss, the majority (98.2 %; 7,757,607; mean = 77,576.1 ± 14,293.5 per sample) of all generated sequences (7,898,164) were retained.

The filtered ASV table was then collapsed at each taxonomic level (phylum, class, order, family, genus) to perform separate differential abundance analyses via ANCOM-BC (Lin & Peddada, 2020). While no compositional dissimilarities ($p_{adj} > 0.05$) were observed between clusters at the phylum-level, a panel of salivary bacterial signatures significantly ($p_{adj} < 0.05$) distinguished CL-1 from CL-2. These included the proportions of 5 classes, 8 orders, and 12 families of microbes (Supplemental Table S1). More interestingly, we also found that the relative abundances (Supplemental Table S1) of 13 genera were differentially enriched in the two groups (Fig. 2a).

Compared to CL-2, CL-1 harbored greater amounts of the genera *Lachnospiraceae_[G-2]* (W = 3.7; $p_{adj} = 0.014$), *Lachnospiraceae_[G-3]* (W = 6.0; $p_{adj} < 0.001$), *Neisseria* (W = 3.8; $p_{adj} = 0.012$), *Parvimonas* (W = 3.9; $p_{adj} = 0.009$), *Peptococcus* (W = 5.6; $p_{adj} < 0.001$), *Peptostreptococcus* (W = 5.9; $p_{adj} < 0.001$), *Porphyromonas* (W = 4.0; $p_{adj} = 0.005$), *Ruminococcaceae_[G-1]* (W = 3.5; $p_{adj} = 0.028$), and *Saccharibacteria_(TM7)_[G-6]* (W = 4.1; $p_{adj} = 0.007$). Conversely, CL-2 was enriched in the genera *Alloscardovia* (W = 4.9; $p_{adj} < 0.001$), *Bifidobacterium* (W = 3.9; $p_{adj} = 0.006$), *Lactobacillus* (W = 4.0; $p_{adj} = 0.004$) and *Mitsuokella* (W = 3.6; $p_{adj} = 0.025$).

3.5. Variations in MetaCyc modules between salivary microbial profiles

Third, the full PICRUSt2 pipeline was launched to infer functional pathways from the 585 ASVs (mean NSTI = 0.2 ± 0.4) retained after permutation filtering. Overall, 315 MetaCyc pathways were imputed and annotated before testing the groups for β-dissimilarities by PERMANOVA and differential abundance analysis via ANCOM-BC. Pathway analysis revealed statistically significant omnibus differences (PERMANOVA; $R^2 = 0.055$; $p < 0.001$) in bacterial metabolic functions between salivary microbial profiles (Supplemental Figure S5), which were then resolved into 12 differentially abundant ($p_{adj} < 0.05$) MetaCyc pathways (Fig. 2b).

Of particular interest, we observed prominent deviations between clusters with respect to the metabolism of a sour-eliciting compound, with CL-1 showing higher methanogenesis from acetate (METH-ACETATE-PWY; W = 5.6; $p_{adj} < 0.001$) or its production from L-lysine (P163-PWY; W = 5.1; $p_{adj} < 0.001$) and hexitol (P461-PWY; W = 4.1; $p_{adj} = 0.009$) fermentation. Conversely, CL-2 was enriched in pathways involved in the formation of vitamin K2, namely the superpathways of menaquinol-8 (PWY-5838; W = 6.9; $p_{adj} < 0.001$) and demethylmenaquinol-8 (PWY-5861; W = 4.7; $p_{adj} = 0.001$) biosynthesis.

3.6. Differences in oral responsiveness, hedonics, familiarity, and frequency of consumption of actual foods between salivary microbial profiles

Salivary microbial profiles were then evaluated for differences in acuity for oral stimulations evoked by the five liquid and five solid foods

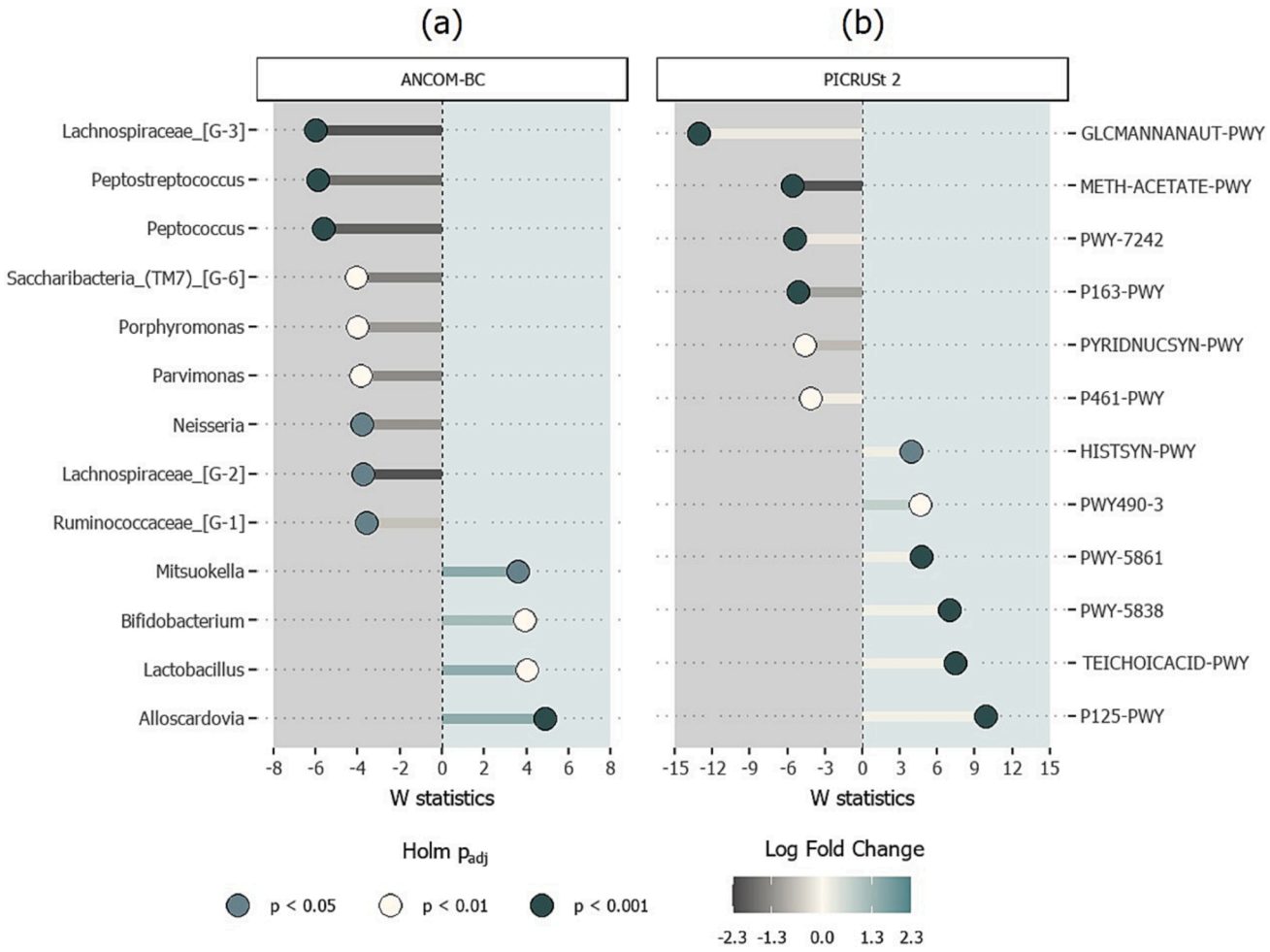

Fig. 2. Pool of bacterial genera (a) and imputed MetaCyc (b) pathways housed in significantly different ($p_{adj} < 0.05$) proportions by salivary microbial profiles. In each panel (a; b), the colored vertical bands comprise the taxa (a) or the inferred bacterial functions (b) found to be differentially abundant between CL-1 (left; light gray) and CL-2 (right; cadet blue). The ANCOM-BC main statistic (W statistics) and its relative effect sizes (log fold change) are represented by the length and the color of the horizontal bars, respectively. Holm's adjusted p. values ($p < 0.05$; $p < 0.01$; $p < 0.001$) are also provided, and illustrated by the dark cyan- ($p < 0.05$), white- ($p < 0.01$), and dark slate grey-filled ($p < 0.001$) circles.

used in the current study. As a result, clusters differed ($p < 0.05$) in their responsiveness to oral sensations elicited by 7 out of the 10 foods. In liquid products (Fig. 3), CL-2 perceived innately disliked tastes (sour in PR-02; bitter in PR-03) to a greater extent ($p < 0.05$) than CL-1, and this effect went along with enhanced perceptions of sour- (grapefruit in PR-02) and bitter-evoking (olive in PR-04) flavors. Similar trends were observed in solid foods (Fig. 4), with CL-2 showing heightened acuity ($p < 0.05$) for astringency (PR-08) and for sour- or pungent-evoking flavors such as lemon (PR-07) and ginger (PR-10), respectively. In addition, CL-2 gave higher intensity ratings when experiencing a sweet-evoking flavor such as caramel (PR-06).

To exclude the effect of potential confounders underlying such findings, salivary microbial profiles were examined for differences in liking, familiarity and frequency of consumption. Further, variations in psychophysical responses to the five extraoral stimuli employed within the gLMS training (section 2.4.2) were tested to confute possible idiosyncratic uses of the scale. Overall, while the clusters showed no differences ($p > 0.05$) in liking and familiarity scores (Table 3), CL-2 reported consuming a few energy-dense food products (PR-07: lemon candy; PR-08: dark chocolate) more frequently ($p < 0.05$; Table 3). Importantly, all recalled intensities evoked by the gLMS orienting extraoral stimuli were rated equally ($p > 0.05$) by both groups (Supplemental Figure S6), thus corroborating the reliability of the observed variations in sensory perception.

3.7. *Differences in habitual dietary intakes by salivary microbial profiles*

Lastly, salivary microbial profiles were assessed for variations in habitual dietary intake, taking into account the large number of nutrients ($n = 93$) plus total energy (Kcal) retrieved from the food diaries (Table 4). Although the dietary habits of both clusters largely overlapped ($p > 0.05$), a few differences emerged. Specifically, CL-1 reported habitually consuming higher amounts of several beneficial nutrients either from plant- or animal-based sources. These included vegetable proteins ($p = 0.029$), monounsaturated fatty acids ($p = 0.043$), and vitamins such as folic acid ($p = 0.049$), menadione ($p = 0.014$) and pantothenic acid ($p = 0.014$). Moreover, CL-1 also showed an almost significant ($p = 0.050$) higher habitual intake of ascorbic acid. Conversely, CL-2 was found to habitually consume larger quantities of simple carbohydrates ($p = 0.010$).

4. Discussion

Here, we probed whether homogeneous patterns of bacterial cohabitation in the salivary microbiota could reflect variations in orosensory acuity and habitual eating habits. Overall, unsupervised data-driven clustering of the genus-level Aitchison distances objectively resolved into two distinct salivary microbial profiles, distinguished by α- and β-diversity metrics and by a spectrum of differentially abundant

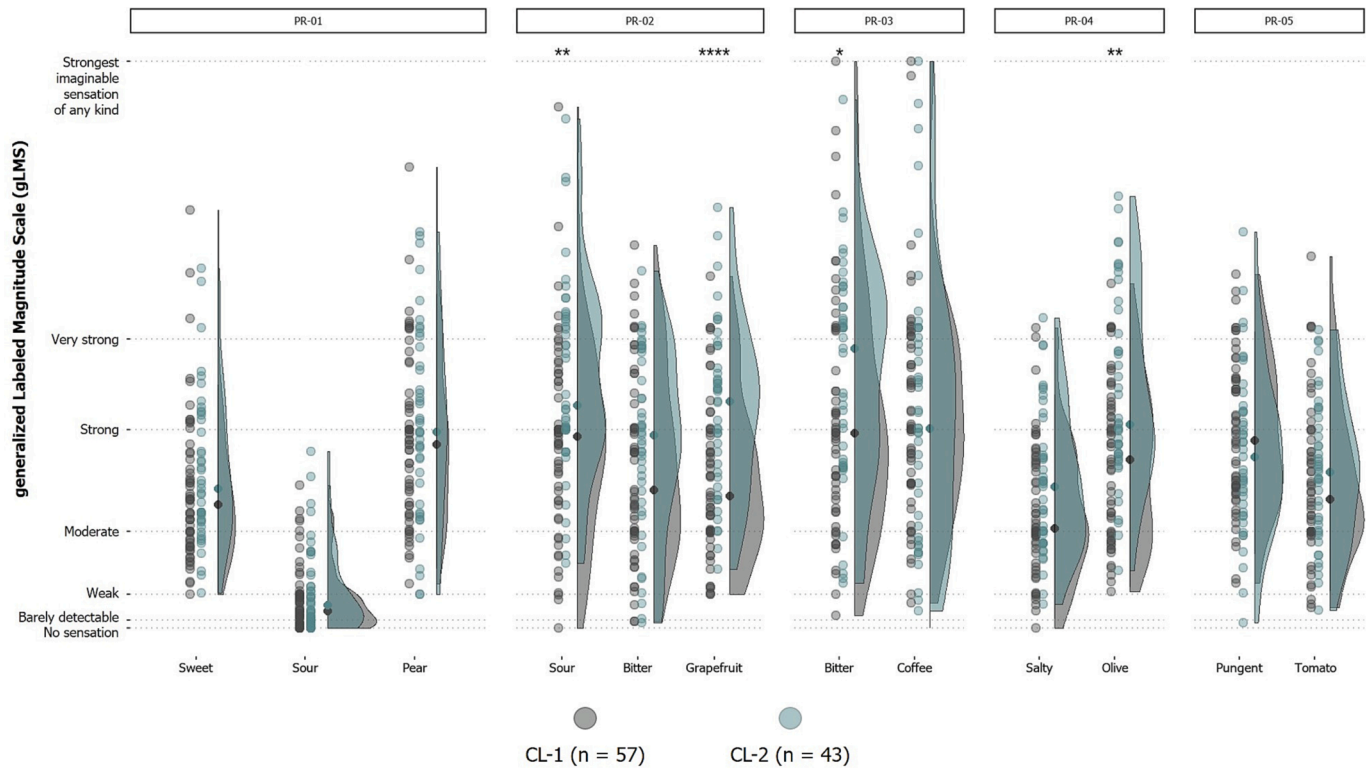

Fig. 3. Variations in acuity for oral sensations evoked by the five liquid foods as a function of salivary microbial profiles (CL-1: light gray; CL-2: cadet blue). Distribution (the "cloud") of raw observations (the "rain") plus the median (filled circle) ± IQR (perpendicular black line) are provided. * = $p < 0.05$; ** = $p < 0.01$; **** = $p < 0.0001$.

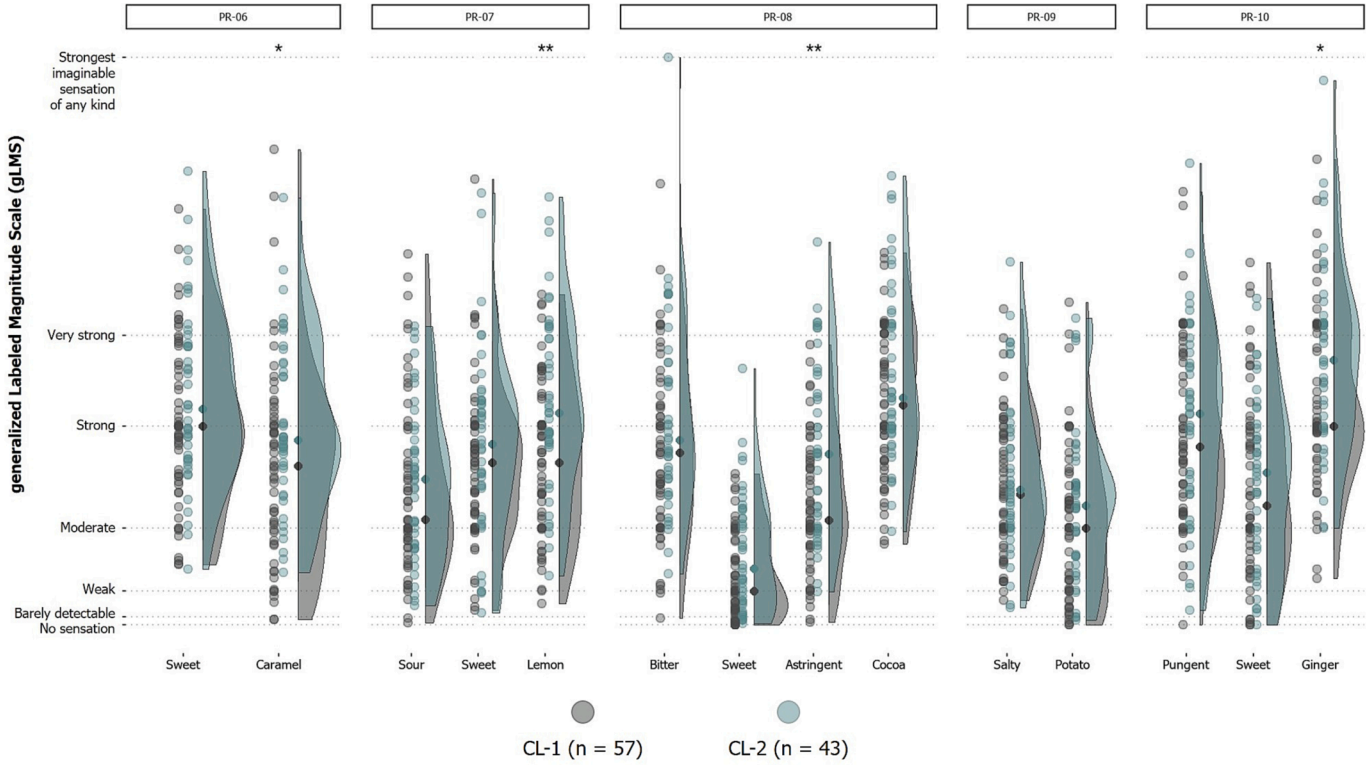

Fig. 4. Variations in acuity for oral sensations evoked by the five solid foods as a function of salivary microbial profiles (CL-1: light gray; CL-2: cadet blue). Distribution (the "cloud") of raw observations (the "rain") plus the median (filled circle) ± IQR (perpendicular black line) are provided. * = $p < 0.05$; ** = $p < 0.01$.

Table 3
Liking, familiarity and frequency of consumption ratings (median ± IQR) given by salivary microbial profiles (CL-1; CL-2) to the ten food matrices (Sample). Values in bold are considered statistically significant ($p < 0.05$).

Sample	Liking		p.value	Familiarity		p.value	Consumption		p.value
	CL-1	CL-2		CL-1	CL-2		CL-1	CL-2	
PR-01	68.2 ± 11.7	67.6 ± 19.5	0.883	4 ± 2	4 ± 1	0.533	2 ± 0	2 ± 0	0.749
PR-02	41.4 ± 20.6	45.3 ± 28.2	0.633	2 ± 2	2 ± 1.5	0.130	2 ± 1	2 ± 1	0.659
PR-03	39.2 ± 24.7	28.1 ± 28.9	0.093	4 ± 2	5 ± 2	0.611	5 ± 2	5 ± 2	0.949
PR-04	64.7 ± 27.9	68.2 ± 22.9	0.633	3 ± 2	2 ± 1	0.233	2 ± 1	2 ± 1	0.843
PR-05	64.7 ± 19.1	68.2 ± 21.9	0.362	5 ± 1	5 ± 1	0.694	3 ± 1	3 ± 1.5	0.566
PR-06	77.5 ± 17.5	78.4 ± 11.9	0.423	5 ± 1	5 ± 1	0.274	4 ± 3	4 ± 2	0.369
PR-07	67.9 ± 16.8	72.1 ± 15.4	0.052	3 ± 2	4 ± 2	0.122	2 ± 1	2 ± 1	**0.011**
PR-08	63.6 ± 27.1	63.5 ± 23.5	0.471	4 ± 1	5 ± 1	0.158	3 ± 2	4 ± 1	**0.038**
PR-09	74.4 ± 11.4	74.9 ± 13.4	0.337	4 ± 1	4 ± 2	0.359	2 ± 1	2 ± 0.5	0.921
PR-10	43.9 ± 42.2	47.8 ± 38.3	0.089	2 ± 2	2 ± 2	0.376	1 ± 1	1 ± 1	0.681

Table 4
Variations in habitual dietary intakes between salivary microbial profiles (CL-1; CL-2). Data are tabulated as median ± IQR, and p.values observed after Wilcoxon rank sum test are highlighted in bold whether statistically significant ($p < 0.05$).

	CL-1 (n = 57)	CL-2 (n = 43)	p.value
Carbohydrates (g)			
Simple carbohydrates	69.8 ± 20.3	80.6 ± 28.1	**0.010**
Proteins (g)			
Vegetable proteins	31.4 ± 11.1	27.1 ± 11.8	**0.029**
Fats (g)			
Monounsaturated fatty acids	21.2 ± 8.3	19.1 ± 5.0	**0.043**
Vitamins			
Ascorbic acid (mg)	152.5 ± 116.5	121.6 ± 74.5	0.050
Folic acid (mg)	306.6 ± 163.6	264.8 ± 142.5	**0.049**
Menadione (mcg)	154.9 ± 112.1	102.6 ± 98.5	**0.014**
Pantothenic acid (mg)	1.8 ± 1.0	1.5 ± 0.9	**0.014**

bacterial members and predicted metabolic functionalities. Intriguingly, the clusters further differed in their responsiveness to oral sensations or flavors that elicit alarming chemosensory properties, pleasure-oriented tendencies and endorsement of prosocial behaviors, and habitual consumption of beneficial dietary components or simple carbohydrates.

4.1. Confirming previously observed networks of salivary bacteria

At first, we observed compositional commonalities with the healthy salivary microbiota and with previously reported networks of microbes suspended in saliva. As common in healthy individuals (e.g., Ruan et al., 2022; Takeshita et al., 2016), we found that the phylum- and genus-level salivary microbiota of our cohort were dominated by *Firmicutes* and *Streptococcus*, respectively. Furthermore, although the depth of our sequencing approach did not permit taxonomically annotating the majority of ASVs at the species level, we detected multiple similarities with the genera assigned to the 68 core residents of the salivary microbiota (Ruan et al., 2022).

Notably, we also identified known networks of taxa governing the genus-level microbial consortia of the clusters. Indeed, salivary microbial profiles showed both *Streptococcus* and *Rothia* as dominant genera (Supplemental Figure S2), thus falling into one of the five "*stomatotypes*" (Willis et al., 2018) observed by Zaura et al. (2017) in a large cohort (n = 268) of similarly aged (18–32 y/o) adults. Similarly, our findings are consistent with previously reported patterns of co-occurrence and co-exclusion of bacterial genera in health (De Filippis et al., 2014; Valentino et al., 2022). As a result, *Streptococcus* positively related to *Gemella* and *Granulicatella*, whereas *Atopobium* and *Megasphaera* exhibited contrasting behavior and co-occurred with *Actinomyces*, *Stomatobaculum*, and *Lachnoanaerobaculum* (Supplemental Figure S7). Taken collectively, our findings reinforce ample evidence pointing out the existence of core salivary bacteria whose intimate relationships function in safeguarding host homeostasis (De Filippis et al., 2014; Marsh & Zaura, 2017; Ruan et al., 2022; Takeshita et al., 2016; Zaura et al., 2017).

4.2. Differences in chemoperception between salivary microbial profiles

Interestingly, we found that salivary microbial profiles differed in terms of oral sensations (astringent, bitter, sour) or flavors (grapefruit, olive, lemon, ginger) linkable to warning chemosensory signals. Notably, CL-1 (hereafter hyporesponsive cluster) perceived alarming taste qualities, trigeminal sensations, and flavors to a lesser extent than CL-2 (hereafter hyperresponsive cluster), and this was true for both liquid (Fig. 3) and solid foods (Fig. 4). Importantly, such differences did not correspond to differences in demographics, dietary styles, nearly all the psychological traits, and food-related attitudes considered other than liking and familiarity for the ten stimuli between groups. Moreover, we excluded systematic use of the gLMS, as both clusters rated the five extraoral stimuli used for scale orientation as equally intense. Thus, it appears plausible that physiological rather than external cues explain how salivary microbial profiles behave differently in response to oral stimulation.

Consistent with a previous report on the same cohort (Menghi et al., 2023), enhanced acuity for warning sensations did not translate into higher phenotypic responses to PROP. As we have previously argued (Menghi et al., 2023), such an unexpected finding is likely related to two potential drawbacks on using paper strips (rather than water solutions) to assess PROP Taster Status: a) the tendency of impregnated strips to overestimate the percentage of individuals with enhanced acuity for PROP, though poorly responsive (Lawless, 1980); and b) discrepancies on the amount of PROP tasted by each participant due to potential inconsistencies in its amount throughout the strip (Zhao et al., 2003) and/or by difficulties in adhering to the artificial tasting procedure (Menghi et al., 2023). Nevertheless, the hyperresponsive cluster (CL-2) tended to be populated by fewer PROP NTs (and more MTs as a percentage) than the hyporesponsive cluster (CL-1; Table 2; $p = 0.084$) and to systematically rate (Fig. 3; Fig. 4) all sensations as more intense (although not always statistically significant).

4.3. Habitual dietary intakes might be affected by mutualisms between salivary microbiota, oral responsiveness and psychological traits

We showcased a complex crosstalk between host related non-genetic (microbiota), biological (sensory perception) and psychosocial factors underlying the differences in habitual dietary habits between members of the hypo- and hyperresponsive clusters. Specifically, hyporesponsive individuals (CL-1) harbored a richer and more complex salivary microbiota and reported higher intakes of beneficial nutrients (vegetable proteins, monounsaturated fatty acids, vitamins) compared to the

hyperresponsive group, who was found to habitually consume higher quantities of simple carbohydrates. Also, hyperresponsiveness to warning sensations was parallel to higher craving for sweet foods and levels of agreeableness.

Our findings are broadly consistent with previous reports linking oral bacterial diversity, chemoperception, and psychological traits to dietary habits, albeit never together in a single study. As in the current study, salivary microbial richness and evenness have been associated with higher habitual intake of nutrients from plant-based sources (Hansen et al., 2018) and lower daily sugar consumption (Esberg et al., 2020), whereas higher acuity for bitterness and sourness has recently been shown to hinder the choice of sour- and bitter-eliciting phenol-rich foods (Pagliarini et al., 2021). Further, individuals with high craving for sweet foods were found to be prone to selecting more frequently chocolate bars over apples (Roininen & Tuorila, 1999) and to consume more high-fat sweet snacks (Zandstra et al., 2001).

Conversely, results from agreeableness appear to be less consistent with previous knowledge, as prosocial personalities usually tend to be associated with healthier food choices (see for a review Esposito et al., 2021). Nevertheless, such personality traits also co-occurred with high sweet (Meier et al., 2012) and low bitter (Sagioglou & Greitemeyer, 2016) taste preferences, which are proxies for the observed dietary patterns. Thus, as the role of agreeableness on eating habits remains controversial and inconsistent across studies (Pfeiler & Egloff, 2020), further studies are motivated to conclusively elucidate its influence on diet.

In an attempt to further explain the differences in dietary habits between salivary microbial profiles, potential interplays between oral taxa, inferred bacterial functions, and chemosensory abilities can be deduced. Firstly, we associated hyporesponsiveness to warning sensations and healthier dietary habits with a number of bacterial genera, mostly belonging to the class *Clostridia*. Remarkably, these results are consistent with a recent study on the same cohort (Menghi et al., 2023), in which gut commensal *Clostridia* (families *Lachnospiraceae* and *Ruminococcaceae*) were more abundant in individuals with generalized hypogeusia to oral sensations elicited by the same range of foods used here. Moreover, salivary members of the family *Lachnospiraceae* were previously found to be inversely correlated with sour acuity (Duarte-Coimbra et al., 2023), whereas taxa from the family *Peptostreptococcaceae* (Sandell & Collado, 2018) and 2 ASVs from the genus *Porphyromonas* (class *Bacteroidia*; Yousaf et al., 2022) were found to be enriched in PROP-insensitive individuals, who are typically unresponsive to chemosensory warning signals (Dinnella et al., 2018; Nolden et al., 2020; Piochi et al., 2021). Similar salivary bacterial consortia have also been evidenced with respect to olfactory performances (Valentino et al., 2022), with an unclassified genus of the family *Lachnospiraceae_XIV* and the genus *Porphyromonas* being inversely related to orthonasal olfactory acuity. Thus, in line with the notion that individuals would be similarly responsive across various (taste, olfaction, chemesthesis) sensory modalities (Piochi et al., 2021; Puputti et al., 2018), we suggest that a *Clostridia*-enriched salivary microbiota might relate to lower chemosensory abilities.

This can be further speculated based on both past research and our findings. Indeed, *Clostridia* are known to be producers of free catecholamines (Asano et al., 2012), whose pharmacological reuptake inhibition acutely blunts bitter sensitivity (O'Driscoll. et al., 2006), and this could act as a suppressor of taste function (Huang et al., 2009). Moreover, hyporesponsive (relative to hyperresponsive) individuals (CL-1) showed higher proportions of MetaCyc modules attributable to the biosynthesis of a sour-eliciting compound (acetate), whose increased concentration nearby the taste buds could promote sensory adaptation (Leung & Covasa, 2021). Given that hyporesponsiveness to a taste quality usually exacerbates its intake (e.g., Cattaneo, Riso, et al., 2019; Menghi et al., 2023) and that dietary outcomes associated with salivary *Clostridia* members are consistent with a previous report (Cattaneo, Riso, et al., 2019), we urge further research to elucidate the links between *Clostridia*, sensory perception, and dietary habits.

Secondly, interesting host-microbe interactions that might have influenced the dietary habits of the hyperresponsive group (CL-2) also emerged. We found that this cluster simultaneously housed more cariogenic bacterial genera (*Bifidobacterium*, *Lactobacillus*) and habitually consumed larger amounts of simple carbohydrates than the hyporesponsive group (CL-1). These results overlap with those of Esberg et al. (2020), who noted the same bacteria to be enriched in individuals with high sugar consumption, and reinforce previous evidence suggesting synergisms between oral microbes sharing the same nutritional requirements for survival in the salivary milieu (Marsh & Zaura, 2017).

More interestingly, hyperresponsive individuals (CL-2) also harbored greater amounts of some taxa from the *Actinobacteria* phylum (families *Bifidobacteriaceae* and *Eggerthellaceae*; genera *Alloscardovia* and *Bifidobacterium*). While the salivary members of this phylum have previously been shown to be negatively correlated with taste (especially saltiness) perception (Feng et al., 2018), some authors have observed opposite trends in the tongue *dorsum* microbiota (Cattaneo, Gargari, et al., 2019; Duarte-Coimbra et al., 2023). Moreover, higher proportions of the genus *Actinomyces* were detected in the salivary microbiota of PROP STs compared to NTs (Yousaf et al., 2022), and we recently documented that the abundances of a pro-inflammatory gut taxon of the *Actinobacteria* phylum (genus *Eggerthella*) were associated with hypergeusia to chemosensory stimuli in the same individuals involved here (Menghi et al., 2023). Thus, our results suggest that an enrichment of microbes belonging to this phylum might relate to enhanced orosensory acuity.

Nevertheless, the direction of this association remains largely unclear, as it has been inferred so far from small sample sizes, from oral niches known to house distinct microbial communities (Feng et al., 2018), and from poorly consistent bioinformatics and taste assessment procedures across studies. Thus, to conclusively address this issue, future research should devote additional effort to: a) include larger sample sizes; b) collect microbial samples from multiple oral niches potentially communicating with taste transduction systems (saliva and tongue *dorsum*); c) ensure homogeneous (higher) sequencing depth and downstream (compositionally-aware) data treatments; d) test taste function via real foods to increase the ecological validity of outcomes; and e) use methods capturing suprathreshold intensities, as advocated to best relate to actual food perception (Puputti et al., 2018; Webb et al., 2015).

4.4. Strengths, limitations and conclusions

For the first time, homogeneous patterns of salivary bacterial cohabitation have been linked to systematic variations in orosensory responsiveness to liquid and solid foods, psychological traits, and habitual eating habits. The strengths of the current study revolve around the high external validity of outcomes, which was ensured by a large data collection protocol, a wide range of oral sensations and food products tested, and by a substantial background homogeneity among individuals preventing our conclusions from being strongly biased by underlying confounders.

Nevertheless, the results should also be interpreted in light of some limitations. First, this study provides a limited picture of the potential bacterial networks inhabiting the salivary environment, albeit those found have been proven to be consistent with the literature (Zaura et al., 2017). Second, the homogeneity and relatively small size of our cohort make our findings poorly generalizable to other age groups and/or ethnically diverse populations. Third, while corroborated by acceptable NSTI mean values (0.2 ± 0.4), results from PICRUSt2 should still be taken with caution, as the soundness of functional prediction is limited by its inability to infer taxa-specific pathways (Douglas et al., 2020). However, these data was still pivotal to speculate on potential mechanistic links between taste function, salivary microbiota and eating habits. Fourth, our dietary outcomes may be partly influenced by

psychological biases associated with self-reporting (Thompson & Subar, 2017), although significant overlap with current knowledge was evidenced. Lastly, given the cross-sectional nature of this report, it is not possible to infer a causal relationship underlying our findings. Thus, we can not exclude the possibility that diet itself may be an active contributor to the associations found here. In this sense, future longitudinal intervention studies will prove critical in clarifying this open question.

In conclusion, this work depicts a complex scenario in which microbe-microbe and microbe-taste interactions play in tandem with host psychology to shape dietary behavior, and derives putative underlying mechanisms that require empirical confirmation. Specifically, we observed that a *Clostridia*-enriched microbiota corresponded to lower responsiveness to warning oral sensations and higher habitual intake of beneficial nutrients, and we speculated the ability of such taxa to produce free catecholamines (Asano et al., 2012) and/or an increased microbial biosynthesis of acetate to be foundational to this link. Conversely, a salivary microbiota harboring more cariogenic bacteria and members of the *Actinobacteria* phylum led to opposite sensory- and diet-related outcomes. Taken together, given that peculiar co-occurring salivary bacterial networks were associated with specific patterns of orosensory acuity and dietary habits, the current study also motivates future investigations to test the hypothesis of the existence of a "core" taste-related salivary microbiota.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.113072.

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