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REVIEW

Candidate Phyla Radiation, an Underappreciated Division of the Human Microbiome, and Its Impact on Health and Disease

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SUMMARY Candidate phyla radiation (CPR) is an emerging division of the bacterial domain within the human microbiota. Still poorly known, these microorganisms were first described in the environment in 1981 as "ultramicrobacteria" with a cell volume under 0.1 μm^3 and were first associated with the human oral microbiota in 2007. The evolution of technology has been paramount for the study of CPR within the human microbiota. In fact, since these ultramicrobacteria have yet to be axenically cultured despite ongoing efforts, progress in imaging technology has allowed their observation and morphological description. Although their genomic abilities and taxonomy are still being studied, great strides have been made regarding their taxonomic classification, as well as their lifestyle. In addition, advancements in next-generation sequencing and the continued development of bioinformatics tools have allowed their detection as commensals in different human habitats, including the oral cavity and gastrointestinal and genital tracts, thus highlighting CPR as a nonnegligible part of the human microbiota with an impact on physiological settings. Conversely, several pathologies present dysbiosis affecting CPR levels, including inflammatory, mucosal, and infectious diseases. In this exhaustive review of the literature, we provide a historical perspective on the study of CPR, an overview of the methods available to study these organisms and a description of their taxonomy and lifestyle. In addition, their distribution in the human microbiome is presented in both homeostatic and dysbiotic settings. Future efforts should focus on developing cocultures and, if possible, axenic cultures to obtain isolates and therefore genomes that would provide a better understanding of these ultramicrobacteria, the importance of which in the human microbiome is undeniable.

KEYWORDS candidate phyla radiation, dysbiosis, genome analysis, human microbiome, imaging, taxonomy

INTRODUCTION

The last 15 to 20 years have revealed the human microbiome as a determinant of homeostasis and health in humans. The evolution of molecular methods has revealed the colonization of several human anatomical sites, including the gastrointestinal tract, oral cavity, urogenital tract, respiratory tract, and skin, with a symbiotic microbial ecosystem. This ecosystem encompasses all domains of life, namely, eukaryotes represented by fungi and parasites, bacteria, archaea, and viruses. The composition and diversity of the human microbiome has a well-established impact on human health, and its dysbiosis has been linked to numerous pathologies as an etiological agent or as an outcome of the pathology.

To date, numerous bacterial taxa from the human microbiome remain uncultivated. However, the rise of next-generation sequencing (NGS) has allowed unprecedented knowledge of the human microbiome, particularly regarding uncultivated and/or fastidious taxa. Major bacterial phyla include *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia*. However, recent studies have revealed that the Candidate Phyla Radiation (CPR) superphylum represents an unexpectedly large proportion of the human microbiome. Specifically, the phylum "*Candidatus* Saccharibacteria" has been described as a ubiquitous member of the oral microbiome and is also reported in smaller amounts in the gut and vaginal microbiome. Other CPR phyla, namely, "*Candidatus* Absconditabacteria," "*Candidatus* Parcubacteria," and "*Candidatus* Gracilibacteria," have also been reported in the

human microbiome. Although only a small proportion of human microbiome studies available on PubMed have also investigated CPR in the human microbiome (0.3%), recent studies are increasingly reporting the detection of CPR in healthy as well as dysbiotic conditions. However, these studies have mostly investigated the "*Candidatus* Saccharibacteria" phylum and seldom the "*Candidatus* Absconditabacteria and Parcubacteria" phyla, although the CPR superphylum is highly diverse taxonomically. In this review, we aim to define the CPR and the current state of knowledge regarding their taxonomy, lifestyle, and detection methods using four databases (PubMed, Google Scholar, Google, and Web of Science) with designed queries. In addition, we present their distribution within the human microbiome, as well as the pathologies and microbiota-directed therapies affecting their prevalence and relative abundance.

CPR: HISTORICAL PERSPECTIVE

From Unassigned Sequences to "Ultramicrobacteria"

Ultramicrobacteria were first reported by Torrella and Morita, who used this term for microorganisms with a volume under 0.1 μm^3 (1). This term includes "ultramicrocells," which refers to cells with a reduced size from their physiological state, as opposed to "ultramicrobacteria," referring to cells with a small size in their physiological state, including CPR (2). The first sequence of the TM7 16S ribosomal subunit was published 15 years later by Rheims et al. (3) in a study aiming to describe the microbial diversity of a German peat bog sample. The term TM7 was in fact coined from that study, where it referred to the Torf, mittlere Schicht clone 7, which translates to peat, middle layer clone 7. The existence of these sequences was then confirmed in a large-scale phylogenetic study based on the 16S rRNA gene (rRNA), which showed the existence of 36 divisions or putative divisions, of which the TM7 division represented 20 to 25% (4). This little-known division at the time accounted for a nonnegligible part of the microbial dark matter (5). Other candidate phyla divisions, including OD1 (newly renamed "*Candidatus* Parcubacteria") and OP11 (newly renamed "*Candidatus* Microgenomates"), were subsequently detected using PCR techniques and by studying clonal libraries of environmental samples and analyzing rRNA gene sequences (6).

The evolution of technology has allowed the reconstruction of partial TM7 genomes from human oral samples, notably via the use of single-cell sequencing combined with whole-genome amplification (7). Since then, the number of genome drafts of unknown organisms from different microbiomes has been steadily increasing (8, 9). The first complete genomes of TM7 were obtained in 2007 when the name "*Candidatus* Saccharibacteria" was proposed for the first time to define the TM7 phylum (10).

The initial studies reporting the presence of CPR were molecular-method-based descriptive studies. The first culture attempts of these mysterious microorganisms were reported later through the soil-slurry system, which demonstrated the possibility of cocultivation between TM7 and bacteria (11, 12). Subsequent studies started tackling the taxonomic nomenclature and classification of CPR with the suggestion of "*Candidatus* Patescibacteria" as a superphylum, including the candidate phyla OD1, OP11, and "*Candidatus* Gracilibacteria" (9, 13). The year 2015 was one of significant progress for research on candidate phyla with the notable coining of the term Candidate Phyla Radiation (CPR), as well as their description as a monophyletic group of different candidate phyla, including "*Candidatus* Parcubacteria" (OD1), "*Candidatus* Microgenomates" (OP11), WWE3, "*Candidatus* Berkelbacteria" (ACD58), "*Candidatus* Saccharibacteria" (TM7), WS6, "*Candidatus* Peregrinibacteria" (PER), Kazan, CPR1, CPR2, CPR3, SR1, "*Candidatus* Gracilibacteria" (BD1-5), and SM2F11 (14). Of note, only seven phyla have been reported in the human microbiome to date, namely, "*Candidatus* Saccharibacteria," "*Candidatus* Absconditabacteria," "*Candidatus* Parcubacteria," "*Candidatus* Gracilibacteria," "*Candidatus* Microgenomates," OP10, and WS3 (Table 1). The first coculture of human TM7 was also performed in 2015 with a bacterial host, *Schaalia odontolytica* strain XH001 (formerly named *Actinomyces odontolyticus*) (15). Furthermore, microscopic detection of ultrasmall cells

TABLE 1 Heatmap representative of the CPR phyla found in different human habitats[a]

	"*Candidatus* Saccharibacteria"	"*Candidatus* Absconditabacteria"	"*Candidatus* Parcubacteria"	"*Candidatus* Gracilibacteria"	"*Candidatus* Microgenomates"	OP10	WS3
Oral microbiota	82	44	1	9	1	1	0
Ancient oral microbiota	4	2	0	0	0	0	0
Respiratory microbiota	3	2	0	0	0	1	0
Digestive microbiota	27	9	7	0	0	0	1
Microbiota of female reproductive system	8	1	0	1	0	0	0
Skin microbiota	6	1	0	0	0	2	0
Blood microbiota	0	0	2	0	0	0	0
Microbiota of male reproductive system	0	0	1	0	0	0	0
Urinary microbiota	0	0	1	0	0	0	0
Eyes microbiota	1	0	0	0	0	1	0

[a]This heatmap was made according to the number of studies describing the presence of CPR in each habitat.

subsequently to restricted pore filtration (0.2 μm) was matched and assigned after sequencing to previously described CPR sequences (16).

The development of new culture techniques based on size-based selection, aminoglycoside resistance and cell sorting represented another major turning point in the understanding of CPR (15, 17, 18). Indeed, this method allowed the cultivation of CPR from the human microbiome and led to the obtention of new complete human CPR genomes of the TM7 and SR1 phyla. In addition, new hosts for these fastidious microorganisms were identified (17). This timeline is represented in Fig. 1.

Molecular Classification and Taxonomy

Molecular characteristics: what do genomic data reveal? CPR have highly reduced genomes presenting a lack of known metabolic pathways (13). Analysis of their coding genes revealed a huge deficiency in the membrane and amino acid biosynthetic pathways, a lack of electron transport chains, ATP synthase, tricarboxylic acid cycles, carbon cycles, and an absence of essential ribosomal subunits for bacteria (9, 14–16, 19–22). Conversely, CPR possess glycoside hydrolases and are thus able to hydrolyze complex sugars. In addition, the presence of nitrite reductase illustrates a potential role of these microorganisms in denitrification or microbial nitrate reduction (23). Regarding their ribosomes, all known CPR lack rpL30, which is usually found in symbiotic bacteria. In addition, the WS6, WWE3, TM7 and "*Candidatus* Microgenomates" phyla lack rpL9, which is universal in other bacteria (14). This finding suggests that the evolution of ribosomes in CPR cells differs from that of bacteria (14). This hypothesis is also supported by the presence of insertion sequences in the ribosomal genes of CPR cells. Different studies have also shown that they have intron sequences with a strong self-splicing capacity within their 16S rRNA, 23S rRNA, and tRNA genes (13, 14, 24, 25).

Taxonomy: what does the rhizomal reclassification of CPR tell us? From a taxonomic standpoint, CPR represent >26% of the bacterial domain (26). This superphylum consists of at least 73 highly divergent phyla (18, 27). The increased knowledge recently garnered about this axenically uncultivated division is largely due to technological progress in sequencing methods, as well as the improvement in analytical

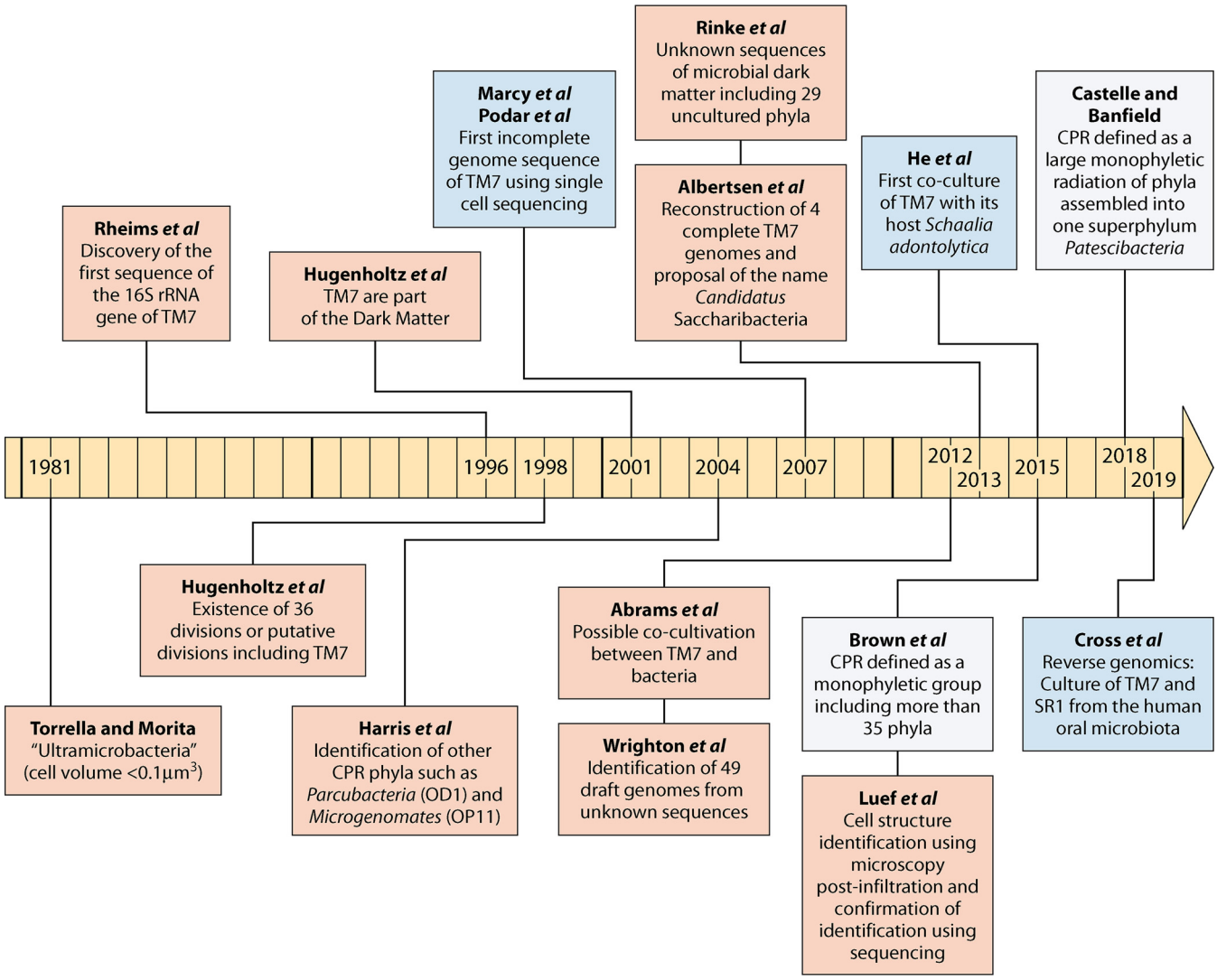

FIG 1 Historical timeline of the discovery of Candidate Phyla Radiation (CPR). The orange boxes represent studies on environmental samples, the blue boxes represent studies on human samples, and the white boxes represent work using genomic sequences from different origins. The references cited are as follows: Torrella and Morita (1), Rheims et al. (3), Hugenholtz et al. (4), Hugenholtz et al. (5), Harris et al. (6), Marcy et al. (7), Podar et al. (174), Abrams et al. (12), Wrighton et al. (199), Rinke et al. (9), Albertsen et al. (10), He et al. (15), Brown et al. (14), Luef et al. (16), Castelle and Banfield (70), and Cross et al. (17).

methods and, more specifically, advances in bioinformatics. However, the classification of this group is still considered a challenge for evolutionists and microbiologists (28).

The first inclusion of CPR in a phylogenetic tree occurred in 1996 and was based on 16S rRNA gene sequences (3). The initial classification of CPR as a new candidate phylum was then achieved in 1998 through an extensive phylogenetic study also based on the 16S rRNA gene (4). The topology of this tree shows the emergence of a new extension representing CPR next to bacteria and separated from archaea and eukaryotes. A similar classification was also obtained using other conserved coding genes, including ribonucleotide reductase, elongation factor 1, topoisomerase IIA, thymidylate synthase, DNA-dependent RNA polymerase subunit II, and DNA polymerase (14, 28, 29). The 16S-based phylogeny also allowed efficient classification of CPR putative species within a single phylum. The "*Candidatus* Saccharibacteria" phylum, the most studied phylum within the CPR division, encompasses the majority of available human genomes, all sequenced from oral samples (26). Moreover, taxonomic analysis revealed the existence of at least 6 different groups or clades within the "*Candidatus* Saccharibacteria" phylum (G1 to G6). To date, all available complete "*Candidatus* Saccharibacteria" genomes are classified within the G1 and G6 clades (26, 30).

The recent evolution in taxonomic concepts has not only improved the classification of CPR but also that of bacteria in general. For instance, 94,759 bacterial genomes (including CPR genomes) were recently reclassified based on a concatenation of 120 unique ubiquitous proteins (one copy/genome). This phylogeny resulted in a tree consisting of 6 major monophyletic groups, one of which represents the CPR (the superphylum "*Candidatus* Patescibacteria") as a separate phylum from the other bacterial phyla (31). Recent retrospective studies have shown that the use of 16S rRNA is not adequate for an accurate and sensitive classification of CPR due to their high divergence. This divergence is probably due to the rapid evolution of CPR linked to their obligatory symbiotic (or parasitic) lifestyle. The universal primers currently used to amplify bacterial 16S rRNA are not sufficiently sensitive for the detection of CPR (14, 32). In addition, in contrast to the bacterial 16S rRNA gene, some CPR 16S rRNA sequences present introns or insertion sequences (13, 32). These variations led to the use of other sequence types to classify CPR, such as ribosomal proteins (31, 32). The advantage of using ribosomal proteins for such analysis is that they are located in a small genomic region. This localization reduces the error rate, as well as the disruption of the topology of a phylogenetic tree (29, 32, 33). Furthermore, this tool can be applied to organisms that do not possess 16S rRNA gene sequences or for which SSU rRNA is unavailable. These proteins also present the advantage of being very specific, since they exhibit differences in their composition between life domains, probably due to the specificity in the lifestyle of each domain (33, 34). The alignment, concatenation and phylogenetic analysis of this set of proteins provides a much deeper representation of the divergence and evolutionary history of organisms, which is more suitable for the classification of CPR due to their high divergence (32).

Classification and evolutionary studies on CPR are ongoing. Therefore, the selection of appropriate sequences for that purpose is crucial. Following comparative analyses of the superfamily repertoire of protein domains of CPR and bacteria, Meheust et al. (35) showed the existence of 106 protein families that are abundant in the majority of CPR but rare in bacteria. Bokhari et al. (36) showed that all CPR have a conserved unique FSF (superfamily fold protein) core composed of four protein domains, all of which are involved in cell communication. However, these researchers also highlighted the absence of a protein domain unique to the CPR. Studies using phylogenomic trees have shown that CPR and bacteria are two paraphyletic groups and not two distinct divisions. In fact, the topology of phylogenomic trees, based on the presence/absence of protein families of CPR superphyla alongside bacteria with reduced genomes (such as intracellular bacteria), has revealed that CPR represent a deep branching of the bacterial node. In fact, according to recent analyses (29, 35, 36), CPR are classified within the bacterial kingdom, with which they share a high evolutionary relationship. CPR and bacteria with reduced genomes share the common genomic characteristic of having a low frequency in protein families (35, 36). Therefore, CPR are considered a bacterial superphylum. This idea was also supported by the finding that proteins known to be present in archaea and eukaryotes and absent in bacteria are also absent in CPR, such as the ribosomal protein L19e. Moreover, CPR only added 1.22% to the bacterial protein repertoire, which was not sufficiently significant to consider CPR as a new domain (36).

Interestingly, distinct metabolism-based analyses have shown that CPR are completely distinct from other bacteria. This separation can be explained by their reduced or absent biosynthetic and metabolic activity (35). In fact, numerous previous studies have suggested that CPR have a particular evolutionary pathway. A recent analysis of the total genomic content of representatives of all life domains and divisions existing in the 21st century has confirmed that CPR have particular genomes and evolutionary histories, since CPR genomes have a unique rhizome mosaic profile consisting of sequences from heterogenous origins (28, 37), in contrast to the classical mosaic of bacteria, which shows a homogeneous profile (28). This idea was supported by the possibility of sequence transfer between organisms. Rhizome-based classification, which is based on whole-genome sequence analysis, has shown that the CPR is a

genetically independent division (28). They represent a 5th TRUC (Thing Resisting Uncompleted Classification) in the rhizome of life next to the three existing domains of life and the giant viruses that make up a fourth TRUC (38).

Evolutionary history of human CPR superphyla through rhizomal representation. Evolution can take place through sequence fusion, exchange, degradation, or *de novo* production of new genes known as ORFans. As organisms can evolve through an early loss of metabolic function, two possible hypotheses can be applied to the evolution of CPR. (i) Bacteria and CPR evolved from a common protogenote. Subsequently, CPR followed a particular path characterized by the reduction of their genome. (ii) CPR evolved from bacteria and underwent tremendous genomic modifications as a result of their unique lifestyle (35). Here, we inferred the evolutionary history of a representative genome from each CPR superphylum through rhizomal analysis. Rhizomes represent the domain origin of each coding gene (28, 39, 40) and are particularly adapted for evolutionary representation, since they account for the possibility of sequence transfer and show the degree of mosaicism of the studied genome (37, 38). For this purpose, we selected from NCBI a representative genome from each phylum already described in humans ("*Candidatus* Saccharibacteria," "*Candidatus* Absconditabacteria," and "*Candidatus* Gracilibacteria"). The genome with the lowest number of scaffolds from each nonhuman phylum was also selected.

A rhizomal representation of each selected genome was performed exactly as previously described (28). These analyses highlighted that CPR present a particular mosaic profile, which includes a mixture of sequences from bacteria, CPR, and ORFans, as well as sequences of archaeal and eukaryotic origin. Interestingly, the CPR mosaic is divided into three different profiles (Fig. 2): the first profile with a predominance of bacterial sequences compared to the CPR (phyla "*Candidatus* Absconditabacteria," "*Candidatus* Gracilibacteria," and "*Candidatus* Saccharibacteria"); the second profile with a predominance of specific CPR sequences (phyla "*Candidatus* Doudnabacteria," "*Candidatus* Dojkabacteria," WWE3, and Kazan); and the third profile with an equivalent percentage of bacterial and CPR genes (phyla "*Candidatus* Parcubacteria," "*Candidatus* Wirthbacteria," "*Candidatus* Peregrinibacteria," "*Candidatus* Microgenomates," and "*Candidatus* Berkelbacteria"). Here, we suggest that the scenario of this transfer is abundant between these phyla and bacteria due to their environment. CPR have been described as obligate exosymbiotic organisms, generally with anaerobic bacteria, thus explaining the large percentage of genes of bacterial origin in some phyla. Microorganisms that share the same ecological niche have a much higher possibility of transfer (41). CPR can be exposed to bacterial stresses caused by different mechanisms in humans, and it is known that stress results in genomic rearrangement, including sequence transfer.

In addition, we noticed the presence of sequences of archaeal (0.24 to 5.67% for "*Candidatus* Wirthbacteria") and eukaryotic (0 to 0.44%) origin. Similarly, mosaicism studies of archaeal genomes highlight a nonnegligible percentage of sequences with a CPR origin (42). Moreover, it has been reported that CPR hosts present sequences from different origins in their genomes (28, 37). We can also suggest an alternative hypothesis of another, thus far undescribed, physical contact between CPR and nonbacterial microorganisms. In addition, we detected a gene of viral origin in "*Candidatus* Gracilibacteria" and "*Candidatus Absconditabacteria*" (SR1), suggesting that CPR might be in contact with eukaryotes that harbor viruses (e.g., amoebae), as recently described using next-generation sequencing (42).

METHODS FOR STUDYING CPR IN THE HUMAN MICROBIOME

Detection of CPR Sequences Using Molecular Methods

Since CPR have not been axenically cultured to date (17), most detection methods are based on molecular techniques. Because "*Candidatus* Saccharibacteria" is the most studied phylum among all CPR, different studies have designed standard/real-time PCR systems to screen samples of interest. These primer/probe systems have been designed based on the 16S rRNA gene (43) or 23S rRNA gene (37). PCR is an efficient tool to not

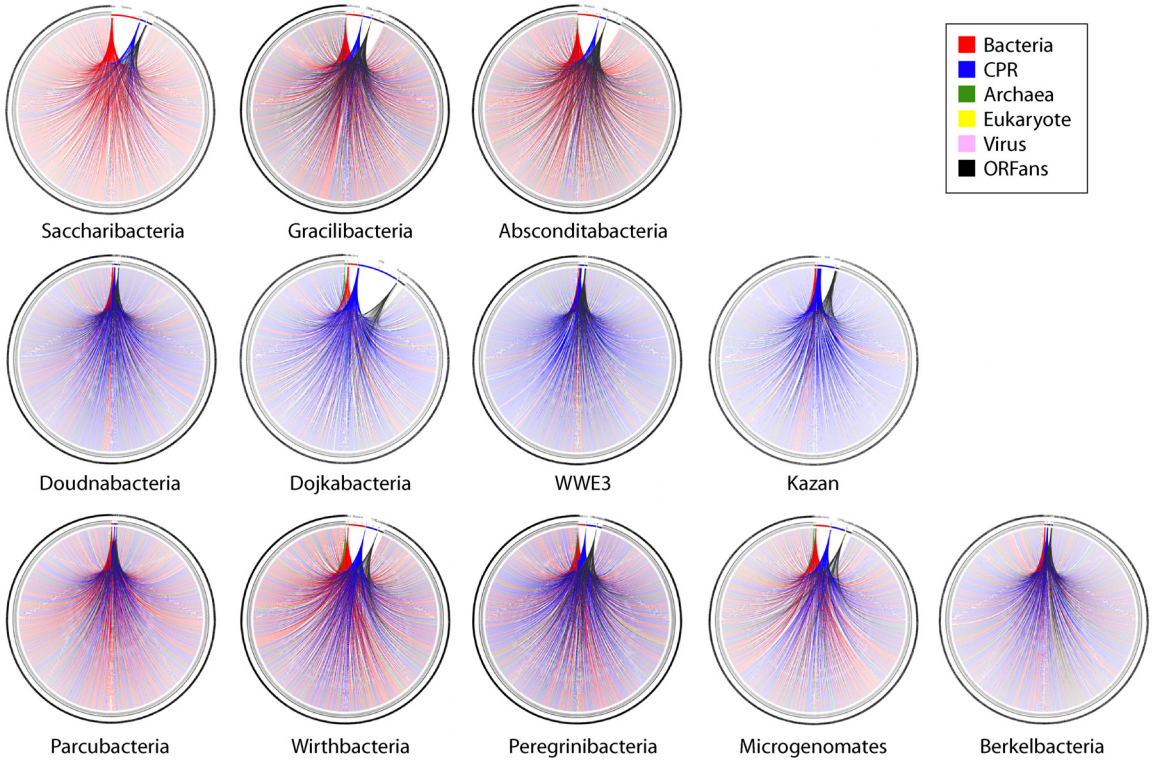

FIG 2 Rhizomes of genomes illustrating the mosaicism of each Candidate Phyla Radiation (CPR) phylum. Each gene is represented by a curve colored according to the origin: bacterial origin in dark red, CPR origin in pink, Eukarya origin in yellow, virus origin in orange, archaeal origin in dark blue and ORFans in gray. The figures were constructed using the Circos tool. The following genomes were included: *Candidatus* Berkelbacteria GCA_016432625.1, *Candidatus* Dojkabacteria HGW GCA_002840365.1, candidate division WWE3 bacterium RIFOXYA1 GCA_001773015.1, *Candidatus* Peregrinibacteria GCA_016699145.1, *Candidatus* Nanosynbacter lyticus GCA_000803625.1, candidate division Kazan bacterium GCA_001029795.1, *Candidatus* Doudnabacteria RIFCSPLOWO2 GCA_001780055.1, *Candidatus* Wirthbacteria CG2_30_54_11 GCA_001873755.1, *Candidatus* Gracilibacteria GN02-873 GCA_003260345.1, *Candidatus* Parcubacteria Gr01-1014_8 GCA_007376385.1, *Candidatus* Chazhemtobacterium aquaticus GCA_009936135.1 and candidate division SR1 bacterium GCA_015259585.1.

only screen samples but also to select positive samples prior to NGS. Interestingly, quantification is mostly achieved using standard PCR by diluting the DNA and observing the contrast of the obtained bands (44). In fact, only one TaqMan system specific for "*Candidatus* Saccharibacteria" has been described (37), while standard PCR systems can be used with SYBR green technology (43). Moreover, some studies have quantified "*Candidatus* Saccharibacteria" using 16S amplicon sequencing (Table 2).

Presently, new CPR taxa are defined based on genome similarity with existing taxa. Shotgun sequencing of samples of interest (whether environmental or clinical) is carried out prior to the recovery of uncultivated organism genomes through mapping on a reference genome (17) or *de novo* assembly (45, 46). These metagenome-assembled genomes (MAGs) are recovered from metagenomic data sets, whereas single-cell amplified genomes (SAGs) (15, 17) can also be recovered using purification methods prior to sequencing. Target organisms can be sorted through filtration and/or flow cytometry. However, this type of assembly increases the error rate. Phylogenetic and taxonomic analysis of MAGs could change their classification. Moreover, according to clustering with other described genomes and/or the percentage of the core genome, the classification may be altered (47). For example, as shown in a recent study, the authors taxonomically classified a "*Candidatus* Gracilibacteria" MAG as an SR1-Absconditabacteria species (47), thus confirming the importance of culture-based methods to recover accurate genomes. Here, we show the importance and necessity of screening additional clinical and environmental samples and sequencing as many CPR as possible to increase their genomic database to facilitate their intraphylum classification.

Recently, cocultivation of CPR has shown significant effectiveness in addressing this issue. Many coculture methods have managed to purify and coisolate many CPR members,

TABLE 2 Summary data recapitulating the relative abundances and frequencies of CPR phyla within human niches

	RA (%) or F (%)[a]														
	"*Candidatus* Saccharibacteria TM7"		"*Candidatus* Gracilibacteria GN02"		"*Candidatus* Absconditabacteria SR1"		"*Candidatus* Parcubacteria OD1"		OP10		"*Candidatus* Microgenomates OP11"		WS3		
Human niche	RA	F	RA	F	RA	F	RA	F	RA	F	RA	F	RA	F	Reference(s)
Oral cavity	0.47–3	85–100	0.03–0.2	0.54	0.02–0.4	26–100	N0	N0	N0	N0	N0	45	NA	NA	26, 116, 119, 120, 124, 125, 134–136, 141, 155, 196, 197
Respiratory tract	0.4–2.4	NA	NA	NA	Tr	NA	Tr	NA	NA	NA	NA	NA	NA	NA	26, 136, 193
Digestive tract	0.18–0.43	0.08–15	NA	NA	0–1.3	0–7.6	N0	Tr	NA	NA	NA	NA	Tr	Tr	116, 134, 144, 179, 192
Female genital tract	0–5	17	Tr	NA	Tr	NA	NA	NA	NA	NA	NA	NA	NA	NA	26, 148, 149, 198
Skin	Tr	0.33	NA	NA	Tr	NA	NA	NA	Tr		NA	NA	NA	NA	150
Blood	Tr	NA	NA	NA	NA	NA	Tr	NA	NA	NA	NA	NA	NA	NA	152, 165
Male genital tract	NA	NA	NA	NA	NA	NA	0–0.1	NA	NA	NA	NA	NA	NA	NA	136
Urine	NA	NA	NA	NA	NA	NA	0–0.2	NA	NA	NA	NA	NA	NA	NA	136
Eyes	0–0.9	NA	NA	NA	NA	NA	NA	NA	0–0.009	NA	NA	NA	NA	NA	136

[a]Relative abundance (RA) and frequency (F) values are all presented as percentages. Tr, trace; NA, not applicable; N0, near zero.

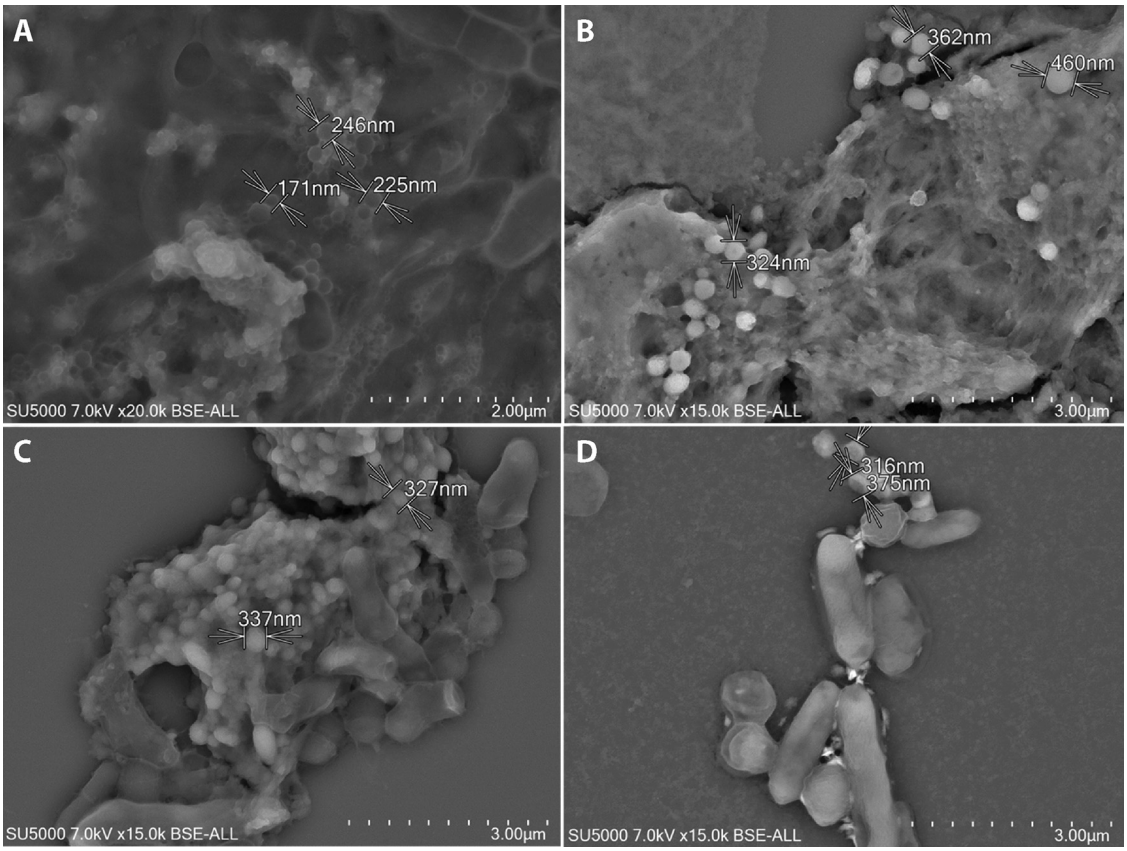

FIG 3 Microscopic observations of images consistent with the structure of CPR in human saliva (A, B), dental plaque (C), and human digestive tract (D). Two saliva and one dental plaque samples, as well as two stool samples, were fixed in a 2.5% glutaraldehyde solution overnight at 4°C. Stool samples were then diluted to 1:10 in 2.5% glutaraldehyde. Fixed oral samples and diluted stool samples were stained using 1% phosphotungstic acid for 3 min and were cyto-centrifuged onto glass slides and sputtered with a 5-μm-thick platinum layer (ion sputter MC1000, Hitachi, Japan). Micrographs were then acquired on an SU5000 SEM (Hitachi, Japan) using a backscatter electron detector at 7 kV in high vacuum mode. Micrographs were recorded at magnifications ranging from $\times$3,000 to $\times$35,000.

thus broadening the genomic data available for CPR and improving our knowledge about its physiology and physiopathology (15, 17, 18, 37, 44, 48, 52).

Morphology and Imaging of CPR

Study of CPR using TEM (i.e., CryoTEM and microscomics). CPR are routinely characterized by their small size (Fig. 3). In fact, their initial detection (specifically that of the candidate phyla OD1 and OP11) occurred in environmental ecosystems after 0.2- and 0.1-μm filtration prior to molecular identification (49). Electron microscopy is therefore particularly suited for their detection. A microscomics study, in which the goal was to characterize the microbial composition of the fecal microbiota using transmission electron microscopy (TEM), detected CPR-like structures in human stool samples for the first time (50). In fact, the microscomics method consisted of the creation of a structural repertoire of all cell-like objects in a biological sample using TEM. Using TEM and similar approaches, such as scanning electron microscopy (SEM) and cryoelectron tomographic imaging (CryoTEM), CPR from the candidate phyla WWE3, OP11, and OD1 have been described in the environment as small cells with sizes ranging from 0.1 μm (16) to 0.7 μm (10). Most coculture studies from human samples have shown that CPR and, in particular, TM7 are coccus-shaped (15, 44, 51), with a size between 0.2 and 0.3 μm (Fig. 3). Other shapes have been described within the candidate phylum Saccharibacteria as a part of culture-based studies. A study by Murugkar et al. described a shape dependent on the size of the cells, with coccus-shaped cells $<$0.2 μm and coccobacilli with a size from 0.1 to 0.2 μm (44). Although they are most often described as cocci or

TABLE 3 FISH probes used for TM7 detection

Probe name	Targeted gene	Reference
TM7-567	16S rRNA gene	17
TM7-580	16S rRNA gene	58
TM7-567	16S rRNA gene	18
SYTO 9	NA[a]	NA
TM7-567	16S rRNA gene	15
EUB 338	NA	NA

[a]NA, not applicable.

coccobacilli (44), the authors have described them as filaments (5, 48) with rounded poles (48). However, it is noteworthy that CPR in biological samples cannot be distinguished from other small structures with a similar size, such as viruses or vesicles, using SEM. Complementary methods are thus necessary to confirm the SEM observations.

Using electron microscopy, TM7 isolates have been demonstrated to modify their shape in response to nutrient deprivation and oxygen input in their environment, shifting from a round (shell) to a filamentous shape (51). This shift was also observed for the host of TM7x, *S. odontolytica* XH001, which, in response to infection with TM7x, has a response similar to that of exposure to oxygen. Cells bud into a "bowling pin" shape prior to elongation and swelling into hooded cells. The bulging observed at the cell surface using SEM represents the TM7x cell, which appears as a thickening of the cell surface (48). TM7x cells have a cell wall reminiscent of that of Gram-stain positive bacteria, which seemingly contain dark irregular surface deposits that could be inorganic deposits or storage granules in their cytoplasm (48).

Study of CPR using FISH. Although CPR are small cells, they can be observed using optical microscopy, specifically fluorescence *in situ* hybridization (FISH). Using FISH, Marcy et al. reported that TM7 represent 0.7 to 1.9% of the subgingival microbiota (7). Two specific probes targeting the 16S rRNA gene have been designed for "*Candidatus* Saccharibacteria," namely, TM7-567 and TM7-580 (Table 3). Using FISH, TM7 have been described in the oral cavity as filamentous (48, 55, 56), with lengths ranging from 4 to 75 μm (56, 57). Interestingly, a study has described filamentous cells with a length of 30 μm (56). Conversely, isolates of TM7x and BB001 seem coccus-like when associated with a bacterial host (18), whereas those of AC001, PM004 and TM7_905 can either be cocci or bacilli (18, 57). However, it should be noted that several of the TM7 primers/probes have been reported to match oral and vaginal bacteria (58).

Study of CPR using optical microscopy. Although CPR cannot be observed using optical microscopy without staining due to their small size, TM7-infected *S. odontolytica* XH001 colonies can be visualized by $\times$10 or $\times$20 magnification (53). In fact, infected colonies presented an irregular shape with rough edges and loss of the convex shape of uninfected colonies, which had a circular shape, potentially allowing quantification of host-attached TM7x. This irregular colony shape was also observed with TM7-infected *Arachnia propionica* colonies (59).

Fastidious Cultivation of "*Candidatus* Saccharibacteria"

To date, microbiologists have been unable to isolate "*Candidatus* Saccharibacteria" in axenic conditions. Most culture-dependent studies report an enrichment of "*Candidatus* Saccharibacteria" in environmental samples prior to identification of the microbial flora of the samples using molecular methods (60, 61). However, researchers have successfully achieved the coculture of "*Candidatus* Saccharibacteria" with its bacterial host in environmental (62), as well as human settings (18, 30, 44, 63). The culture of human CPR has only been performed from oral samples in coculture with a bacterial host (Fig. 4). For instance, the most notable isolated strain, "*Candidatus* Saccharibacteria TM7x" strain HMT952, was isolated in coculture with *S. odontolytica* subsp. *actinosynbacter* strain XH001 (Fig. 4). All cultured CPR strains and their hosts are shown in Fig. 4.

Aminoglycoside resistance applied to culture. Due to the resistance to aminoglycosides exhibited by CPR, a culture protocol was attempted based on this characteristic

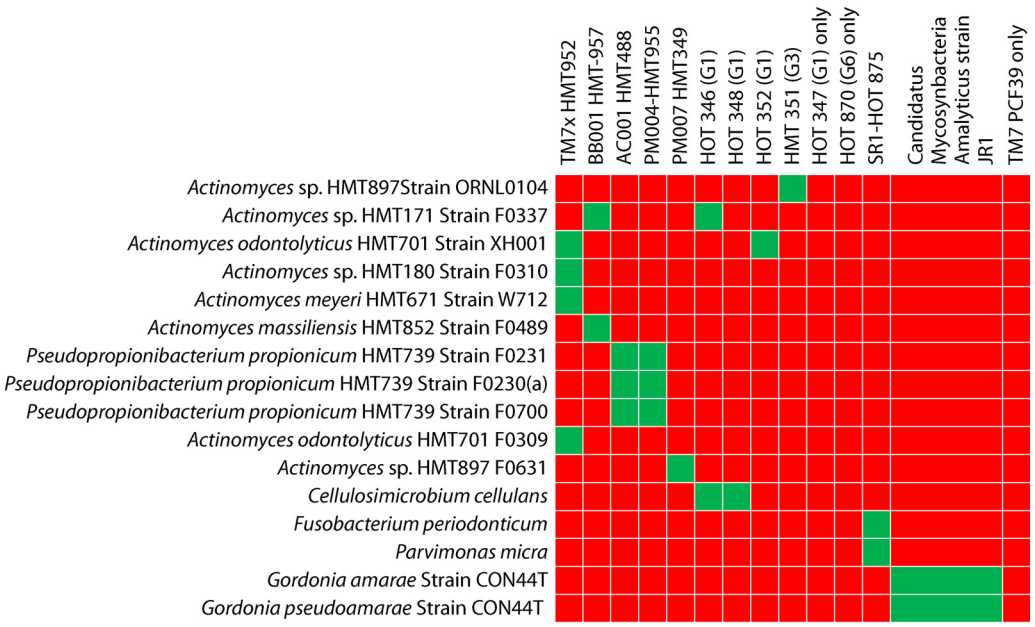

FIG 4 Heatmap representative of the cultivated CPR strains and their respective bacterial hosts. Red: failed coculture attempts, green: successful coculture.

using two aminoglycosides: streptomycin (15) and kanamycin (48) (Fig. 5). To achieve this goal, He et al. used increasing concentrations of streptomycin ranging from 100 to 500 μg/mL in SHI medium, a medium formulated to replicate the physicochemical characteristics of saliva. One milliliter of saliva was inoculated in liquid SHI medium with 100 μg/mL streptomycin at 37°C anaerobically after low-speed centrifugation to remove eukaryotic cells. This initial culture was subcultured at a 10-fold dilution every 24 h in SHI medium with increasing concentrations of streptomycin to reach 500 μg/mL. Although the authors were able to obtain colonies of TM7x in coculture with *S. odontolytica* XH001 on agar plates consisting of SHI agar supplemented with 300 μg/mL streptomycin at 37°C under anaerobic conditions, they did not succeed in the axenic culture of TM7x after centrifugation and resuspension in a small volume of SHI medium. In fact, various physicochemical treatments, including repeated passage of the culture through a 28-gauge needle, filtration of the culture through a 0.22-μm-pore-size filter, and heat treatment, did not produce TM7x colonies with or without nutrient addition.

Kanamycin was also successfully used for TM7 culture from dental plaque (48). The principle of this method was to reduce selection pressure by inhibiting the growth of Gram-stain negative bacteria. Dental plaque was seeded in Fastidious Anaerobe Broth (FAB) for 48 h in anaerobic conditions prior to subculture in FAB supplemented with 100 μg/mL kanamycin for 7 days under similar conditions. The subculture was inoculated on Fastidious Anaerobe Agar (FAA), leading to the growth of distinct TM7 colonies after 7 days of incubation under anaerobic conditions.

Size-based selection and coculture. Coculture of CPR has also been achieved by exploiting the main phenotypic characteristics of their small size. "*Candidatus* Saccharibacteria" microcolonies have been isolated on nutrient-rich agar alongside other colonies from environmental samples (11). Moreover, filtration of oral samples was used to obtain host-free CPR cells prior to infection of selected hosts (18, 30, 44, 64) carried out in a coculture with *S. odontolytica* using a small volume of dental plaque and saliva, which were filtered at 0.2 and 0.45 μm, respectively, prior to ultracentrifugation to obtain purified CPR (Fig. 5). The pellet was subsequently used to infect *S. odontolytica* monocultures. Successful infection of the host was confirmed using microscopy and molecular biology. Seven new species of "*Candidatus* Saccharibacteria" were discovered as a result in coculture with *S. odontolytica* and/or with *Pseudopropionibacterium propionicum*.

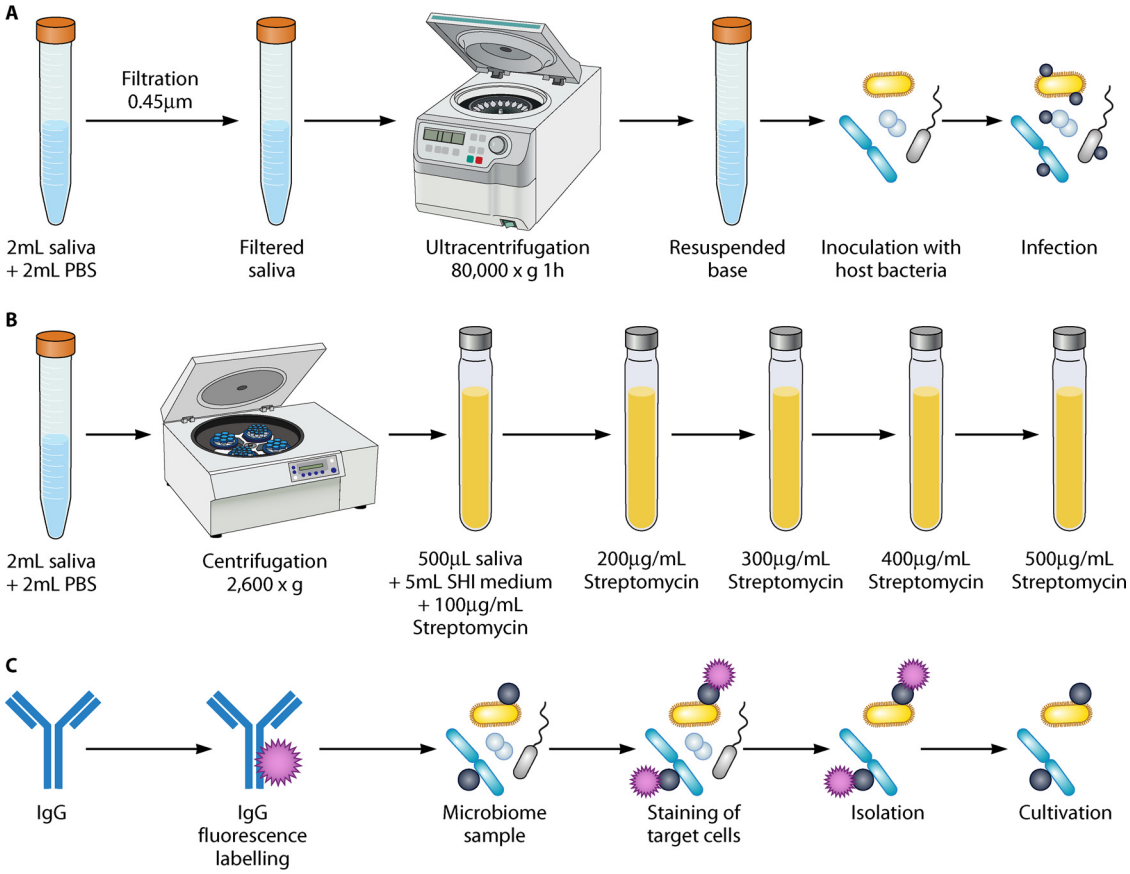

FIG 5 Successful culture attempts for isolating CPR from the oral microbiota. (A) Purification of CPR using active filtration at 0.2 μm and ultracentrifugation prior to host infection (18). (B) Coculture based on selection through aminoglycoside resistance (15). (C) Reverse genomics (17).

A similar procedure was successfully achieved by Murugkar et al. in 2020 (44) using 0.2-μm filtration of dental plaque samples to purify CPR prior to infection of different host bacteria in different atmospheres. Subculture, monitored by microscopy and molecular biology, was carried out every 48 h either in fresh sterile medium or in medium containing the host bacteria. However, the authors also attempted to obtain a stable coculture on solid medium with inconclusive results, as very few colonies were positive for CPR on agar.

A study from 2018 improved upon this protocol by selecting XH001/TM7x colonies grown on a rich solid medium according to the aforementioned protocol. These colonies were seeded in large volumes of liquid medium prior to 0.45-μm filtration and two rounds of ultracentrifugation, resulting in free CPR cells that were used to reinfect pure host cultures. XH001/TM7x coculture must necessarily be subcultured several times to establish a viable and stable experimental system where the host and TM7 are in equilibrium. It is noteworthy that infection with TM7x induces a reduction in the growth and cell division rate of its host XH001. Interestingly, the addition of fetal bovine serum (FBS) to RPMI medium increases the growth of TM7 (63), as shown by the significant increase obtained with a 2% concentration of FBS, as well the elongated XH001 cells, a shape typical of infection or stress, observed with a concentration of 10.5% FBS.

Cell-sorting-based culture techniques: the example of reverse genomics. "*Candidatus* Saccharibacteria" culture methods all share the characteristic that they are based on cell selection prior to cultivation. Several techniques aimed at culturing or sequencing ultramicrobacteria have thus been developed around the concept of cell sorting. Initially, these methods were designed for environmental ultramicrobacteria, including CPR (2). Cell sorting can be achieved using serial dilutions as performed for single-cell sequencing (65, 66), which has also been applied to culture previously uncultured oral bacteria (67). Sorting

can also be performed using flow cytometry as previously applied to identify ultramicrobacteria in the environment (2, 68). Similarly, the reverse genomics method (Fig. 5) uses flow cytometry to sort CPR using antibodies designed specifically to target epitopes from two transmembrane peptides, namely, PBP2 and CpsC (17). MiSeq sequencing was then used to identify and quantify the sorted taxa prior to reseeding on solid and liquid media under anaerobic or hypoxic conditions. Oral samples, namely, saliva and subgingival fluid from healthy participants or patients with periodontitis, were cultured in several media, such as brain heart infusion (BHI), oral treponeme enrichment broth (OTEB), membrane tryptone glucose extract (MTGE), and tryptic soy broth (TSB). Amplified cultures were sorted using the designed antibodies prior to sequencing and reseeding on solid medium, and the authors succeeded in identifying five phylotypes of TM7 with various hosts, such as *Cellulosimicrobium cellulans*, *Actinomyces* sp. strain HOT 171 or *S. odontolytica* XH001. All cultured TM7 cells were epibiont cocci of $<$0.5 μm in diameter. The oxygen sensitivity of some TM7 phylotypes was highlighted, since the exposure of the culture to oxygen reduced the abundance of TM7. This method led to the identification of SR1 HOT 875, which represents one-third of the known phylotypes in the human oral cavity, in coculture with two hosts, *Fusobacterium periodonticum* and *Parvimonas micra*.

Study of CPR biofilms. Biofilm formation has been associated with TM7 growth *in vitro*. Indeed, *S. odontolytica* XH001 in combination with TM7x showed increased biofilm formation in XH001 monocultures (54). The study of dental biofilms (48) of patients undergoing orthodontic treatment highlighted TM7 as a long filament in coculture with *Actinomyces oris* and *Fusobacterium nucleatum* and as bacilli and cocci with *Porphyromonas gingivalis*, *Prevotella intermedia*, *P. micra*, and *Streptococcus gordonii*. Moreover, as part of a study attempting to recreate oral cavity biofilms *in vitro*, the authors also showed that a 5% sucrose concentration increased the TM7 abundance, suggesting that this strategy should be used for future studies of coculture or axenic culture of TM7.

Conservation and storage. Glycerol can be used as a cryoprotectant for cocultures prior to freezing at −80°C. However, this cryoprotectant is not suitable for all CPR-host cocultures. For instance, *S. dontolytica*-TM7 cocultures can be efficiently stored using glycerol, whereas 5% dimethyl sulfoxide is better suited for cocultures with *Arachnia propionica* (44).

CPR LIFESTYLE

Metabolism

The metabolic activity of CPR remains poorly studied, since most genomes are incomplete, and there is no pure culture to date. The genes involved in metabolism constitute a very small percentage of the total coding genes in CPR (ranging between 24 and 27% for some studied genomes) (15, 32). They are therefore considered auxotrophic microorganisms, since they are generally unable to produce the metabolites necessary for their growth (30, 69). For instance, most CPR are unable to *de novo* synthesize their cell envelope, nucleotides, and amino acids (13, 70). Exceptions, such as "*Candidatus* Perigrinibacteria" species, are able to synthesize nucleotides, amino acids, cofactors, and membrane components such as peptidoglycans (13). In addition, CPR lack respiratory pathways, including NADH dehydrogenase and complex II/IV of the phosphorylation chain. They are also unable to produce cobalamin and achieve complete glycogen synthesis, since they lack glucose-6-phosphatase-encoding genes (13).

Conversely, the genomes of CPR are packed with genes essential for their unique lifestyle. Studies of their metabolic pathways based on bacterial databases have shown that peptidoglycan synthesis pathways are almost complete in most available genomes (13, 35). Glycosyltransferase-encoding genes are detected in CPR genomes (13). These enzymes are implicated in the production of glycoproteins, saccharides, and polysaccharides, which are metabolites involved in attachment to the bacterial cell surface and its regulation. Furthermore, "*Candidatus* Saccharibacteria" present sortase-encoding genes, as well as UDP-*N*-acetylmuramyl tripeptide synthetase, which promotes linkage with Gram-stain positive bacteria and thus CPR-bacterium

interactions (7). Moreover, the cell membrane of CPR contains highly synthesized metabolic components. They have high PM values (index of quantification of the biosynthetic capacity), such as teichoic acid and bactoprenyl diphosphate. High activity levels of dGTP, GTP, and TTP are also observed (71). In addition, an arginine deiminase system (ADS) was found in most mammalian "*Candidatus* Saccharibacteria" strains but was absent in their environmental counterparts (72). This system appeared to be acquired as part of the adaptation to the mammalian niches (73). More specifically, it confers protection against acid stress which is a common occurrence in the oral cavity of mammals (74, 75). Moreover, the ADS system appears to also play a role in the selection of the bacterial host, since TM7x, which carries this system, was systematically associated with bacterial hosts devoid of it.

Lifestyle

Almost all recent studies have confirmed the inability of CPR to live independently. They consistently need a bacterial host to grow (15, 70). Evidence of this lifestyle is provided by genomic markers. For instance, "*Candidatus* Saccharibacteria" present dependency markers, such as Holliday junctional resolyase (13), spermidine synthase, and 5-adenosylmethionine decarboxylase proenzyme, which are considered evidence of a symbiotic association (15). Several studies have reported that eukaryotes can also be CPR hosts, particularly the phylum "*Candidatus* Parcubacteria" (21, 70). The relationship between CPR and their hosts is still not well studied. It is difficult to determine whether it is a symbiotic or parasitic relationship (28). However, it seems that each CPR member is adapted to a specific bacterial species as a host and cannot be associated easily with another bacterium. For example, the G1, G3, and G6 clades of the superphylum "*Candidatus* Saccharibacteria" have been described as exosymbionts of rod-shaped cells, whereas "*Candidatus* Saccharibacteria" (G5) and "*Candidatus* Absconditabacteria" (SR1) are associated coccus-shaped bacteria (26). Several CPR may have the same bacterial host. Likewise, a bacterium may be a host for different CPR, and host specificity seems to occur *in vivo*, as shown using electron microscopy and FISH (15, 17, 44). This CPR-host relationship is mediated by the type 4 secretion systems encoded in CPR genomes (26). Different functions have been established for this system (16), including bacterial lysis, transfer of plasmid/genetic components, metabolite and nutrient production, host cell adhesion, DNA uptake, protein secretion, and locomotion (16, 76).

Interestingly, a few studies have suggested that the symbiotic lifestyle of CPR may not be obligatory. It has been reported that the size limit of a genome for an organism to live independently is 0.5 Mb, which is much smaller than the genomes of most CPR (77–80). Moreover, analysis of their COGs shows that they have a different profile than parasitic bacteria and facultative/independent bacteria. In addition, some CPR, such as "*Candidatus* Parcunitrobacter nitroensis," have been described in the environment as host-independent, free-living CPR (13).

It has been suggested that the relationship between CPR and their host is epiparasitic. This would be the first occurrence of epiparastism described in bacteria (81–83). However, an epiparasitism relationship has already been reported in other life domains, namely, in eukaryotes (84) and nanoarchaea, which, unlike "*Candidatus* Vampirococcus" and *Micavibrio* (which both cause rapid cell death of their hosts), can inhibit the growth of their archaeal host (70) and do not invade but stay on the surface of the host (51). Conversely, CPR/host viability can be maintained if nutrients are added to the enriched broth (15).

All metabolic pathways of CPR are still predicted from bioinformatics analyses since there is not yet a pure culture procedure. However, following the first pure coculture of a "*Candidatus* Saccharibacteria" strain, TM7x, with its host in 2015, two stages have been described in their relationship: the first is a parasitic phase that leads to massive death of the bacterial host. This death may be caused by a rapid multiplication of TM7 strains or a surinfection of one bacterial cell with several TM7 strains. This phenomenon may be followed by a second phase: a stable, long-term infection of the bacterial host due to its rapid adaptation (15, 51). Such a scenario was subsequently reported with increased viability of *S. odontolytica* in four subcultures with TM7x. Moreover, this

adaptation occurs through genetic changes in the bacterial host. In fact, several mutations of genes encoding membrane transporters and their associated regulators have been reported after stable infection with TM7x, which partially protects against TM7 infection (53, 85). In addition, this protection does not inhibit the effect of TM7 infection, such as the reduced growth rate and the increase in the bacterial stress response (30). Thus, "*Candidatus* Saccharibacteria" can modulate the microbial environment by inhibiting the growth of other bacteria or completely killing others.

Given their auxotrophic state, the lifestyle of CPR may be supported by a simple retrieval of the necessary metabolites from the bacterial host through the pilus (type 4 especially) (30). For example, a comparison between a host infected with CPR, and the same uninfected host shows that there is a significant increase in some metabolites among the bacteria, such as acetate (32), isoleucine, valine, acyl carrier protein, and 5-methyl-tetrahydrofolate. Moreover, transcriptomic studies have highlighted the upregulation of some genes in the host in the presence of CPR, such as the gene encoding *N*-acetylglucosamine (essential for cell envelope synthesis) (71), and genes responsible for the stress response, such as the prevent-host death family protein, the toxin component GNAT family, the addiction module toxin RelE family, and the YefM TA system (15).

Metabolites are not the only molecules that can be transferred between CPR and their hosts. This physical association also increases the possibility of lateral transfer of DNA sequences between them (28). Analysis of their genomes shows that they are highly mosaic due to sequence transfer between organisms sharing the same ecological niche (28). Lateral sequence transfer has been shown to involve *Chloroflexi*, *Actinobacteria*, *Bacilli*, *Clostridia*, and *Fusobacterium* spp. (7).

Quorum-Sensing-Based Communication

Due to their necessary biosynthetic functions and their symbiotic/parasitic lifestyle, CPR can emit and detect communication molecules, probably to inform and control their environment. Based on an *in silico* study conducted by Charles et al. on 2,503 CPR genomes, a rich repertoire of quorum sensing (QS) proteins was found (86). These homologous proteins encoded by the CPR genomes were recognized as interspecies QS signals and receptors and were divergent from the reference protein sequences (bacterial, eukaryotic, archaeal, and viral QS protein sequences). They were reported to predict three modes of communication: quorum sensing, signal presence, and attentiveness to external signals. The AI-2 QS system is responsible for the latter modality, which is necessary for the regulation of the relationship between TM7x and its host *S. odontolytica* subspecies *Actinosynbacter*, XH001. This QS operon is highly upregulated in the TM7-infected state of *Actinosynbacter* XH001, as reported in a previous study in 2018 (54). AI-2 is an active QS in the regulation of biofilm formation of oral cavity bacteria (87), allowing CPR to enhance the biofilm formation of their host, as shown in the aforementioned study for the first time. It has been shown that this QS system controls virulence factors, particularly for oral bacteria present, such as *P. gingivalis* (88, 89), *Aggregatibacter actinomycetemcomitans* (90, 91), *Streptococcus intermedius* (92, 93), *Streptococcus mutans* (94, 95), and *S. gordonii* (96).

Moreover, TM7x can help its bacterial host evade the immune system through the regulation of biofilm formation via the AI-2 QS system (97–99). Furthermore, this matrix is associated with a decrease in tumor necrosis factor alpha (TNF-α) and therefore the proinflammatory response (100). According to He et al. (15), the association of XH001 and TM7x, which are involved in the phagocytosis process, evasion of innate immunity, and invasion of eukaryotic host cells (101, 102), also promotes the evasion of its host through the expression of choline-binding proteins in XH001. Therefore, CPR-bacterium communication promotes host immune system evasion.

Antibiotic Resistance

Despite their low metabolic capacity, CPR genomes present enzyme-encoding genes that enable antibiotic resistance (AR). A recent study on 4,062 CPR genomes reported high AR genes in all tested genomes using an adapted AR screening strategy for nonbacterial microorganisms (103). This rich reservoir of 30,545 AR genes has been

associated with 14 antibiotic families. Their AR profile was conserved according to the detected families of antibiotics with the presence of glycopeptides, beta-lactams, MLS, tetracycline, and aminoglycoside in all the CPR superphyla tested. These AR genes have a wide divergence, with 89 corresponding enzymatic activities detected using the bacterial AR databases in the analysis. They confer resistance through enzymatic activity by antibiotic inactivation, antibiotic target protection or alteration (103).

The most abundant AR genes are glycopeptide-resistant enzymes consisting mostly of vancomycin resistance genes. Meheust et al. (35) previously reported the presence of vancomycin resistance domain W (vanW) in CPR genomes, suggesting a Gram-stain-positive bacterium-like membrane structure (76). Similar to other life domains (archaea [104], bacteria [105], and eukaryotes [106]), CPR genomes present β-lactamase-encoding genes (103), which could be a valuable feature (107) in microorganisms with such reduced genomes. All beta-lactamase classes were detected (A, B, C and D) with a higher prevalence of the subclass B3 metallo-β-lactamase. In addition, the intron sequences in their RNAs can code for several genes depending on the splice site (14). This RNA structure induces aminoglycoside resistance, which can be used as a key factor for their culture. For instance, He et al. reported that TM7x had an atypical substitution in its 16S rRNA genes (equivalent mutation to the *Escherichia coli* gene at position 912: U instead of C), which is associated with aminoglycoside resistance. Moreover, CPR encodes aminoglycoside-resistant enzymes, of which the most abundant is aminoglycoside acetyltransferase, which inactivates aminoglycoside through acetyltransferase activity (103).

Overall, this rich AR gene reservoir found in the 13 tested CPR superphyla led to their classification into three major AR profiles: the "*Candidatus* Microgenomates" group, "*Candidatus* Parcubacteria" group, and other CPR phyla (Table 4). According to this classification, AR enzyme-encoding genes could be involved in metabolic pathways distinct from those of AR function, depending on the CPR phylum and the environment they inhabit.

To better understand whether human-associated CPR have a specific AR profile, we selected human CPR genomes included in the study by Maatouk et al. (103) (84 genomes), to which 27 genomes were added due to updates in the database up to 16 July 2021. They were all detected in the human oral cavity, except one that was detected in the vagina. Almost all tested genomes presented AR genes (107 genomes), despite the poor assembly of most genomes and the high number of contigs per genome. A profile similar to that of the "*Candidatus* Saccharibacteria" superphylum presented by Maatouk et al. (103) was found, as expected, due to the prevalence of "*Candidatus* Saccharibacteria" members (98 "*Candidatus* Saccharibacteria" genomes with seven corresponding to the "*Candidatus* Gracilibacteria" superphylum and six corresponding to the "*Candidatus* Absconditabacteria" superphylum) among the CPR detected in the human microbiota (103). The AR profile of "*Candidatus* Gracilibacteria" was similar to that previously observed, whereas the AR profile of "*Candidatus* Absconditabacteria" was different with an inversion of the aminoglycoside resistance to glycopeptide resistance ratio.

Defense System: CRISPR-Cas and Diversity Generation Retroelements

Although CPR genomes are particularly small, a few candidate phyla (Parcubacteria and Katanobacteria) encode the CRISPR-Cas12c/d defense system (108). This system joins with a scoutRNA (short-complementarity untranslated RNA) and a crRNA (CRISPR RNA) to form a functional complex against DNA. This complex participates in the cleavage of double-stranded DNA. In addition, scoutRNA is required to process precRNA with the Cas12c system. This processing is mediated by a specific mechanism that leads to the maturation of the cRNA. These functions are essential for CPR, since they lack the RNase 3 enzyme that cleaves dsRNA (109). Interestingly, the lack of some features in the CPR genomes may help them survive with other microbes. For instance, the superphylum "*Candidatus* Patescibacteria," which consists of more than 20 CPR phyla, does not present phage membrane receptors and therefore may evade viral infection even in the absence of a CRISPR defense system (110). This alternative resistance strategy may be linked to the ultrasmall size of CPR cells (110). However, an association of bacteriophages with other CPR has been predicted *in silico* for TM7

TABLE 4 Various antibiotic resistance genes with enzymatic activities in CPR genomes detected in humans[a]

Antibiotic family	Antibiotic resistance gene	*Candidatus* Saccharibacteria	*Candidatus* Gracilibacteria	*Candidatus* Absconditabacteria	Total
Aminoglycoside	aac	37	1	3	41
	gna	9	0	0	9
	aph	2	0	0	2
Beta-lactam	A	28	0	1	29
	B2	1	0	0	1
	B3	11	1	1	13
	C	1	0	0	1
	D	53	0	0	53
Colistin	mcr	1	0	0	1
Glycopeptide	vanA	1	0	0	1
	vanC	15	0	0	15
	vanG	2	0	0	2
	vanH	12	0	0	12
	vanI	1	0	0	1
	vanK	49	0	1	50
	vanS	72	1	2	75
	vanT	3	0	0	3
	vanX	23	0	0	23
	vanY	13	2	1	16
	vanZ	7	1	0	8
MLS	erm	98	3	4	105
	vat	2	5	2	9
	lnu	1	0	0	1
	cfr	1	1	1	3
	llm	1	0	0	1
MLS/Phenicol	cip	1	1	0	2
Nitroimidazole	nim	5	1	0	6
Pyrazinamide	Pnc	3	0	0	3
Tetracycline	tet(T)	59	2	4	65
	tet(W)	7	0	0	7
	tetQ	5	0	0	5
	tet(M)	5	0	0	5
	tet(Q)	4	0	0	4
	tetO	3	0	0	3
	tetB(P)	3	2	1	6
	tet(S)	2	0	1	3
	tet(O)	2	0	0	2
	tet(32)	2	2	0	4
	tet34	1	0	0	1
	tet(36)	1	0	1	2
	tetW	1	1	0	2
	tetM	1	0	0	1
	tet(44)	1	2	0	3
	tet	0	1	0	1
Total		550	27	23	600

[a]The shading reflects the numbers of resistance genes detected in CPR genomes, with the lightest shade representing the lowest number of genes and the darkest shade representing the highest number of genes detected.

("*Candidatus* Saccharibacteria") (111) and SR1 ("*Candidatus* Absconditabacteria") (112). Moreover, this association has also been reported *in vitro* for WWE3, OP11 ("*Candidatus* Microgenomates") and OD1 ("*Candidatus* Parcubacteria") using cryogenic TEM (16).

It is noteworthy that CPR genomes may encode CRISPR spacers divergent from those of known bacteria. Therefore, the detection of phage sequences is remarkably challenging to achieve in metagenomes, which is also true for divergent QS systems (86) and AR-encoding genes (103). Therefore, the absence of such sequences cannot be inferred from the lack of detection. Diversity generation retroelements have been reported in CPR. These elements that modify DNA sequences target proteins involved in attachment, defense, and regulation (13, 113).

CPR AS COMMENSALS IN THE HUMAN MICROBIOME

Human Niches of CPR

CPR have been reported in different niches within the human microbiome, namely, the oral cavity, the gastrointestinal tract, the male and female genital tracts, skin, eyes, and even blood. However, it is noteworthy that they have only been established as commensals within the oral microbiota. The low percentages at which they have been detected to date in other human niches make it unclear whether they are residents of the studied ecosystem or merely transient populations.

Oral cavity. A large number of publications have described CPR as a ubiquitous member of the human oral microbiota with a higher relative abundance of "*Candidatus* Saccharibacteria" (TM7) than in other human microbiomes (26). Despite their recent

discovery, CPR have existed in the human microbiota for thousands of years. Various candidate phyla have been identified in ancient human dental calculus, including in the mouths of hunter gatherers even prior to the agricultural revolution (114). Indeed, different groups of the "*Candidatus* Saccharibacteria" phylum have been detected in ancient dental calculus. The TM7-G1 group was detected in high proportions in 48,000-year-old Neanderthal dental calculus alongside traces of TM7-G3, TM7-G6, and "*Candidatus* Absconditabacteria" (26). The TM7-G3 group and the "*Candidatus* Absconditabacteria" phylum were also found in medieval dental calculus (1200 CE) (26). Altogether, the phylum "*Candidatus* Saccharibacteria" represented 4.6% $\pm$ 4% of the microbial population of the secular dental calculus microbiota dating back to more than 7,550 years (115). To date, "*Candidatus* Saccharibacteria" is among the most represented phylum in the oral cavity of healthy modern humans. In fact, the candidate phylum Saccharibacteria represented approximately 3.1% $\pm$ 5.7% of the microbial diversity in saliva samples and 0.6% $\pm$ 1.2% of the bacteria in dental plaque samples in a cohort of 200 healthy individuals using 454 pyrosequencing (116). This finding was confirmed using FISH, which showed that TM7 represented 0.7 to 1.9% of the subgingival microbiota of a 40-year-old male (7). Furthermore, "*Candidatus* Saccharibacteria" have been detected in other sites of the oral cavity, including the mesial sulci of all teeth, human tongue (dorsum and coating), keratinized and attached gingiva, palatine tonsils, hard palate throat, buccal mucosa, teeth (surface and caries), and mouth rinse (Table 1). Intraindividual and interindividual diversity is observed within the "*Candidatus* Saccharibacteria" phylum in the oral microbiota (117). Indeed, TM7 from the human oral cavity was divided into six groups, G1 to G6 (30, 118), the distribution and proportion in the microbiota of which are variable. Two phylotypes of "*Candidatus* Saccharibacteria" were also identified in six pooled saliva supernatants from healthy individuals (117). Moreover, using pyrosequencing of 16S rRNA amplicons from the saliva of five healthy adults, Lazarevic et al. showed that there was a higher intraindividual than interindividual variation within the phylum "*Candidatus* Saccharibacteria," in contrast to the other phyla of the human salivary microbiota (119). Although the phylum "*Candidatus* Saccharibacteria" is part of the core human oral microbiota (120), other CPR phyla have also been detected, notably the phylum "*Candidatus* Absconditabacteria" (SR1). It is not clear whether this phylum is a part of the core oral microbiota (121–123), but its presence in different sites of the mouth, including saliva (17, 124–126), tooth biofilm (124), dental plaque (123), subgingival fluid (17), mouth rinse (120), supradental plaque (127), tongue coating (128), and dental microbiota (129), has been shown in several studies using reverse genomics methods (130), pyrosequencing (120, 123–125, 127), 16S rRNA amplicon sequencing (128, 129), and quantitative PCR (123) (Table 1 and Fig. 6). Furthermore, the phylum "*Candidatus* Absconditabacteria" was abundantly found in the healthy oral cavity and represented up to 4% of the oral microbial population together with the phylum "*Candidatus* Saccharibacteria" (116, 131).

Other CPR phyla, such as "*Candidatus* Gracilibacteria" (GN02), "*Candidatus* Parcubacteria" (OD1), and "*Candidatus* Microgenomates" (OP11 and OP10), have been detected in the healthy human oral microbiota in lower proportions than TM7 and SR1 (26, 116, 120, 132–136). Interestingly, a study conducted using 16S rRNA amplicon pyrosequencing on oral specimens from 12 healthy individuals highlighted a prevalence of 58.3% for "*Candidatus* Gracilibacteria" (GN02), thus suggesting that this phylum might ultimately be more prevalent than expected (120).

Gastrointestinal tract. CPR are commensals of the human gastrointestinal tract (GIT), with the "*Candidatus* Saccharibacteria" phylum being the most abundant within this ecosystem. In fact, many studies have highlighted the presence of "*Candidatus* Saccharibacteria" in the human fecal microbiota (Table 1 and Fig. 6). Segata et al. demonstrated using 16S amplicon sequencing that the "*Candidatus* Saccharibacteria" and "*Candidatus* Absconditabacteria" phyla were found in 13.6 and 1.4% of the human stool samples studied, respectively (116). Interestingly, the microscomics method provided visual

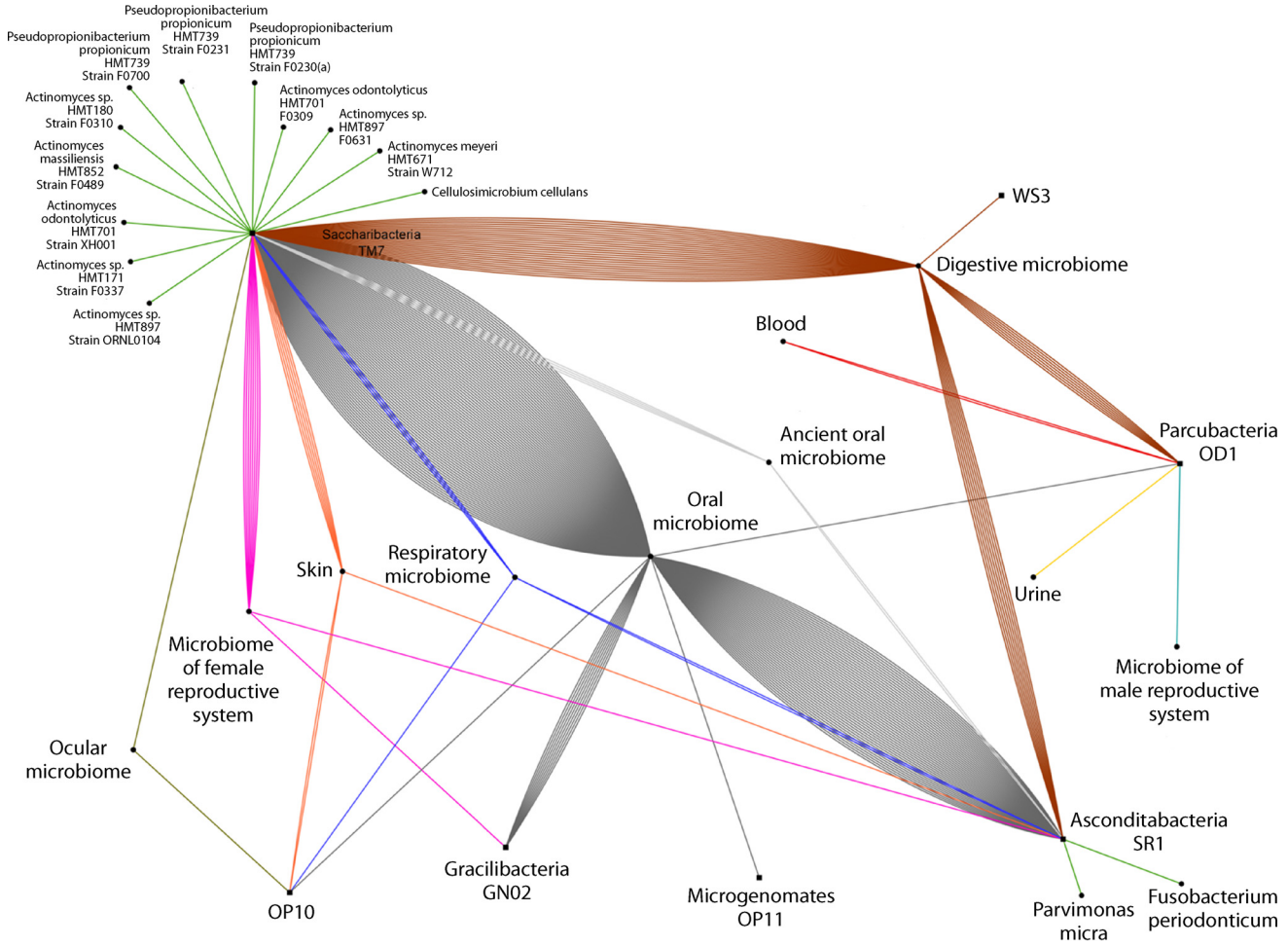

FIG 6 A network representation highlighting the presence of CPR in the different human microbiota and the known bacterial hosts associated with some phyla. This representation was generated using the Cytoscape tool version 3.8.

confirmation of the presence of CPR-like structures in human fecal samples (50), as mentioned above.

CPR are also found along the different anatomical sites of the digestive tract. For instance, the TM7 phylum has been identified in the human esophageal microbiota (141, 142) with a three times higher abundance than that in the mouth (3.3% versus 1.1%) (141) using 16S amplicon sequencing (V3-V4 region) in a cohort of 27 dental and esophageal disease-free individuals. Moreover, this phylum was found in as many as 96.7% of human esophageal samples, suggesting that it is a ubiquitous member of this flora (141, 142). Moreover, the TM7 and SR1 phyla could also colonize the stomach, duodenum, colon and ileum (26, 137, 143, 144), especially the "*Candidatus* Saccharibacteria" phylum, which is part of the human digestive core microbiome (144). The human lower digestive tract (duodenum, ileum, and colon) was also a niche for CPR, as the phyla "*Candidatus* Saccharibacteria," "*Candidatus* Absconditabacteria" and "*Candidatus* Parcubacteria" have been detected in such samples (30, 56, 134, 144–146).

Genital tract. Recent studies have highlighted the presence of CPR in the human genital tract. Three different phyla, namely, "*Candidatus* Gracilibacteria" (which was the most represented), "*Candidatus* Saccharibacteria" and "*Candidatus* Absconditabacteria," have been detected in the female genital tract (26, 147, 148), whereas only the "*Candidatus* Parcubacteria" phylum has been detected in the male genital tract (136) (Table 1 and Fig. 6). In the female genital tract, the abundance of the different phyla is variable along the different anatomical sites. The "*Candidatus* Gracilibacteria" phylum is the most represented midvagina, whereas the TM7-G5 subphylum is the most abundant

in the posterior vaginal fornix (26, 147). Furthermore, the presence of "*Candidatus* Saccharibacteria" has also been described in the human uterine cervical microbiota (149). CPR are thus commensals within the human genital microbiota in males as well as in females.

Other human niches. New human ecological niches of CPR are being explored (Table 1 and Fig. 6). In particular, the human skin microbiota (retroauricular crease and anticubital fossa) contains sequences of the "*Candidatus* Saccharibacteria," "*Candidatus* Absconditabacteria," and OP10 phyla (26, 136, 147, 150, 151). Moreover, different phyla have been found in low abundance in other human microbiomes, such as the "*Candidatus* Saccharibacteria" and "*Candidatus* Parcubacteria" phyla in human blood (152–154), "*Candidatus* Parcubacteria" in urine, and "*Candidatus* Saccharibacteria" and OP10 phyla in eyes (136), which thus represent new ecological niches to be further explored in the future.

Impact of Physiological Situations on the Prevalence of CPR in the Human Microbiome

Impact of sex and age on CPR from the human microbiota. Sex and age are among the physiological conditions that play a role in the variation of human microbiota. Specifically, the candidate phylum Saccharibacteria was significantly more prevalent (at the phylum and genus levels) in the oral microbiota of women than men in an Arab population (120). Furthermore, the abundance of "*Candidatus* Saccharibacteria" seemed linked to the age of the individual. In fact, an increase in the proportion of TM7 has been described between 3-year-old children and 18-year-old young adults (155). There seems to be a peak in the abundance of TM7 in young adults (18 to 19 years), as another study showed a 6.1% TM7 increase in the oral microbiota of young adults compared to older adults (23 to 74 years) (156). Similarly, the "*Candidatus* Absconditabacteria" phylum was more predominant in 22- to 24-year-old adults than in 3- to 6-year-old children (125), reflecting the maturation of the microbiome and age-related human biological changes (155). Conversely, in the gut microbiota, there seems to be a decrease in CPR abundance with age, as an algorithm that measures the replication rate of a microbial population showed higher values in preterm infants (35.8%) than in adults (19.6%) (157).

Impact of geographical distribution and diet on the prevalence of CPR. The geographical location of the population and their diet seem to modulate the richness of CPR in the human microbiota. For instance, urban populations present a higher relative abundance of "*Candidatus* Saccharibacteria" (in particular TM7-G1) than rural populations (127) in the oral and gut microbiota. This phenomenon is likely related to the composition of the diet, as the large amounts of animal protein and processed sugars associated with high-fat diets seem to favor an increased abundance of "*Candidatus* Saccharibacteria" in the human gut microbiota (158, 159). Interestingly, a randomized crossover study showed a diurnal variation in "*Candidatus* Saccharibacteria," specifically an increased relative abundance under early mealtimes (160). Interestingly, "*Candidatus* Saccharibacteria" may be associated with the proinflammatory uptake of bacterial components such as lipopolysaccharide (159) and would therefore be overrepresented in populations fed high-fat and high-sugar diets, since they are associated with the degradation system of these components.

Impact of pregnancy on the prevalence of CPR. Pregnancy leads to microbial changes in the human microbiota, including the abundance of CPR, probably due to the associated hormonal changes. For instance, the abundance of "*Candidatus* Saccharibacteria" has been described to be increased at the end of pregnancy in the microbiota of the posterior fornix of the vagina (161) but decreased in the fecal microbiota (162). However, in the salivary microbiota of pregnant women, no difference was found in the abundance of "*Candidatus* Saccharibacteria" depending on the length of the pregnancy (preterm low birth weight or healthy delivery) (163).

Impact on the immune system. Several studies have shown a potential impact of CPR on the immune system. The role of "*Candidatus* Saccharibacteria" in the intestinal microbiota has been described as predominantly anti-inflammatory (137). It has been shown that a coculture of TM7x with its host *S. odontolytica* XH001 can modulate the

immune response through the inhibition of TNF-α expression by macrophages (15). Conversely, Ye et al. reported that preterm infants presented increased TM7 and proinflammatory cytokine levels. Other CPR, such as "*Candidatus* Absconditabacteria," were also anti-inflammatory, since they were negatively associated with the concentration of proinflammatory cytokines, such as IL-1β, in the human salivary microbiota (164). Similarly, a negative correlation was observed between TNF-α and "*Candidatus* Parcubacteria" in children suffering from celiac disease (165).

CPR AND DYSBIOSIS

Impact of CPR on Inflammatory and Mucosal Diseases

Gingivitis. According to the World Health Organization (WHO), oral diseases affect over 3.5 billion people (166). Gingivitis is an inflammation of the gums described as associated with an increase in "*Candidatus* Saccharibacteria" in the human oral cavity (30, 123, 156), which can return to normal levels posttherapy (30). For instance, TM7-3 has been shown to be 14 times more abundant in the oral cavities of patients with chronic gingivitis than in healthy individuals (156). In addition, a decrease in the proportion of TM7-Rs045 among other non-TM7 taxa can potentially be an indicator of the transition from gingivitis to periodontitis (156). Notably, the level of "*Candidatus* Saccharibacteria" was significantly higher in patients with periodontitis than in patients with chronic gingivitis (156).

Periodontitis. Periodontitis is a pathology affecting the supporting tissues of the teeth and subsequently gum inflammation. To date, according to the WHO (https://www.who.int/fr/news-room/fact-sheets/detail/oral-health), 10% of the world's population is affected by severe periodontitis, possibly leading to tooth loss. The "*Candidatus* Saccharibacteria" phylum has been repeatedly and convincingly associated with this pathology, as an increase in the prevalence and relative abundance of TM7 has been reported in several studies (56, 57, 64, 116, 156, 167–170). In fact, members of the "*Candidatus* Saccharibacteria" phylum were detected in 65.5% (38/58) of patients with periodontitis versus 5.5% (1/18) of healthy individuals (56). This increase was also highlighted using FISH in a study describing both an increase in the abundance and size of "*Candidatus* Saccharibacteria," specifically that of TM7-I025 (which formed filamentous structures of a particularly large size, unprecedented in CPR) in patients with chronic periodontitis (21.0 $\pm$ 2.2 μm in patients with chronic periodontitis versus 5.0 μm $\pm$ 1.0 μm in healthy individuals) (57, 135). Moreover, the relative abundance of "*Candidatus* Saccharibacteria" during periodontitis development could reach up to 21% of the microbial population in dental plaque versus 1% in healthy individuals (56, 64, 168, 171). Furthermore, the prevalence of "*Candidatus* Saccharibacteria" seems to be correlated with the severity of the disease, with a higher prevalence in aggressive periodontitis than in chronic periodontitis (172). Similarly, patients with refractory periodontitis presented with a higher prevalence of "*Candidatus* Saccharibacteria" than patients responding to therapy (173). This increase in "*Candidatus* Saccharibacteria" in the dental plaque of periodontitis patients could be explained by the decrease in oxygen availability within this ecosystem, thus supporting the growth of CPR and possibly that of their host (168). In fact, the increase in "*Candidatus* Saccharibacteria" in periodontal diseases was correlated with an increase in virulence factors (iron acquisition, pili biosynthesis, and oligopeptide transport) (167) and Gram-stain negative bacteria (*Selenomonas*, *Prevotella*, *Treponema*, *Tannerella*, and *Haemophilus*), including members of the red complex of periodontitis (168). This complex consists of a group of bacteria associated with severe periodontal disease, including *P. gingivalis*, *Treponema denticola*, and *Tannerella forsythia* (64, 167). Interestingly, TM7 is more abundant in mild periodontitis than in severe periodontitis (56), which suggests that the growth of the "*Candidatus* Saccharibacteria" phylum is favored in the early stages of the disease rather than in the advanced stages, probably due to the appearance of bacteria competing with TM7 (51). Interestingly, TM7 species has been associated with reduced inflammation in a periodontitis murine model (64). However, this phenomenon has been controversial, since two studies dating back to 2003 and 2007 showed a decrease in TM7 in the oral microbiota of patients with periodontitis using various methods, such as 16S

amplicon sequencing, quantitative PCR (56), and cell separation using flow cytometry prior to amplification and sequencing (174). It is noteworthy that another CPR phylum, namely, "*Candidatus* Microgenomates" OP11, clone X112, has been associated with periodontitis with a significant increase in the mesial sulci (135).

Halitosis. CPR and, in particular, the "*Candidatus* Saccharibacteria" phylum are associated with the development of mucosal diseases such as periodontitis (56, 175) and halitosis (176, 177). The tongue of preschool children who developed halitosis presented a dysbiotic microbiota characterized by an increase in the presence of "*Candidatus* Saccharibacteria" (176). Furthermore, TM7-G1 (HMT 352) has been defined as a biomarker of halitosis, allowing the correct prediction in 91.7% of cases (176).

Inflammatory bowel disease (Crohn's disease and ulcerative colitis). Inflammatory bowel disease (IBD) presently affects approximately 10 million people of all ages worldwide. These diseases have an important impact on human health due to their derived ailments, including diarrhea, abdominal pain, asthenia, fever, and other extradigestive symptoms. All of these symptoms disrupt the daily life of the affected individual (https://www.handirect.fr/maladies-inflammatoires-chroniques-de-lintestin-une-journee-dediee/). As previously mentioned, the association of CPR in oral inflammatory diseases such as gingivitis or periodontitis has been extensively described. "*Candidatus* Saccharibacteria" play a role in promoting inflammation in gastrointestinal dysfunction (137), probably because this phylum is associated with the alteration of the mucus layer of the intestinal barrier (137), which may lead to leaky gut syndrome resulting in intestinal immune infiltration (137). A higher TM7 diversity has been associated with IBD patients, specifically in the gut microbiota of Crohn's disease (CD) patients (137) and those suffering from ulcerative colitis (178). Indeed, a shift in the environmental conditions toward TM7 adaptation due to the loss of selection pressure has been demonstrated and could explain the broad spectrum of TM7 species in the CD group (137). Moreover, a substitution in the 16S rRNA gene inducing antibiotic resistance, commonly found in CPR as mentioned above, was described in 100% of patients with ulcerative colitis, 97.3% of patients suffering from Crohn's disease and only 65.2% of controls (137).

Eosinophilic esophagitis. Eosinophilic esophagitis is a rare disease with a prevalence of 1 in 2,000 people, affecting people of all ages (https://apfed.org/about-ead/egids/eoe/). The study of the esophageal microbiota of adults with or without eosinophilic esophagitis using shotgun metagenomics has shown no significant difference between the two groups (179). Nevertheless, the use of proton pump inhibitors in these patients was significantly associated with five taxa, including the "*Candidatus* Absconditabacteria" phylum (179). Therefore, a stomach pH increase is likely to increase the proportion of CPR in the esophageal microbiome, specifically the "*Candidatus* Absconditabacteria" phylum (179).

Vaginosis. Bacterial vaginosis is one of the most common causes of vaginal discharge and odor, affecting 29% of women (180). The potential association of "*Candidatus* Saccharibacteria" in bacterial vaginosis has been highlighted (148). Candidate division TM7 AF125206 has been found in higher proportions in the vaginal fluid of women with bacterial vaginosis than in that of healthy women (148). Therefore, this TM7 strain could be a marker of vaginosis or could lead to the development of inflammatory diseases of the vaginal mucosa (148).

Otitis. Otitis is an infection or inflammation of the back of the eardrum that is usually treated with amoxicillin. A decrease in the "*Candidatus* Saccharibacteria" and *Actinobacteria* phyla has been described in treated patients suffering from otitis at the end of treatment, as well as 1 month after treatment, compared to the levels in baseline patients (181). This finding suggests that otitis is related to an increase in the TM7 proportion within the oral microbiota (181).

CPR and Infectious Diseases

Caries. In 2017, the Global Burden of Disease defined permanent tooth decay as the most common condition. In addition, according to the WHO, >530 million children present with baby tooth decay (182).

Various candidate phyla have been associated with caries status. Most studies describe the "*Candidatus* Saccharibacteria" phylum as more abundant in children with

dental caries. It was highlighted that the "*Candidatus* Saccharibacteria" phylum (notably TM7b and TM7c) (133) and TM7-G1 (127) were associated with the development of caries in children (127, 129, 133) and could thus be biomarkers of recurrent caries status in the human mouth (129). However, although less reported (a single study), a significant decrease was also reported in children with dental caries (0.11%) compared to healthy children (0.33%) (183). The high relative abundance of TM7 in caries patients has been associated with bacterial agents (129), as well as with immunological markers such as EGF and GSF2 (133). The "*Candidatus* Absconditabacteria" phylum was also associated with severe early childhood caries (129), since it is involved in H_2S production (184). Notably, sulfate-reducing bacteria, such as species of the *Desulfovibrio* genus, have previously been isolated from human caries.

Conversely, the "*Candidatus* Gracilibacteria" phylum was significantly associated with healthy individuals and was completely absent from the oral microbiota of patients with caries (129, 185). This phylum appears to be beneficial in maintaining a stable microbiota in relation to caries status (185), since it has been shown to promote the growth of bacteria involved in oral biofilm formation under healthy conditions (129).

Idiopathic traveler's diarrhea. Traveler's diarrhea is a major health problem for international travelers, affecting one-third of tourists traveling from industrialized to developing countries (186, 187). The impact of this pathology is notable because it can additionally lead from the expected intestinal symptoms (vomiting, nausea, abdominal pain, and frequent bowel movements) to more serious consequences, such as reactive arthritis, irritable bowel syndrome, and Guillain-Barré syndrome (188).

A study of fecal samples of patients with idiopathic traveler diarrhea showed a correlation between the proportion of "*Candidatus* Saccharibacteria" in the microbial dark matter (which represents the unassigned sequences generated using next-generation sequencing [189]) and development of the disease (187). Moreover, seven members of the dark matter, including "*Candidatus* Saccharibacteria," were detected in the idiopathic traveler's diarrhea group but were completely absent in healthy individuals (187). Furthermore, the authors demonstrated a correlation between a TM7 increase (especially the TM7z species) and that of the *Actinomyces* genus in patients presenting this pathology (187). This positive correlation could be explained by the observation that *Actinomyces* species could be potential hosts of TM7z, as described by He et al. for TM7x and *S. odontolitica* strain XH001 (15).

***Helicobacter pylori* infection.** *H. pylori* is a commensal species of the gastric mucosa, the overgrowth of which can cause digestive infection. Using 16S amplicon sequencing to conduct a follow-up posteradication of *H. pylori* (T0, 6 months, 12 months, and 18 months), a significant decrease in the relative abundance of "*Candidatus* Saccharibacteria" was observed between the initial follow-up and 6 months after eradication (140). However, the relative abundance was significantly increased between the initial follow-up and 18 months after eradication.

***Schistosoma japonicum* infection.** Schistosomiasis, a disease affecting approximately 240 million people worldwide (190), can lead to fibrosis, granulomas and organ failure (191). It seems that CPR could also play a role in parasitic diseases. Indeed, using HiSeq technology on the fecal microbiota of patients with *S. japonicum* infection, the relative abundance of "*Candidatus* Saccharibacteria" was significantly increased (5.9% versus 1%) in patients compared to healthy individuals (192). In addition, different phyla were detected in these patients, notably, OD1, SR1, and WS3. Moreover, the authors suggested the use of the "*Candidatus* Saccharibacteria" phylum as a new biomarker of *S. japonicum* infection.

COVID-19. A study aimed to compare the nasal and oropharyngeal microbiota of different groups of patients suffering from different forms of COVID-19, namely, COVID-positive patients in intensive care, paucisymptomatic COVID-positive patients, COVID-negative individuals, and COVID-negative patients who were positive for other coronaviruses (193). This study describes a significant increase in "*Candidatus* Saccharibacteria" in the COVID-negative and paucisymptomatic COVID-positive groups compared to the other two groups in the study (193). Moreover, a significant TM7 decrease was observed in the COVID-negative

TABLE 5 Impact of probiotic administration on CPR prevalence

Probiotic(s)	Impact on CPR	Niche	Reference
Limosilactobacillus reuteri	↗ SR1	Human saliva	124
Lactobacillus acidophilus + *Bifidobacterium longum*	↘ TM7	Human gut	185
Lactobacillus acidophilus + *Lacticaseibacillus casei* + *Lactococcus lactis* + *Bifidobacterium bifidum*+ *Bifidobacterium animalis* subsp. *lactis*	↘ TM7	Human gut	138
Bifidobacterium breve (two strains)	Negative correlation between TNF-α and "*Candidatus* Parcubacteria"	Human gut	165

group compared to patients with other coronavirus infections. This finding suggests a negative correlation between the severity of COVID-19 infection and the abundance of "*Candidatus* Saccharibacteria."

Impact of Therapeutic Intervention on CPR in the Human Microbiota

Impact of probiotic bacteria on CPR in the human microbiota. It is well known that CPR are episymbionts of host bacteria (15). However, to date, no study has described a probiotic bacterium as a CPR host. Nevertheless, probiotic supplementation can modify the composition of the human microbiota, including the relative abundance of CPR. *Lactobacillus reuteri* supplementation promoted an increased relative abundance of "*Candidatus* Absconditabacteria" in the human salivary microbiota (124). Moreover, supplementation with *Lactobacillus acidophilus* combined with *Bifidobacterium longum* decreased the proportion of the "*Candidatus* Saccharibacteria" and *Proteobacteria* phyla in the human gut, which could be restored to baseline subsequent to digestive lavage (139).

TM7 has been associated with obesity and adiposity markers. A randomized trial attempting to treat obesity using a mixture of probiotic bacteria (*Lactobacillus acidophilus* LA-14, *Lacticaseibacillus casei* LC-11, *Lactococcus lactis* LL-23, *Bifidobacterium bifidum* BB-06, and *Bifidobacterium animalis* subsp. *lactis* BL-4) revealed a decrease in the proportion of "*Candidatus* Saccharibacteria" in the human gut (after 8 weeks of treatment) (138). Similarly, a trial aimed at treating children suffering from celiac disease using either a placebo or a cocktail of bacteria (two different strains of *Bifidobacterium breve*) showed an anti-inflammatory effect of the "*Candidatus* Parcubacteria" phylum in the gut of patients with celiac disease treated with the bacterial cocktail (165).

All of these studies (Table 5) highlight a benefit of probiotic supplementation, which allowed a decrease in dysbiotic levels of "*Candidatus* Saccharibacteria" (185). These findings suggest that probiotics can be used to modulate the abundance and diversity of CPR toward homeostatic levels.

Role of dental modifications on CPR. Due to the high abundance of the "*Candidatus* Saccharibacteria" phylum in the human oral cavity, orthodontic treatment can lead to changes in its prevalence and abundance. For instance, "*Candidatus* Saccharibacteria" were more frequently detected in children who had orthodontic treatment (48), with an increased proportion at the end of the treatment (194, 195). In addition, there was a predominance of "*Candidatus* Saccharibacteria" in children with early mixed dentition compared with children with temporary dentition

PERSPECTIVES

As a part of studies aiming to describe human microbial diversity, CPR has been highlighted as a ubiquitous member of the human microbiota that is highly abundant in the oral microbiota and is detected in several human niches, including the GIT, female and male genital tract, urine, skin, and even blood. Interestingly, more studies are being conducted to specifically investigate CPR in the human microbiome. As the CPR superphylum is highly

diverse taxonomically, it would be interesting to investigate other phyla to clearly map the diversity of CPR within the human microbiome and not only that of the candidate phyla Saccharibacteria, Absconditabacteria, and Parcubacteria, as is mostly seen in the present studies. In addition, we have shown physiological variations in the abundance of CPR, as well as pathological variations, particularly in the context of inflammatory diseases. Although mechanistically not well understood, these findings demonstrate the importance of CPR for human health. This study also underlines the necessity of developing new methodologies of coculture and possibly axenic culture to properly characterize CPR and to provide further understanding of their impact on human health.

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Didier Raoult, M.D., Ph.D., who specializes in infectious diseases, is a professor of microbiology at the Faculty of Medicine of Marseille, Aix Marseille University. In 1984, he created ex nihilo his research laboratory, the Rickettsia Unit. This unit has now become the Research Unit in Infectious and Tropical Emergent Diseases (URMITE), collaborating with the CNRS (National Center for Scientific Research), the IRD (Institute of Research for Development), and INSERM (National Institute of Health and Medical Research). In 2011, he became the director of the University Hospital Institute Mediterranée Infection, which is a 600-person medical institute focused on infectious diseases. This facility includes the largest diagnostic and research microbiology laboratory in France, which includes the culturomics team he created in 2011. In the last 30 years, he has cultured approximately 23% of the bacteria isolated for the first time in humans, including *T. whipplei* and over 800 previously uncultured species. His current research interests include tackling the culture of Candidate Phyla Radiation in the human microbiome. As of 2022, Prof. Raoult has published over 3,100 indexed publications.