Bradymonabacteria, a novel bacterial predator with versatile survival strategies in saline environments

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Research

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Abstract

Background: Bacterial predation is an important selective force for microbial community structure and dynamics. However, only a limited number of predatory bacteria were reported, and their predatory strategies and evolutionary adaption remain elusive. We recently isolated a novel group of bacterial predator, Bradymonabacteria, representative of the novel order *Bradymonadales* in δ-*Proteobacteria*. Compared with other bacterial predators (e.g. *Myxococcales* and *Bdellovibrionales*), the predatory and living strategies of *Bradymonadales* remain largely unknown.

Results: Bradymonabacteria preyed on diverse bacteria but had a high preference on *Bacteroidetes* based on individual co-culture of Bradymonabacteria with 281 prey bacteria. Genomic analysis of 13 recently sequenced Bradymonabacteria indicated that these bacteria had conspicuous metabolic deficiencies, but they could synthesize many polymers, such as polyphosphate and polyhydroxyalkanoates. Dual-transcriptome analysis of co-culture of Bradymonabacteria and prey suggested the potential contact-dependent predation mechanism. Comparative genomic analysis with other 24 bacterial predators indicated Bradymonabacteria had different predatory and living strategies. Furthermore, we extracted *Bradymonadales* from 1552 public available 16S rRNA amplicon sequencing samples and found that *Bradymonadales* was widely distributed and highly abundant in saline environments. Phylogenetic analysis showed there may be six proposed subgroups for this order and each subgroup occupied a different habitat.

Conclusions: Bradymonabacteria have unique living strategies, which are different from so-called “obligate” or “facultative” predators. We propose a framework to categorize the current bacterial predators into 3 groups: (i) highly prey-dependent predators, (ii) facultative prey-dependent predators, and (iii) prey-independent predators. Our findings provide an ecological and evolutionary framework for *Bradymonadales* and highlight their potential ecological roles in saline environments.

Background

Bacterial predators are proposed as an indispensable selective force for bacterial communities [1–4]. Predation by bacteria could release nutrients [5] and affect biogeochemical cycling. In contrast to phages, bacterial predators do not need to be in high concentrations to drive significant bacterial mortality in the environment [6, 7]. And bacterial predators have a higher efficiency to kill prey in low-nutrient medium compared with phage [8]. However, these studies mostly based on the predators Bdellovibrio and like organisms (BALOs), little ecological roles are known on other bacterial predators.

Predatory bacteria are classified as two categories, obligate or facultative predators, based on their prey independent/dependent living strategies [9]. Obligate predators are constituted of several genera collectively known as BALOs [10]. These predatory bacteria can attack their prey by penetrating the cell wall [11], dwelling in the periplasm and then killing their quarry [12]. Therefore, their lifestyle depends on their prey in environments, and BALOs lose viability within several hours if prey is not available [8, 13]. Facultative predators contain several genera [9], such as Myxococcus, Lysobacter, and Herpetosiphon [14]. These predators kill their prey by secreting antimicrobial substances into the surrounding environment [9]. In general, facultative predators were considered as they can be cultured as bacteria pure culture. However, the obligate predators could also be pure cultured on complex microbial extract-based media [15]. Indeed, most so-called obligate predators have a host-independent lifestyle [12, 16]. As a result, the definition “obligate predator” doesn’t fully describe their lifestyle. Thus, it is needed to develop a framework to categorize the current bacterial predators.

Bradymonabacteria are representative of the novel order Bradymonadales, which phylogenetically located in the δ-Proteobacteria [17]. The first type species of Bradymonadales was isolated in 2015 [17] (i.e., Bradymonas sediminis FA350^T). To date, 9 strains within the Bradymonadales have been isolated belonging to 7 candidate novel species, and Bradymonabacteria are also bacterial predators [18]. Interestingly, the δ-Proteobacteria contain three orders of predatory bacteria, Myxococcales, Bdellovibrionales (also categorized into Oligoflexia in 2017 [19]), and Bradymonadales. Myxococcales and Bdellovibrionales are facultative and obligate predators, respectively. Also, they have different distribution patterns in environment. Myxococcales were mainly found in soil and sediments niches [20, 21] while

Bdellovibrionales were aquatic. However, how Bradymonadales adapts to predation lifestyle, and whether they have specific living strategies or ecological importance remain largely unknown.

Here, we analyzed Bradymonadales predation range on diverse bacteria and their predatory morphological and physiological characteristics. By using comparative genomic analysis of Bradymonadales and other predatory bacteria, we revealed the genetic and metabolic potential of this group. To assess the diversity and frequency of occurrence of the various ribotypes of known predators (Bradymonadales, Myxococcales and Bdellovibrionales) on a global scale, we surveyed published 16S rRNA gene amplicon datasets from a number of ecosystems representing a broad range of geographic location, climatic zone, and salinity. Our study provides an ecological and evolutionary framework for Bradymonadales and highlights their potential ecological roles in predation.

Results

Bradymonabacteria are efficient predators of diverse prey bacteria

Totally, 9 strains of bacteria with in the novel order Bradymonadales have been isolated by using enrichment culture method [22]. Among these strains, strain FA350^T [17, 18] and B210^T [23] were the two type strains within different genera in Bradymonadales. And both type strains were used to investigate the predator-prey range of Bradymonabacteria. A total of 281 isolated bacteria were co-cultured with Bradymonabacteria FA350^T [17, 18] or B210^T [23] as lawns on Petri dish, respectively (Fig. 1a, Table S1). Zones of predation were measured (Fig. 1b), and results showed Bradymonabacteria preyed on diverse bacteria but a high preference on Bacteroidetes (90% of tested bacteria could be preyed) and Proteobacteria (71% of tested bacteria could be preyed) (Fig. 1c). Predation on bacteria in the orders Flavobacteriales, Caulobacterales, Propionibacteriales, and Pseudomonadales were broadly distributed, with the mean predation percentage greater than 90%, while, predation of Micrococcales and Enterobacteriales were less efficient.

Transmission electronic microscopy (TEM) and scanning electronic microscopy (SEM) analyses were done to understand the mechanism of predation of strain FA350^T on subcellular level. Lysis of the prey cells can be detected nearby strain FA350^T in both TEM and SEM analyses (Fig. 2). Strain FA350^T was detected to have pili (Figs. 2b and 2 g) and the outer membrane vesicles (OMVs) like structures (Figs. 2d, 2e, 2f, and 2 h). In addition, FA350^T cells contained intracellular particles with low electron-dense (Figs. 2b, 2c, 2d, and 2f), which was polyhydroxyalkanoates (PHAs) tested by Nile blue A staining. Meanwhile, FA350^T cells also contained several electron-dense intracellular granules (black granules) (Fig. 2b, 2c, 2d, and 2f), which indicated the presence of intracellular polyphosphate granules [24]. Both these particles were significantly accumulated during predation (Fig. 2).

Bradymonabacteria are multiple auxotrophs

To explore the metabolic capabilities and predation mechanism of this novel group, we analyzed 13 genomes of Bradymonadales (9 high-quality genomes sequenced from cultured strains and 4 reconstructed from published studies [25]). Genome size of Bradymonabacteria ranged from 5.0 Mb to 8.0 Mb. Average Nucleotide Identity (ANI) analysis of the 9 cultured strains of Bradymonadales revealed 7 different species [26] (Fig. S1b). Other General features of genomes were described in Supplementary Materials (Supplementary Materials Results and Fig. S1a).

Almost all strains (except FA350^T) possessed a minimal Pentose Phosphate Pathway, which lacked key steps for the synthesis of ribose 5-phosphate (Fig. 3, Table S2) [27]. Most of the Bradymonabacteria genomes lacked key enzymes for pyrimidine synthesis, such as aspartate carbamoyltransferase, which catalyzes the first step in the pyrimidine biosynthetic pathway. All genomes lacked the complete purine de novo pathway, which was missing the phosphoribosylaminoimidazole carboxylase catalytic subunit or even completely missing the whole pathway.

Beside the auxotrophs in synthesis of pentose and nucleotide, all the genomes lacked complete pathways for the synthesis of many amino acids, such as serine, methionine, valine, leucine, isoleucine, histidine, tryptophan, tyrosine, and phenylalanine (Fig. 3). For example, all the genomes encoded potential D-3-phosphoglycerate dehydrogenase for the conversion of glycerate-3P into 3-phosphonooxypyruvate for amino-acid synthesis (Fig. 3). However, this pathway appeared to be blocked at the subsequent step because of the absence of phosphoserine aminotransferase in all members of Bradymonabacteria, despite that Bradymonabacteria can continue the subsequent pathways to complete the biosynthesis of cysteine and glycine. Additionally, many cofactors and vitamins that promote the growth of bacteria [22], such as biotin, thiamin, ubiquinone, VB_{12}, and $VB_{6,}$ can not be synthesized by the de novo pathway in almost all the genomes. Notably, all the genomes had an incomplete pathway for type II fatty acid biosynthesis, lacking the key enzymes 3-oxoacyl-[acyl-carrier-protein] synthase I/II (FabB/F) and Enoyl-[acyl-carrier-protein] reductase (FabI/L).

Dual-transcriptome analysis of potential predation mechanism of Bradymonabacteria

To further determine the genes involved in predation, we did dual-transcriptome analysis which Bradymonas sediminis $FA350^T$ with/without preying on Algoriphagus marinus am2 (Fig. S2). Like obligate predators, one way that Bradymonabacteria killed their prey bacteria was likely by using contact-dependent mechanisms. Bradymonabacterial genomes possessed complete Type IV pili (T4P) (Fig. 3), and the attached areas showed more type IV pili than the non-attached areas (SEM, Fig. 2g and 2 h). Dual-transcriptome analysis showed that genes encoding type IV pili twitching motility protein PilT (DN745_17255) were significantly up-regulated during predation (Fig. S3), suggesting these genes may be involved in predation. Bradymonabacteria also had T4b pilins relative homology with those in Bdellovibrio bacteriovorus HD100, in which T4b pilins were necessary for predation [28, 29] (Fig. S4), so T4b pilins may participate in regulating predation. In addition, the group of bacteria had type II and type III secretion systems (The YscRSTUV proteins form a membrane-embedded complex known as “export apparatus” [30]). Dual-transcriptome analysis also supported the prediction that genes encoding type III secretion system inner-membrane protein complex (DN745_01900, DN745_10315, DN745_17280, DN745_03325, and DN745_00480) were significantly up-regulated during predation (Fig. S3), implying these genes may be involved in predation.

Another way that Bradymonabacteria killed their prey bacteria was likely by using secreting antimicrobial substances into the surrounding environment. Similar with most facultative bacterial predators, there were a few potential antimicrobial clusters of secondary metabolites synthesis, such as Lassopeptide [31], in almost all genomes of Bradymonabacteria (Fig. 3). Genes involved in OMVs-like biosynthesis were also detected in most genomes, such as ompA (cell envelope biogenesis protein), envC (Murein hydrolase activator) and tolR (envelope stability) [32]. It was detected that vesicle membrane related genes (DN745_03865, DN745_02930, and DN745_07125) were significantly up-regulated during predation (Table S4, Fig. S3).

Bradymonabacteria are novel predators different from obligate or facultative predators

Comparative genomic analysis with other bacterial predators was done to explore whether Bradymonabacteria had unique living strategy. Two-way cluster analysis showed that Bradymonabacterial genomes features were different from either obligate or facultative predators, which phylogenetically located in a different branch (Fig. 4). The specific multiple metabolic deficiencies of Bradymonabacteria had some similarities with most obligate predators. For example, both of Bradymonabacteria and obligate predators possessed minimal Pentose Phosphate Pathway, lacked key enzymes for pyrimidine synthesis, and lacked complete pathways for the synthesis of many amino acids, cofactors, and vitamins (Fig. 4). However, Bradymonabacteria with multiple auxotrophs could grow on common media (such as marine agar medium) though at a low growth rate [33], which was different from obligate predators.

Unlike most obligate predators, the polyphosphate accumulation pathway, containing a pair of genes (Polyphosphate kinase and Exopolyphosphatase) associated with both polyphosphate formation and degradation [34], was present in most Bradymonabacteria (Fig. 4). Polyphosphate accumulation was also detected in $FA350^T$ cells during predation (Fig. 2).

Different from most of the other predators, potential polyhydroxyalkanoates (PHAs) synthesis from β-Oxidation of fatty acids [35] were observed in most Bradymonabacterial genomes (Fig. 3). In this study, TEM analysis shows strain FA350^T could significantly accumulate PHAs during predation compared with pure culture (Fig. 2). In spite of an incomplete pathway for fatty acid biosynthesis, all the Bradymonabacteria had a high copy number of long-chain fatty acid transporters (fadL) compared to other predators to gain the fatty acids from environments (Fig. 4). In addition, genes associated with alkane synthesis, which was important for maintaining cell membrane integrity and adapt to cold environment [36], were present in most genomes of Bradymonabacteria (Figs. 3 and 4). As a result, we proposed that Bradymonabacteria could be categorized into novel predators different from the so-called obligate or facultative predators (Table 1).

Table 1
The features of 3 different types of bacterial predators

Current predators type	Redefine predators type	Metabolic pathways deficiencies	Pure-cultivable	Storing nutrients as polymers	Predation strategy	Predation specificity
Obligate	Highly prey-dependent	High	Extremely Difficult	None	Contact-dependent	Gram-negative
Bradymonabacteria	Facultative prey-dependent	High	Difficult	Polyhydroxyalkanoates, polyphosphate, and alkane	Contact-dependent	Gram-negative and Gram-positive
Facultative	Prey-independent	Low	Normal	Polyphosphate*	Mostly contact-independent	Gram-negative and Gram-positive

* Polyphosphate accumulation pathway was fund in genomes, but not determined by experiments.

Bradymonadales are mainly distributed in saline environments with a high diversity

To evaluate the global prevalence of the Bradymonadales order, we surveyed recently published 16S rRNA gene amplicon studies that provided a fine taxonomic resolution along with relative sequence abundances. 16S rRNA gene amplicons from 1552 samples were grouped into eight types of environments (Fig. 5a and Table S5). A total of 811 samples were from an inland environment, while others were from the marine environment, with each biotope showing a relatively different microbial community (Figs. 5b and S5). Bradymonabacteria was detected in 348 of 741 marine samples (relative abundance > 0.01%), but only 20 of 544 soil samples (Fig. 5a). All samples were sorted into an ordination diagram based on the similarity of communities (Fig. 5b). Saline biotopes were clearly separated from non-saline ones (Fig. S6), suggesting that saline was a significant factor in shaping microbial communities. For each biotope, the relative abundance of Bradymonadales in the saline environments (i.e. seawater and saline lake sediment) was significantly higher than in the non-saline environment (i.e. no-saline soil and non-saline water) ($P < = 0.0001$, Fig. 5c). The distribution analysis was consistent with the genomic feature analysis (Fig. 2), which several genes encoding sodium symporters and Na^+/H^+ antiporters were found in genomes, and suggesting a beneficial effect of salinity on Bradymonobacteria.

In addition, we compared the relative abundance of Bradymonadales with another two orders of well-known predatory bacteria, Bdellovibrionales and Myxococcales [12, 37, 38]. We found that Myxococcales and Bdellovibrionales were also globally distributed (Fig. S7); however, Myxococcales were more likely distributed in soil and sediment environments, while Bdellovibrionales were more likely distributed in freshwater and seawater (Fig. S7). The total relative abundances of Bradymonadales, Bdellovibrionales, and Myxococcales ranged from 0.7–6.4% of total prokaryotic microbes in all 1552 samples (Fig. S8a). The mean relative abundance of Bradymonadales (0.51%) was similar to Bdellovibrionales (0.62%)

when both were detected in environmental samples (Fig. S8b). In contrast, Bradymonadales was one of the most abundant known predatory bacteria in saline lake sediment and saline lake water (Fig. S8c).

To further determine how salinity affected the relative abundance of Bradymonadales, we used Gaodao multi-pond salterns as a model combined with 16S rRNA gene amplicons, fluorescence in situ hybridization (FISH), and real-time PCR analyses (Figs. S8d and S9). The results showed that Bradymonadales appeared in all tested multi-pond saltern datasets, accounting for an average of 0.74% of all bacterial sequences and more than 1.0% relative abundance within the range of 80 g/L and 265 g/L salinity (Fig. S8d), which was significantly higher than Bdellovibrionales and Myxococcales. The detailed descriptions of effects on the abundance of Bradymonadales were in supplementary materials (Supplementary Materials: Results, Figs. S8d and S9).

To explore the diversity and distinct evolutionary of Bradymonabacteria subgroups in different biotopes, we performed a phylogenetic analysis of nearly full-length 16S rRNA gene sequences of diverse origin by maximum likelihood inferences (Table S6). A total of 187 OTUs were detected and formed six sequence clusters (Fig. 6a). Almost 87.2% representative sequences originated from saline biotopes (such as seawater, marine sediments, salterns, corals, and saline lake). Since Bradymonabacterial subgroups may be selectively distributed in local biotopes, we investigated the relative abundance of each subgroup throughout the 127 representative samples, in which the relative abundance of Bradymonadales was above 1% (Fig. 6b). Five of 6 Bradymonabacterial subgroups showed significantly high abundance patterns in saline environments. Cluster-2 and cluster-6 were mainly observed in seawater biotopes, whereas cluster-3 was mainly observed in marine sediment and saline lake sediment (Fig. 6b), consistent with the environment of the cultured strains. The cluster-5 lineages tended to occur in both freshwater and seawater (Fig. 6b).

Discussion

In all ecosystems, predation is an important interaction among living organisms. Bacterial predators are proposed to play an important role in controlling and shaping bacterial populations in diverse environments [3, 39]. However, in spite of their ecological importance, only a few examples of predatory bacteria have been studied in depth. Recently, many predatory bacteria from various phyla have been isolated from different environments; however, most of their strategies involved in the predatory way of life and living adaption are not clear. Our study systematically analyzed the predatory living adaption, global distribution, and diversity of Bradymonadales, and highlighted the ecological role of Bradymonadales and provided a framework for the categorization of known predatory bacteria.

In our study, based on comparative genomic and physiological analyses, Bradymonabacteria were shown as a novel group of bacterial predators, which had versatile survival strategies different from either the so-called “obligate” or “facultative” predators (Table 1). Bradymonabacteria had multiple metabolic deficiencies. The incomplete pathways might be important for prey-dependent growth, as the precursor compounds could be acquired from predation. In addition, the loss of genes in the fatty acid biosynthesis pathway is notable because fatty acids are integral components of cellular membrane and are considered to be a housekeeping function of cells [40]. These organisms may incorporate exogenous fatty acids from prey bacteria into membrane phospholipids using a high copy number of long-chain fatty acid transport proteins [41] (Fig. 3). Gene loss in these organisms may render them dependent on prey for the lost metabolic functions, and may also provide a selective advantage by conserving the predators’ limited resources [42]. However, the sequenced genomes of Bradymonabacteria were surprisingly large (5.0 Mb to 8.0 Mb, Fig. S1a) and suggest that Bradymonabacteria was far from an obligatory parasite, with seemingly none of the reductive evolution that results from a parasitic lifestyle for bacteria such as Mycobacterium leprae [43]. The large size of genome may be indicative of the vast range of genes required to effectively tolerance to the absence of prey or carry out predation.

Different from most of predators, Bradymonabacterial cells could synthesize many polymers of nutrients, such as polyphosphate, PHA, and alkane. Exopolyphosphatase catalyzes the hydrolysis of terminal phosphate residues from polyphosphate chains, accompanying the production of ATP, thus playing a role in the production of energy [44].

Bradymonadales cells may accumulate polyphosphate in the phosphate-rich zone, using is as an energy source [45]. Meanwhile, PHA granules are synthesized as sinks of excess carbon and are used as carbon and energy reserves in starvation conditions [46]. Under nutrient starvation, maintenance energy and free amino acids can be provided by endogenous substrates such as PHAs and polyphosphate [47, 48], which may be an important feature for the survival of Bradymonabacteria during predation interval. This feature is interesting in bacterial predators, as it is commonly found in animals' predation. For example, the bear can store fat in its body to ensure that it will survive the long winter. In addition, Bradymonabacteria may also synthesize alkane to maintain cell membrane integrity [36] and complements its poor ability of fatty acids synthesis. Thus, those multiple auxotrophs and polymers of nutrients synthesis confer Bradymonabacteria a versatile survival strategy in natural environments, which were different from the current known obligate or facultative predators.

As the bacterial predators, Bradymonabacteria have developed a wide range of mechanisms to fight their preys. Contact-dependent predation mechanisms allow predators to attach to the preys, and then carry out predation. This process is relative energy low-cost, which could avoid the secretory virulence factors to be diluted by surrounding environments [49]. Bradymonabacteria had T4P, which could pull adherent bacteria close association with other bacteria [50]. T4P could also transport bound substrates like DNA [51] into the periplasm and export exoproteins across the outer membrane [52]. Contact-dependent Type III secretion systems were also found in Bradymonabacteria, which were reported to be capable of moving virulence factors across bacterial outer membranes and directly across the host cell membrane into the cytoplasm of a host cell [53]. However, there was no report indicating type III secretion system was involved in the direct fight between bacteria. Whether the type III secretory complexes could penetrate the bacterial cell wall is unknown. Further gene knock out experiments and systematic TEM analysis should be done to identify whether and how type III secretion system work during predation.

Biogeographic analysis suggested Bradymonabacteria was mainly distributed in saline environments, and some other studies also detected Bradymonadales in hypersaline soda lake sediments [25], suggesting saline environments could enrich these bacteria. Genome analysis also shows that Bradymonadales had many genes encoding sodium symporters and Na^+/H^+ antiporters to maintain the osmotic pressure in saline environments. These findings supported the global analysis (Figs. 5 and S8c), suggesting that Bradymonadales might be a dominant bacterial predator in some specific saline environment compared with Bdellovibrionales and Myxococcales. The analysis of the complex intragroup phylogeny of the 6 subgroups of Bradymonabacteria revealed that distinct evolutionary Bradymonabacteria subgroups had arisen in different biotopes, suggesting the occurrence of adaptive evolution specific to each habitat. Patterns related to saline status also suggest that most Bradymonadales are halophiles [17].

Bradymonabacteria had a very high predation efficient on bacteria within phylum Bacteroidetes. Members of the phylum Bacteroidetes are one the most abundant group of bacteria in the ocean. Bacteroidetes are assumed to be attachment to particles and degradation of polymers, and has an important role in the carbon cycle of the oceans [54]. Thus, the high predation efficient on Bacteroidetes might provide Bradymonabacteria important roles in regulating Bacteroidetes communities and affecting the carbon cycle of the oceans. In addition, Bradymonadales were detected in corals samples, as Bradymonabacteria have a wide range of prey including corals pathogens Vibrio harveyii, suggesting Bradymonabacteria may protect the host by consuming potential pathogens [39]. The exact ecological roles of this group in different environments should be determined in further studies.

Conclusion

The unique metabolic pathways of Bradymonabacteria, with conspicuous metabolic deficiencies similar to obligate predators, but with a more effective starvation stress response mechanism, provide the bacteria a unique survival strategy (different from the survival model of so-called "obligate" or "facultative" predators). We propose a framework to categorize the current bacterial predators into 3 groups: (i) highly prey-dependent predators, such as most of the BALOs; (ii) facultative

prey-dependent predators, such as Bradymonabacteria; and (iii) prey-independent predators, such as Myxobacteria and Lysobacter sp. (Table 1). This categorization is helpful for further study of different ecological importance of each type of bacterial predator. The evolution of bacterial predation in these three groups of predators should also be studied in future to better understand the significance of predation to biological evolution.

Our study highlights the ecological role of Bradymonadales in saline environments. Given their substantial sequence and cell frequencies in the saline environment and storing nutrients in polymers in cells during predation, Bradymonadales may have an alternative way of regulating global nutrient cycling. To better understand the impact of Bradymonabacteria predation on regulating biogeochemical cycling, predation mutant and microcosm need to be developed in further study.

Methods

Predation experiments

To explore predation of Bradymonabacteria, we used Bradymonas sediminis FA350^T and Lujinxingia litoralis B210^T as representative strains. All candidate prey strains were obtained from our lab. Cells were centrifuged, washed and concentrated in sea water to a final OD_{600} of 3.0 for predator strains and 6.0 for candidate prey strains. Drops of 5.0 µl of the predator strains suspensions were deposited on the surface of agar plates and allowed to dry. Next, drops of 20.0 µl of the different candidate prey strain suspensions were spotted close to the predator spot. Plates were incubated at 33 °C and images were taken after 48 h with a digital camera. To detect Polyhydroxyalkanoates (PHAs) accumulation, granules were stained with the Nile red component of Nile blue A.

Genome sequencing and comparative genome analyses

To explore the potential metabolic capacity of bacterial predators, we sequenced 3 complete genomes and 6 draft genomes of all currently known Bradymonabacter isolate strains using the methods reported in our previous studies [18, 55]. We also retrieved 37 predator genomes from NCBI (including 4 MAGs). tRNAs and gene prediction were analyzed using tRNAscan and prodigal, respectively. Genome-based metabolic potential of bacterial predators was predicted by BlastKOALA (https://www.kegg.jp/blastkoala/). The average nucleic identities among the 9 cultured Bradymonabacteria strains was calculated using pyani (https://github.com/widdowquinn/pyani), and the percentage of conserved proteins (POCP) between each strain was calculated as described previously by Qin et. al.[56]).

Electronic microscopy analyses

We selected Algoriphagus marinus am2, which is smaller than the predator Bradymonas sediminis FA350^T, as prey. Bradymonas sediminis FA350^T and Algoriphagus marinus am2 were cultured to exponential growth phase separately, then adjusted to the same OD value, and mixed together and co-cultured on Marine Agar medium at 33 °C for 68 h.

For transmission electronic microscopy (TEM) analysis, mixed culture samples were supported on carbonformvar-coated copper grids. Grids were inverted over a drop of 1% uranyl acetate. Thin sections were prepared with the predator-prey co-culture at 68 h incubation. The samples were mixed with 0.5 ml of 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, centrifuged and resuspended in 1 ml of the same solution for 3 h. Cells were washed in cacodylate buffer, fixed with 1% osmium tetroxide and encased in agar. The agar encased cells were then fixed in 2% uranyl acetate and dehydrated in an ethanol series and embedded in Epon resin. Thin sections were cut and stained with uranyl acetate and lead citrate. Specimens were examined with a JEM-1200EX electron microscope operated at 80 kV.

For scanning electronic microscopy (SEM) analysis, mixed culture samples were washed 3 times with PBS, fixed for 1 h in 2.5% glutaraldehyde in sodium cacodylate buffer (0.1 M, pH 7.2). To dehydrate the bacteria, the EM grids underwent a series of washes in increasing concentrations of ethanol (25, 50, 75, and 96%) and placed in a vacuum overnight. Samples were coated with gold and observed using Nova NanoSEM 450.

Dual transcriptomic analyses

To determine the gene expression profiles of type strain FA350^T, a pure culture of FA350^T and a co-culture of FA350^T with the prey Algoriphagus marinus am2 were cultured on Marine Agar medium at 33 °C for 0 h, 68 h, and 120 h, respectively. Each time point was collected in triplicate (n = 3) for further transcriptomic analysis. For transcriptomic analysis, RNA extraction, library construction, sequencing and analyzing were processed as described in our previous study [57]. Sequencing was carried out on a HiSeq sequencer at the Novogene Co., Ltd. (Beijing, China). For transcriptomic analysis of mixed culture samples (dual transcriptomic analysis), total RNA sequences were mapped to the complete genome of FA350^T using the method reported by Westermann et al. [58]. Then, the completely mapped sequences were selected for further analysis.

The phylogenetic analysis of Bradymonabacterial type IV pili

An unrooted, maximum likelihood phylogeny shows relationships between type IVa, type IVb, IVc pili, archaellum (archaeal flagellum), T2SS and T4SS extension ATPases. Protein amino acid sequences were aligned with mafft and used to estimate the maximum likelihood phylogeny with RAxML under the JTT substitution model with gamma-distributed rate variation. The protein amino acid sequences of Bradymonas were annotated by RAST (Rapid Annotation using Subsystem Technology) [59]. The other protein amino acid sequences were obtained from other research supplementary materials [60].

Biogeodistribution database construction

All 16S rRNA gene sequences analyzed in this paper were download from the European Nucleotide Archive (https://www.ebi.ac.uk/ena, ENA), during or before December 2018. As a result, we collected 1,552 samples from 102 projects or studies: 25 from non-saline lake sediments (NSLS), 275 from marine sediments (MS), 499 from non-saline soil (NSS), 45 from saline soil (SS), 216 from non-saline water (NSW), 19 from saline lake sediments (SLS), 466 from sea water (SW), and 7 from saline lake water (SLW) (Table S5).

Microbial community composition

The raw 16S rRNA gene reads were filtered, de-uchime. The quality filtering, chimera detection, dereplication, clustering to OTU and assigning taxonomic information were processed using VSEARCH [61]. SILVA database Ref_SSU release 132 was used as a referent taxonomic database (https://www.arb-silva.de/). Alpha diversity indices (shannon, simpson, goods coverage and Ace) detailing microbial community composition within each sample was calculated using scikit-bio (http://scikit-bio.org/) in python, and alpha diversity indices (chao1) was calculated using package fossil (https://www.rdocumentation.org/packages/fossil) in R. For estimating community dissimilarities, Bray-Curtis distance was calculated by vegan in R based on the relative abundance of each taxon at order level.

Phylogenetic analyses

Both RAxML [62] and FastTree [63] were employed to construct the Bradymonabacteria phylogenetic tree. Given both the topology of phylogenetic tree and a good coverage of all Bradymonabacteria lineages, we established the phylogenetic tree using 187 Bradymonabacteria representative 16S rRNA gene sequences, all longer than 1200 bp (at 98.5% cutoff). These sequences were aligned using mafft. Bradymonabacteria subgroup designations were confirmed when one subgroup with > 10 representative sequences was monophyletic with two different constructing phylogenetic tree programs using maximum likelihood approaches [64]. Environmental types (i.e., saline and non-saline) of each Bradymonabacteria sequence in the tree were collected from GenBank. Genome-based phylogeny of bacterial predators and 9 cultured Bradymonabacteria strains were analysed using core genes [65], and trees were constructed using RAxML [62]. All phylogenetic trees were drawn using ggtree [66]in R.

Quantitative real-time PCR

The environmental DNA samples extracted in the previous step were used for qPCR experiments in order to detect the abundance of bacteria and Bradymonadales in each sample. The primer pair composed of 341F (5'-CCTACGGGAGGCAGCAG-3') and 534R (5'-ATTACCGCGGCTGCTGGCA-3') was used for quantification of bacteria [22]. A Bradymonadales-specific primer set composed of qBRA1295F (5'-CTCAGTTCGGATYGYAGTCTG-3') and qBRA1420R (5'-GTCACCGACTTCTGGAGCAARCG-3'), which was designed in this study and generated an amplicon of 148 bases, was used for quantification of Bradymonadales. Reactions for each sample were carried out in an ABI StepOnePlus thermal cycler under the following conditions: an initial denaturation step at 95 °C for 10 min and then 40 cycles of 15 s at 95 °C and 30 s at 60 °C. The reaction were performed in a total volume of 20 µl, composed of 10 µl 2X Universal SYBR Green Fast qPCR Mix (ABclonal), 0.4 µl of each primer (10 µM), 1 µl of sample and 8.2 µl of MiliQ water. The Plasmid DNA Standard was constructed by introducing the 16S rDNA gene amplified from Bradymonas sediminis FA350^T into the pMD18-T Vector (TaKaRa) following the manufacturer's instructions. The plasmid was isolated and purified using a MiniBEST Plasmid Purification Kit (TaKaRa). DNA copy number was determined by the concentration and relative molecular weight of the Plasmid DNA. For each QPCR assay, the plasmid aliquot was serially diluted to produce concentrations ranging from 10^9 to 10^3 DNA copies/µl to generate calibration curves. Each sample was measured in triplicate, and negative controls (no template NTC) were included.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publications

Not applicable

Data availability

The genomes of cultured Bradymonabacteral isolates have been deposited in the NCBI database under GenBank accession numbers CP042467.1 (*Bradymonadales sp.* V1718), CP042468.1 (*Bradymonadales sp.* YN101), VOPX00000000.1 (*Bradymonadales sp.* TMQ1), VOSL00000000.1 (*Bradymonadales sp.* TMQ2), QRGZ00000000.1 (*Bradymonadales sp.* TMQ3), VOSM00000000.1 (*Bradymonadales sp.* TMQ4), CP030032.1 (*Bradymonas sediminis* FA350^T), QHKO00000000.1 (*Lujinxingia litorali* B210^T) and SADD00000000.1 (*Lujinxingia sediminis* SEH01^T). The genomes of uncultured Bradymonabacteria have been deposited in the NCBI database under GenBank accession numbers PWKZ00000000.1 (*Bradymonadales* bin CSSed10_215), PWTN00000000.1 (*Bradymonadales* bin CSSed11_191), PXAJ00000000.1 (*Bradymonadales* bin T3Sed10_204) and PWZZ00000000.1 (*Bradymonadales* bin T3Sed10_190). The 16S rRNA gene data sets of Gaodao salterns have been deposited in the Sequence Read Archive under accession number SRP217756 for all the samples. The transcriptome sequences for predation of FA350^T have been deposited in the NCBI database under accession numbers PRJNA559243 and PRJNA559253. All Bradymonabacteral isolates have been deposited at the Shandong Infrastructure of Marine Microbial Resources hosted by the Laboratory of Marine Microbiology at Shandong University (http://www.sdum.wh.sdu.edu.cn/search.html?itemId=14). Any Bradymonabacteral isolate is available upon request.

Competing interests

No conflict of interest exists in the submission of this manuscript, and the manuscript has been approved by all authors for publication. The authors declare that they have no competing interests.

Authors' contributions

DSM, GJC, JZ, and ZJD designed the study. SW carried out TEM, SEM, and transcriptome analyses. ZZD carried out FISH and real-time PCR analysis. DSM, QYL, SW, XPW, and RT performed bioinformatic analyses. DSM and ZJD analyzed data and wrote the paper. JYN, AZ and YY improved the paper writing. All authors read and approved the manuscript

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Figures

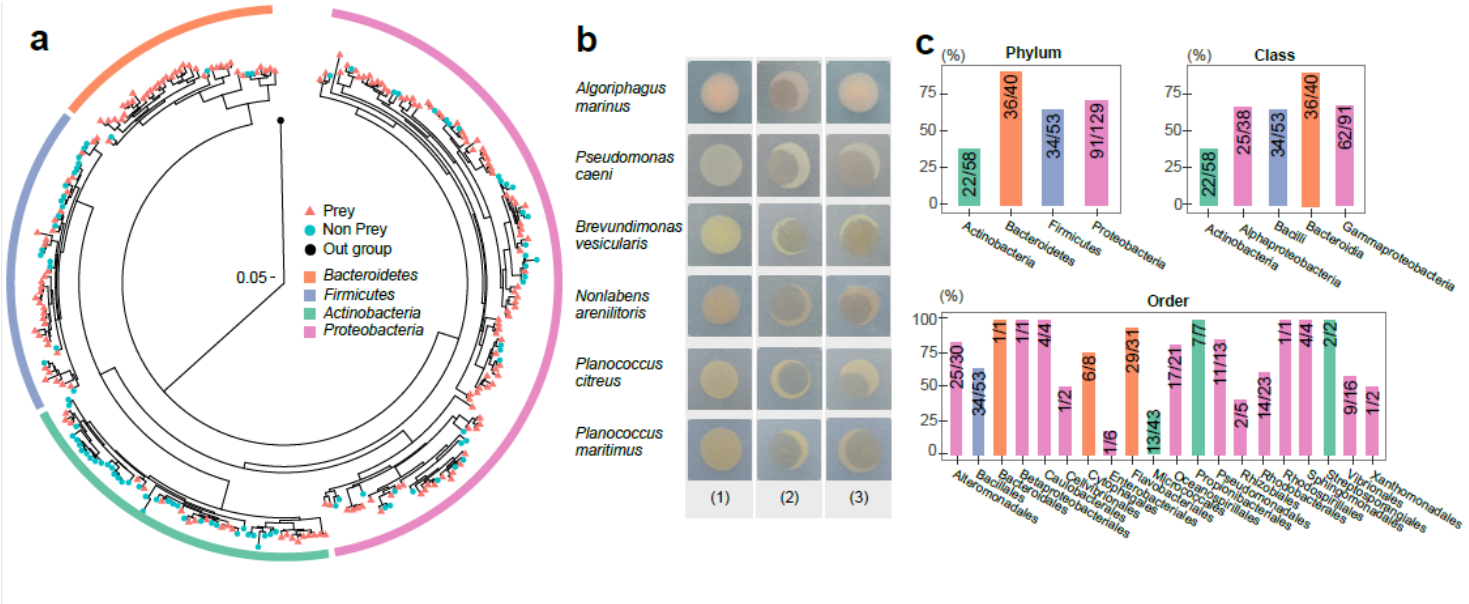

Figure 1

Predation assays for potential prey-organisms. a, Total 281 organisms were selected to test the predation by two type strains of Bradymonadales. Phylogenetic tree was analysed for the tested organisms. Red dot on phylogenetic tree indicate the organism could be preyed by either Bradymonas sediminis FA350T or Lujinxingia litoralis B210T. Green dot indicate the organism could not be preyed by either test predator. The detailed information of organism on the tree is shown in table S1. b, The predation phenotype of Bradymonas sediminis FA350T or Lujinxingia litoralis B210T on prey. Number 1 to 3 indicate

the pure culture of different preys, mixed culture of Bradymonas sediminis FA350T and preys, mixed culture of Lujinxingia litoralis B210T and preys, respectively. c, The percent of organisms which could be preyed on is shown in the bar chart.

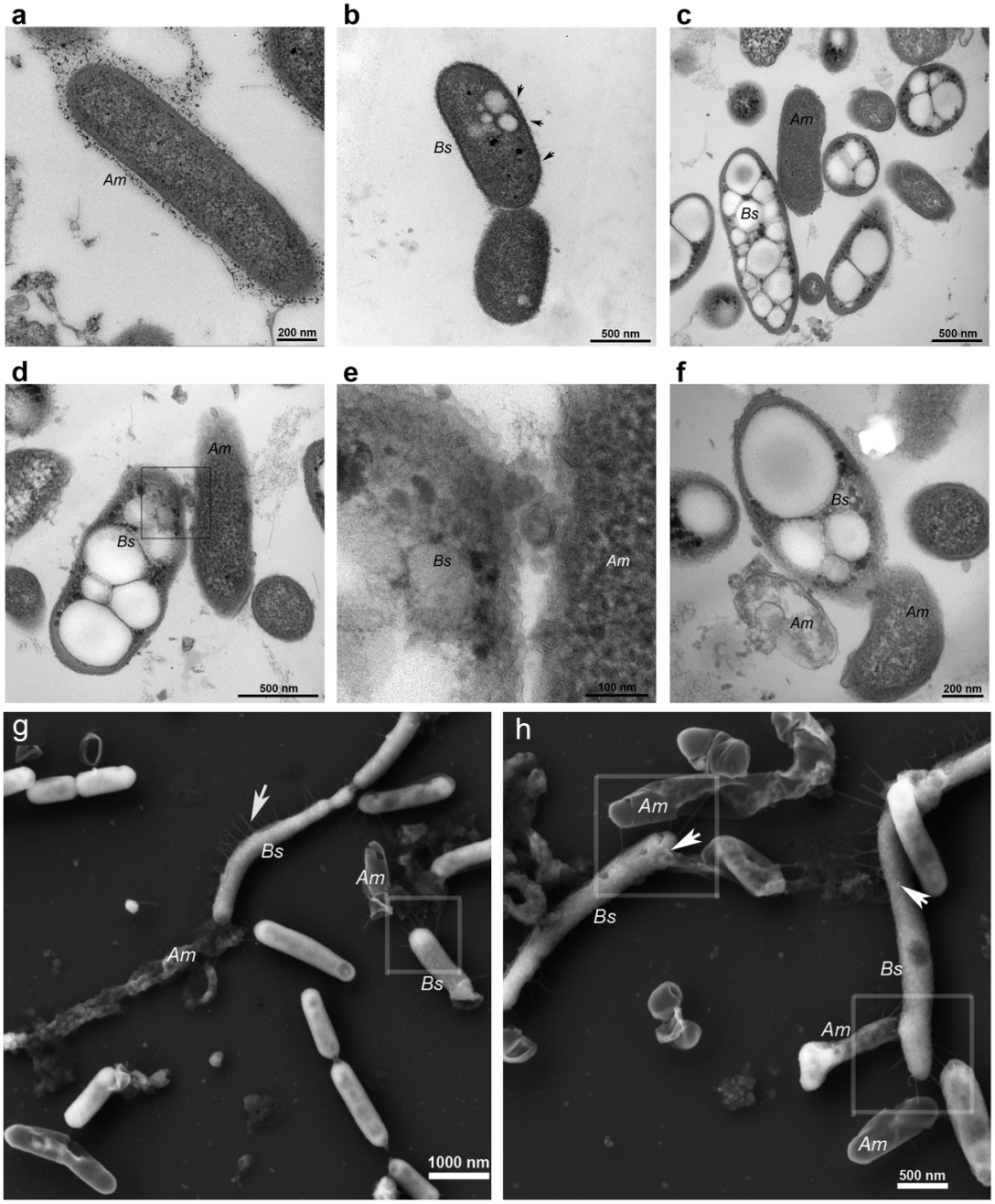

Figure 2

TEM and SEM micrographs of Bradymonas sediminis FA350T (predator) and Algoriphagus marinus am2 (prey). We selected a prey Algoriphagus marinus am2, which was smaller than predator FA350T. a, The free-living prey Algoriphagus marinus am2 (Am) by pure culture. Bar = 200 nm. b, The free-living predator Bradymonas sediminis FA350T (Bs) by pure culture. The white globose granules in the Bs cell indicate accumulation of PHAs, and black arrows indicate type IV pili. Bar = 500 nm. c, Bradymonas sediminis FA350T (Bs) cell co-cultured with Algoriphagus marinus am2 (Am) prey cell. The white globose granules in the cell indicate accumulation of PHAs, and several electron-dense intracellular granules (black granules) indicate polyphosphate. Bar = 500 nm. d, Bradymonas sediminis FA350T (Bs) cell attached to an Algoriphagus marinus am2 (Am) prey cell with outer membrane vesicles-like structures (shown in box area). Bar = 500 nm. e, The enlargement of figure (d) box area. Bar = 100 nm. f, Bradymonas sediminis FA350T (Bs) cell attached to an emptied and died Algoriphagus marinus am2 (Am) prey cell. Bar = 200 nm. g, SEM analysis of Bradymonas sediminis FA350 (Bs) cell co-

cultured with Algoriphagus marines am2 (Am) prey cell. White arrow indicates type IV pili, the box area indicate Bs contact with the emptied Am with type IV pili. Bar=1000 nm. h, SEM analysis of Bradymonas sediminis FA350 (Bs) cell attached to an Algoriphagus marines am2 (Am) prey cell with type IV pili (shown in box area). The white arrows indicate the OMVs-like structures. Bar = 500 nm.

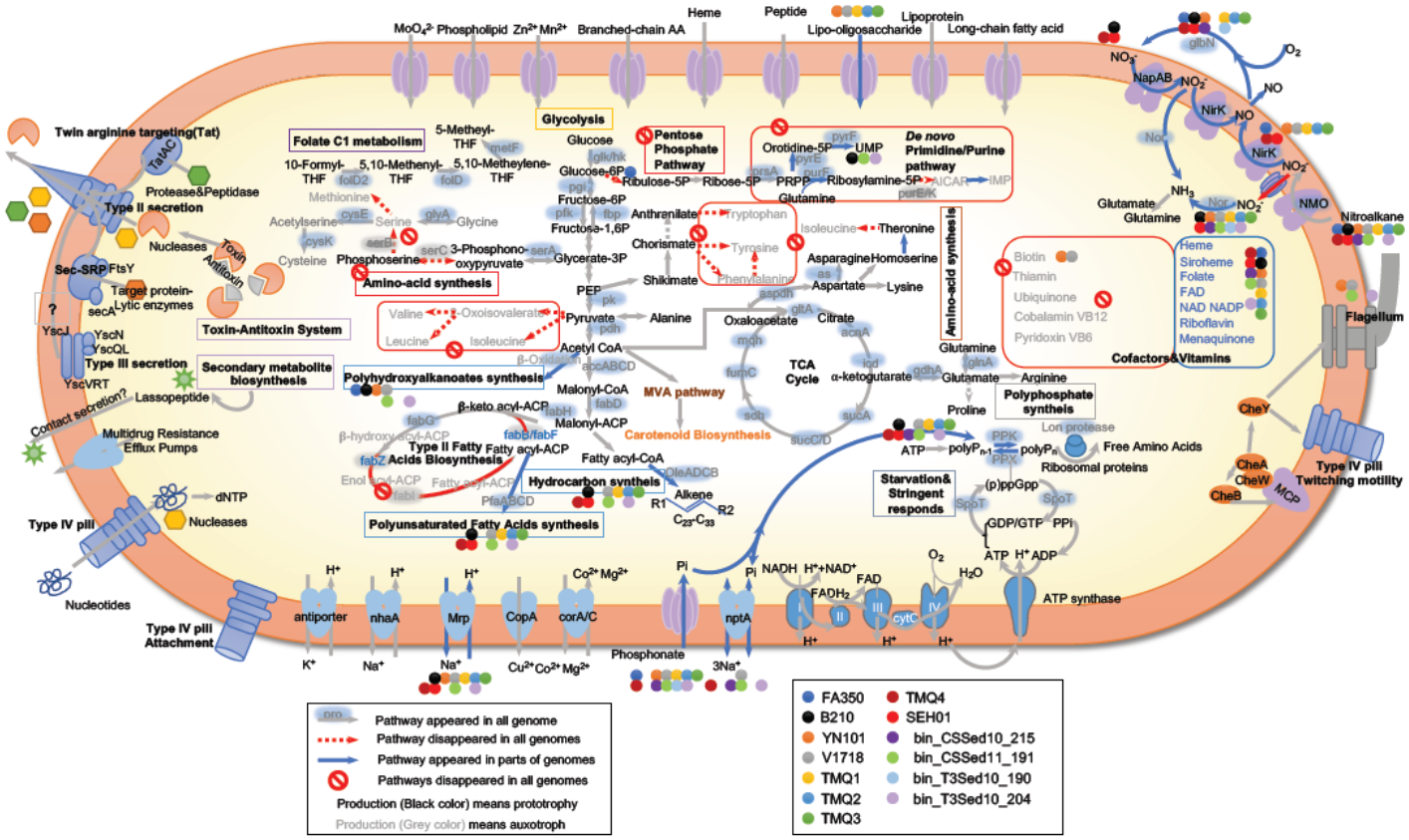

Figure 3

Metabolic capabilities of Bradymonabacteria. Metabolic predictions are mainly generated by referring to the interface KEGG and SEED database. Each subgroup of Bradymonabacteria is depicted as a colored circle (see figure legend). Functional genes (abbreviation by referring to KEGG) encoding the relevant proteins/enzymes are labeled for each metabolic step where colored circles (that is, Bradymonabacteria strain) are depicted to show the potential functions of each subgroup if any. Grey arrows indicate the corresponding genes are detected for the pathways almost in all the genomes, while red arrows indicate the corresponding genes miss from the pathways. Red “no entry” signs indicate the many key genes in pathways missing. All putative transporters and F0F1 ATPases are shown as well as secretion systems, type IV pili, and predicted components of flagella. Process of starvation and stringent-responsive systems remodeling is mediated by the production of the alarmones guanosine pentaphosphate, pppGpp, and guanosine tetraphosphate, ppGpp. Key metabolic predictions are supported by the gene information in Table S2.

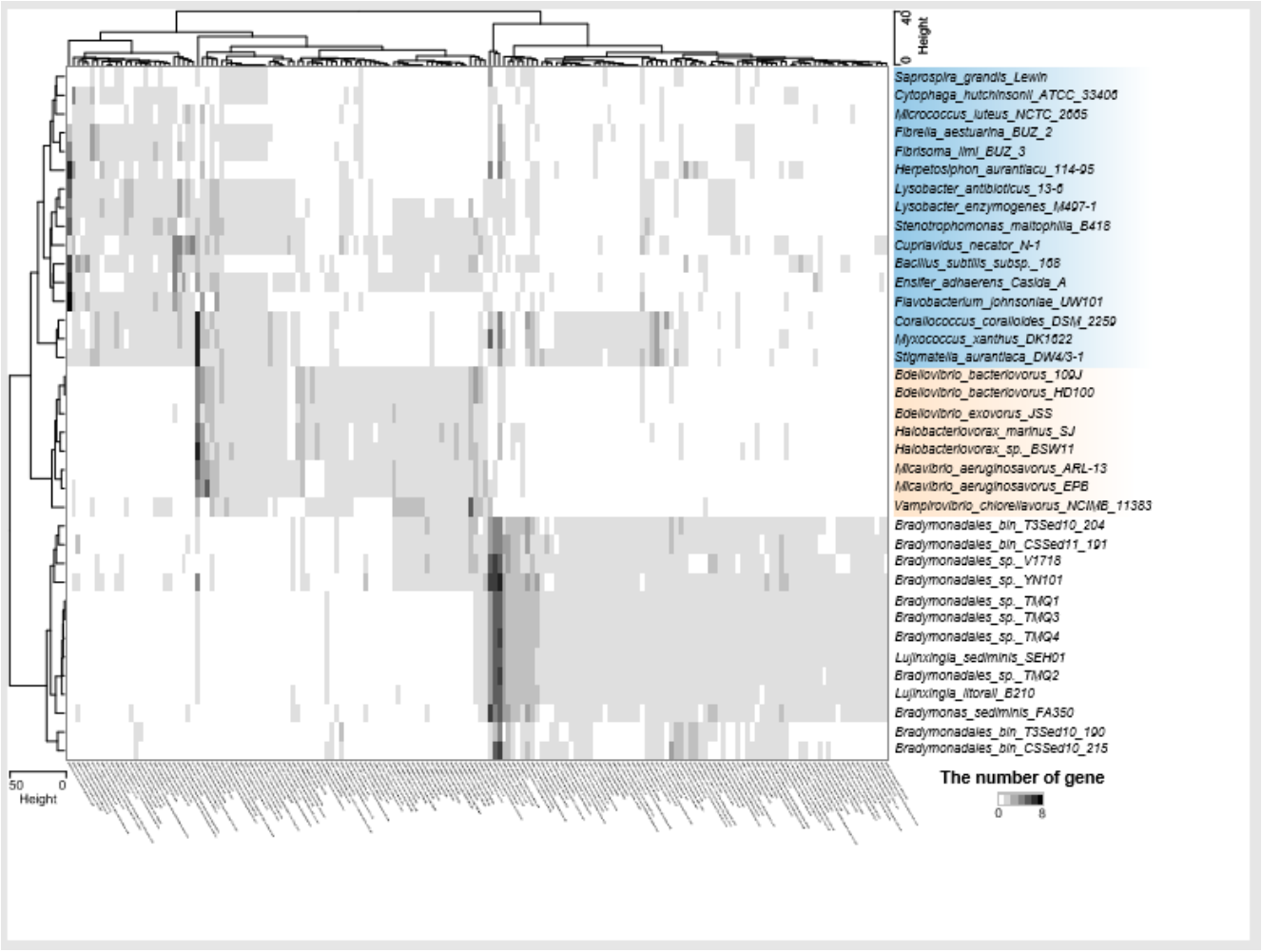

Figure 4

Gene abundance in facultative and obligate bacterial predators. the heatmap is based on two-way cluster analysis of genomic abundance of genes encoding for KEGG protein groups which were specific to either facultative predators or obligate predators. Groups in blue background indicate the so-called facultative predators, groups in yellow background indicate the so-called obligate predators, and groups in red background indicate Bradymonabacteria. Two-way cluster analysis was clustered using ward.D2 method based on euclidean distances. Gene abundance matrix is available in Table S3.

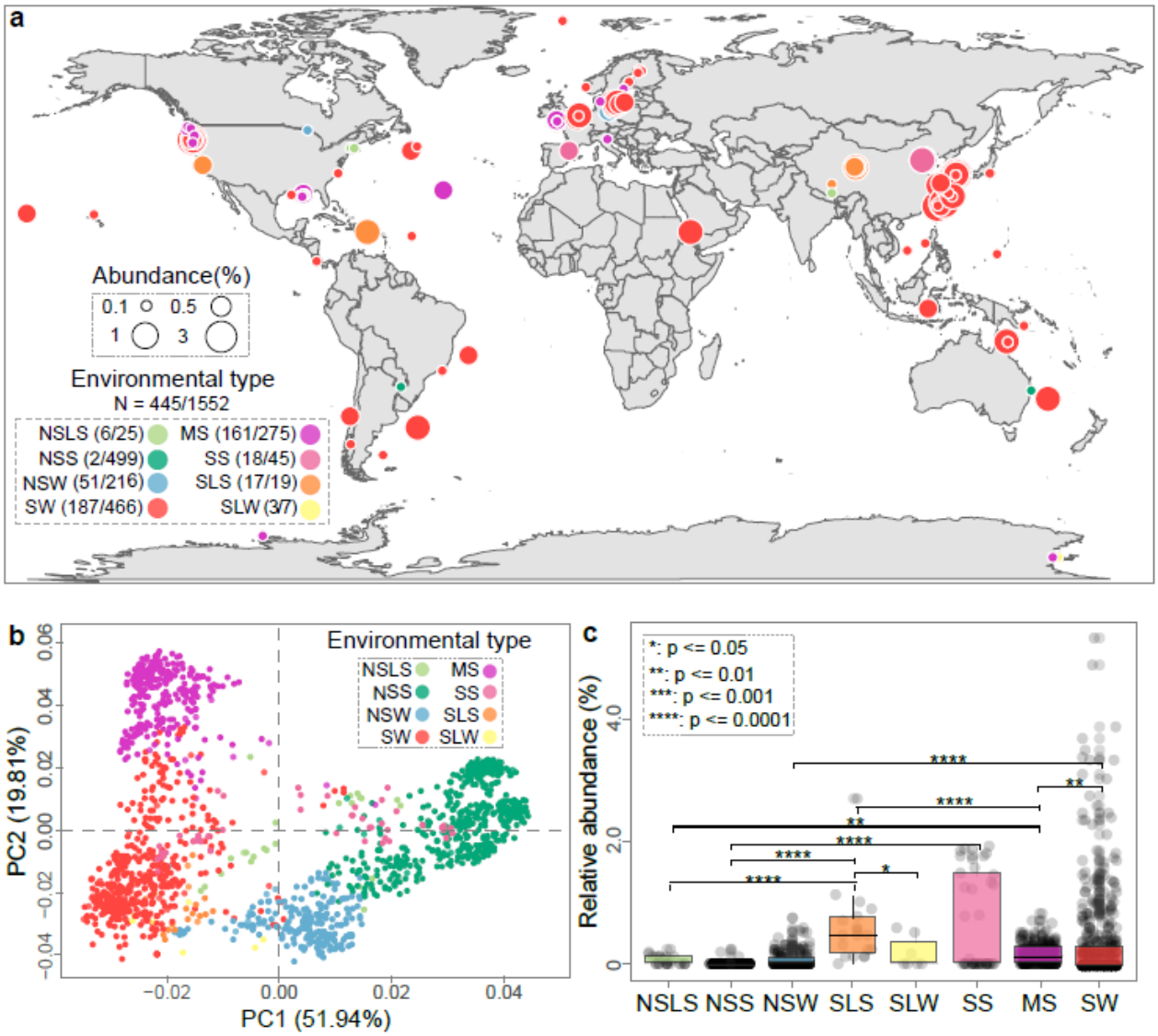

Figure 5

Global distribution and biodiversity patterns of Bradymonabacteria in eight types of biotopes from 1,552 samples. a, Global abundance of Bradymonabacteria. The abundance of 16S rRNA gene sequences of Bradymonabacteria is relative to total prokaryotic sequences in the selected samples. Each node represents one sample. Node color indicates the type of biotopes, and node size represents the relative abundance in corresponding samples. Bold numbers represents the number of samples, which detected Bradymonabacteria. b, Beta-diversity among all biologically independent samples: principal component analysis (PCA) of Bray-Curtis dissimilarity matrix, PC1 versus PC2. Clustering of all samples could be mainly explained by the type of biotopes. c, Relative abundance of 16S rRNA gene sequences among eight types of habitats. This relative abundance of Bradymonabacteria sequences was computed within each habitat (Table S1), and the significant differences among different biotopes was assessed by Kruskal–Wallis test. Abbreviation: NSLS, non-saline lake sediments; NSS, non-saline soil; NSW, non-saline water; SLS, saline lake sediments; SLW, saline lake water; SS, saline soil; MS, marine sediments; SW, sea water.

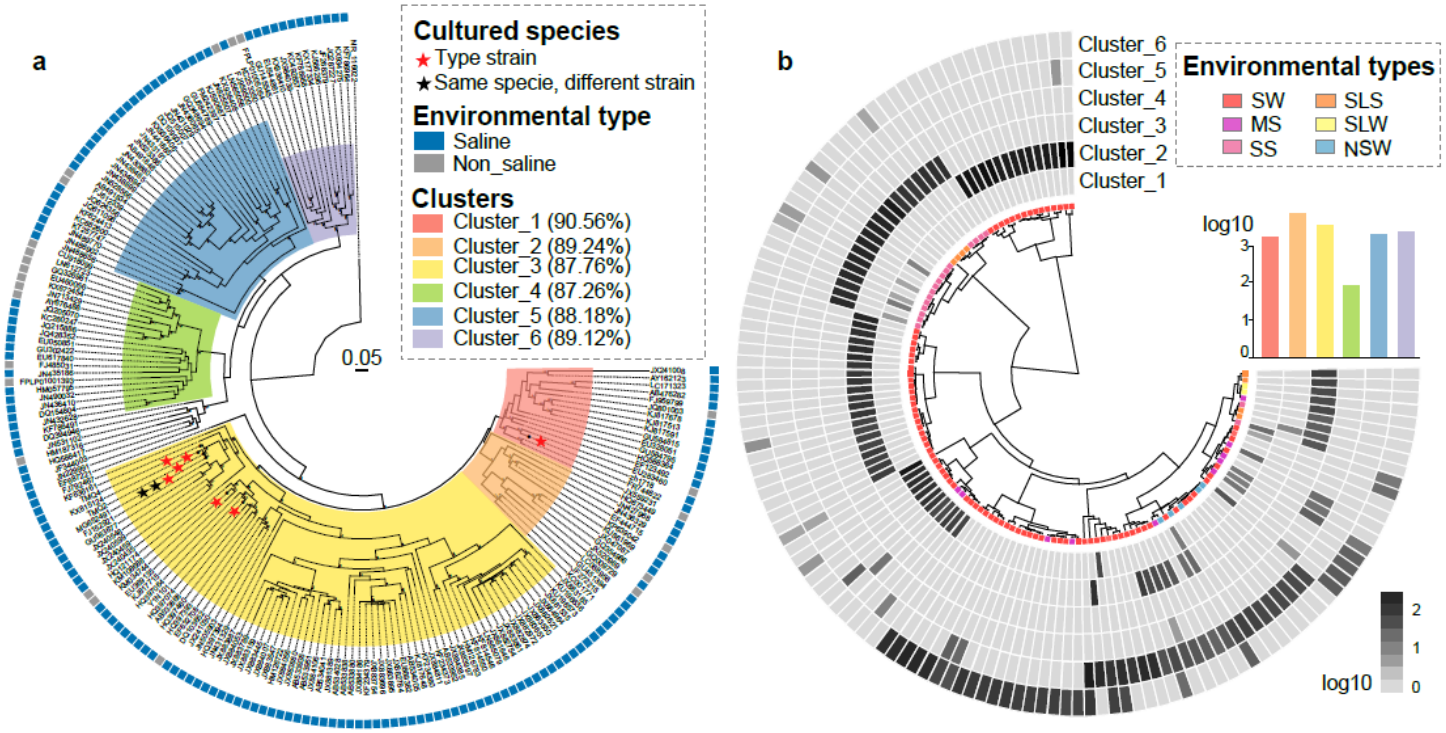

Figure 6

Phylogeny of 6 proposed subgroups of Bradymonabacteria. a, Maximum likelihood phylogenetic tree of Bradymonabacteria based on 187 representative 16S rRNA gene sequences (> 1,200bp) dereplicated at a 98.5% cutoff. Subgroups from Cluster_1 to Cluster_6 were colored within the corresponding leaves in the tree and were provided with similarity of each subgroup. Outer colored square indicate sequence original biotope: non-saline (gray) and saline (blue). Pentagram represents cultured Bradymonabacteria in our lab. All 16S rRNA gene sequences of Bradymonabacteria and the RAxML phylogenetic tree is available in Additional file 1 Material_Fig_3a_seq. b, The coverage of each subgroup of Bradymonabacteria for 127 samples. The abundance of Bradymonabacteria is relative to the total prokaryotic sequences in the corresponding samples. Biotope types are shown by colored nodes which are located under each leaf of the cluster. Bar graph indicates the reads number of each cluster in the 127 samples.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.xlsx
- TableS6.xls
- TableS7.docx
- TableS5.xls
- TableS4.xls
- Table1.docx
- SupplementaryMaterialsforMicrobiome.docx
- TableS3.xls
- TableS2.xlsx