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## *Parabacteroides distasonis*: intriguing aerotolerant gut anaerobe with emerging antimicrobial resistance and pathogenic and probiotic roles in human health

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## ***Parabacteroides distasonis*: intriguing aerotolerant gut anaerobe with emerging antimicrobial resistance and pathogenic and probiotic roles in human health**

Jessica C. Ezeji<sup>a,b,\*</sup>, Daven K. Sarikonda<sup>a,b,\*</sup>, Austin Hopperton<sup>a,b,c</sup>, Hailey L. Erkkila<sup>a,b</sup>, Daniel E. Cohen<sup>a,b</sup>, Sandra P. Martinez<sup>d</sup>, Fabio Cominelli<sup>a,b,e,f</sup>, Tomomi Kuwahara<sup>g</sup>, Armand E. K. Dichosa<sup>h</sup>, Caryn E. Good<sup>e</sup>, Michael R. Jacobs<sup>e</sup>, Mikhail Khoretchenko<sup>j</sup>, Alida Veloo<sup>k</sup>, and Alexander Rodriguez-Palacios<sup>a,b,f,l</sup>

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### ABSTRACT

*Parabacteroides distasonis* is the type strain for the genus *Parabacteroides*, a group of gram-negative anaerobic bacteria that commonly colonize the gastrointestinal tract of numerous species. First isolated in the 1930s from a clinical specimen as *Bacteroides distasonis*, the strain was re-classified to form the new genus *Parabacteroides* in 2006. Currently, the genus consists of 15 species, 10 of which are listed as 'validly named' (*P. acidifaciens*, *P. chartae*, *P. chinchillae*, *P. chongii*, *P. distasonis*, *P. faecis*, *P. goldsteinii*, *P. gordonii*, *P. johnsonii*, and *P. merdae*) and 5 'not validly named' (*P. bouchedurhonensis*, *P. massiliensis*, *P. pacaensis*, *P. provencensis*, and *P. timonensis*) by the List of Prokaryotic names with Standing in Nomenclature. The *Parabacteroides* genus has been associated with reports of both beneficial and pathogenic effects in human health. Herein, we review the literature on the history, ecology, diseases, antimicrobial resistance, and genetics of this bacterium, illustrating the effects of *P. distasonis* on human and animal health.

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## 1 Introduction

The intestinal gut is home to the largest collection of microbes, harboring trillions of bacteria and representing hundreds of species. Most of these species fall into two groups – *Bacteroidetes* and *Firmicutes*. These were among the first phyla that comprise most of the dominant gut commensal bacteria to be defined. Within the *Bacteroidetes* phylum, the relatively new genus *Parabacteroides* (defined as separate from its predecessor, the very broad *Bacteroides* genus, in 2006<sup>1</sup>), now contains at least ten 'valid species,' which are recognized as being real species in clinical settings, and five 'non-valid species,' which are not recognized as being real species in clinical settings by the List of Prokaryotic names with Standing in Nomenclature. Several of these

species were identified in isolates from clinical infections.

Recently, we isolated and fully sequenced multi-drug resistant *P. distasonis* from deep-gut wall tissue lesions in patients with Crohn's Disease that underwent surgical removal of the affected bowel further supporting a potential pathogenic role on gut wall health. Recent studies suggest that *P. distasonis* could exert protective effects against certain diseases, including multiple sclerosis, type II diabetes, colorectal cancer, and inflammatory bowel disease. Furthermore, some reports suggest that this bacterium could even have the potential to serve as a potential probiotic to promote digestive health in humans based on microbiome or animal studies. However, other experimental data show contradictory results, suggesting pathogenic effects

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in various disease models. This suggests that *P. distasonis* may serve a dichotomous role depending on the context.

Herein, we hypothesize and illustrate that the pathologic role of these bacteria in human diseases may depend on the context, including the susceptibility of the host to immune suppression, impaired bacterial clearance, and the promotion of hyperinflammatory responses, together with strain-to-strain differences that may account for differences in antimicrobial resistance to human therapies and *P. distasonis* potential for pathogenicity.

## 2 The new genus *Parabacteroides*

As with several other new genera reclassified from the initially broad genus *Bacteroides fragilis* group, including *Alistipes*<sup>1</sup>, *Parabacteroides* is a relatively new genus with distinctive features shared among other gut commensal bacteria. It is important to highlight that great progress in our understanding of this and other related genera has been facilitated by the taxonomic contributions made by Japanese microbiologists Mitsue Sakamoto and Yoshimi Benno who proposed the genus,<sup>2</sup> and by other international groups cited below. According to the NCBI, the full taxonomy lineage for the genus *Parabacteroides* is: Bacteria; FCB group; *Bacteroidetes/Chlorobi* group; *Bacteroidetes*; *Bacteroidia*; *Bacteroidales*; *Tannerellaceae*; *Parabacteroides*. Of note, the family *Tannerellaceae* is composed of *Parabacteroides* and another new genus, *Tannerella*, with the species *T. forsythia* and *T. pachnodae*.

As of May 1, 2021, the *Parabacteroides* genus (taxonomy ID: 375,288) comprises 15 species (<https://lpsn.dsmz.de/search?word=parabacteroides>), represented by 493 genome assemblies available in the NCBI. Of these species, 10 are listed as 'validly named', and 5 as 'not validly named' (shown in quotation marks)<sup>3</sup>: *P. acidifaciens* (human feces, China, 2019; type strain (TS): 426-9; CGMCC 1.13558; NBRC 113,433),<sup>4</sup> "*P. bouchesdurhonensis*" (healthy human feces, France, 2018; TS: CSUR P3763; Marseille-P3763;),<sup>5</sup> *P. chartae* (wastewater of a paper mill, China, 2011, TS: DSM 24,967; JCM 17,797; NS31-3),<sup>6</sup> *P. chinchillae* (chinchilla feces in a zoo, Japan, 2013; TS: CCUG 62,154; DSM 29,073; JCM

17,104; ST166),<sup>7</sup> *P. chongii* (blood from human with peritonitis, Republic of Korea, 2018; TS: B3181; KACC 19,034; LMG 3006),<sup>8</sup> *P. distasonis* (unclear, human feces, or peritonitis as reported in PATRIC.org, USA, 1933; TS: ATCC 8503; CCUG 4941; CIP 104,284; DSM 20,701; JCM 5825; NCTC 11,152, named in honor to microbiologist A. Distaso),<sup>2</sup> *P. faecis* (human feces; Japan, 2015; TS: 157; CCUG 66,681; JCM 18,682),<sup>9</sup> *P. goldsteinii* (peritoneal fluid, appendix tissue, and intra-abdominal abscess, USA, 2005; TS: ATCC BAA-1180; CCUG 48,944; DSM 19,448; JCM 13,446; WAL 12,034, named in honor to infectious disease clinician Ellie J. C. Goldstein),<sup>2,10</sup> *P. gordonii* (human blood clinical culture, USA, 1930s; TS: CCUG 57,478; DSM 23,371; JCM 15,724; MS-1, named in honor to microbiologist Jeffrey I. Gordon), *P. johnsonii* (human feces, Japan, 2007; TS: DSM 18,315; JCM 13,406; M-165, named in honor to American molecular taxonomist John L. Johnson, who was the first to describe *Bacteroides merdae* (*P. merdae*)),<sup>9</sup> "*Parabacteroides massiliensis*" (healthy human feces, France, 2019; TS:SN4; named after Latin name of Marseille, Massilia),<sup>11</sup> *P. merdae* (human feces, USA, 1978; TS: ATCC 43,184; CCUG 38,734; CIP 104,202; DSM 19,495; JCM 9497; NCTC 13,052; VPI T4-1),<sup>2,12</sup> "*P. pacaensis*" (healthy human feces, France, 2020; TS: Marseille-P4001; named after abbreviation for the region of Provence Alpes Côte d'Azur),<sup>13</sup> "*P. provencensis*" (healthy human feces, France, 2020; TS: Marseille-P3668 T; nomenclature status listed as 'not validly published', named after the region of Provence),<sup>13</sup> and "*P. timonensis*" (human feces from healthy pigmy 39-year-old male, Congo, 2019; TS: CCUG 71,183; CSUR P3236; Marseille-P3236; nomenclature status listed as 'not validly published', named after French hospital *La Timone*).<sup>14</sup>

To provide an example of genetic similarity with other fecal commensals within the *Bacteroidetes*, based on 16s RNA gene sequence similarity, the three founding species of the *Parabacteroides* genus— *P. distasonis* JCM 5825 T, *P. goldsteinii* JCM 13,446 T, and *P. merdae* JCM 9497 T— are phylogenetically closely related to each other (>92.3% DNA sequence similarity) and related to *T. forsythia* with about 90% similarity. However, they are distant from their predecessor genus

*Bacteroides* (83.5–88.8%) and other comparable genera including *Dysgonomonas* (85.9–89.4%), *Paludibacter* (86.9–88.5%), *Porphyromonas* (82.2–86.9%), *Prevotella* (77.2–81.9%) and *Proteiniphilum* (85.9–87.3%).<sup>2</sup>

## 2.1 *Parabacteroides distasonis* as a referent species for the genus

Of all the species listed previously, *P. distasonis* is the reference type strain for the genus *Parabacteroides*. Since the completion of the first genome sequence for the species by investigators at Washington University in St. Louis in 2007, *P. distasonis* strain ATCC 8503 has become the strain comparator for all *Parabacteroides* species.<sup>15</sup> This type strain is an isolate deposited in 1933 and is a gram-negative, non-spore-forming, rod-shaped, strict anaerobic bacterium present in the gastrointestinal (GI) tract of humans and animals.<sup>2,16</sup> A single *P. distasonis* ATCC 8503 cell is  $0.8\text{--}1.6 \times 1.2\text{--}12\ \mu\text{m}$  in size. Its colonies on sheep blood agar plates are 1–2 mm in diameter, appear gray to off-white, and are circular, slightly convex, and smooth in shape.

Recently, studies on *P. distasonis* have displayed evidence in support of *P. distasonis* having a potentially beneficial and commensal nature, while others have displayed evidence for a potential pathogenic role. Although *P. distasonis* is part of the normal gastrointestinal microbiota, it has been isolated from extra-intestinal abdominal infections and abscesses in humans. More recently, *P. distasonis* has been shown to have ambivalent effects on models of inflammatory bowel disease (IBD) in rodents (“murine models”), with reports describing both pro-inflammatory (pathogenic) and anti-inflammatory (beneficial) effects.<sup>17</sup> Thus, *P. distasonis* currently has unclear mechanistic associations with the main forms of inflammatory bowel disease (IBD), namely Crohn’s Disease (CD) and ulcerative colitis (UC), in both humans and animal models<sup>18</sup>.

As a result of this and other contradictory findings, *P. distasonis* has become a highly controversial bacterium in the gastroenterological and microbiological fields of study. In 2020 and early 2021 alone, over twenty studies were published involving *P. distasonis*, many of which produced

controversial findings. Here, we delve into basic concepts on the history, biology, and ecology of the bacterium; its resistance to antibiotics; its genomic features; and its potential clinical relevance in the context of human health. We highlight papers that support both sides of the debate over *P. distasonis*’ pathogenicity and present a summary of potential future perspectives on our analysis of this microbe.

## 3 History, ecology, and identification

### 3.1 Historical overview and reclassification

*Parabacteroides distasonis* is a re-classified bacterium named after A. Distaso, a Romanian bacteriologist who was involved in the description of the *Bacteroides* phylum species in the 1910s.<sup>18,19</sup> Originally, *P. distasonis* was considered part of the *Bacteroides* genus, where it bore the name *Bacteroides distasonis*. Prior to the 1980s, classification of bacteria was based on phenotypic features, which meant that all gram-negative rods with certain phenotypic profiles were designated as being part of *Bacteroides*.<sup>20</sup> For decades, only minor changes in taxonomy were implemented, even though the *Bacteroides* genus increasingly started to contain vast numbers of strains and species (>50) that greatly differed phenotypically from one another.<sup>21</sup>

This began to change in the 1980s, when Woese *et al.* introduced 16S rRNA gene sequencing.<sup>20</sup> After using this method in 1989, Shah & Collins formally proposed that the genus *Bacteroides* be restricted to *Bacteroides fragilis* and related taxa, resulting in the amendment of the description of the genus.<sup>21</sup> This spawned several novel genera over the next decade and into the early 2000s, including *Alistipes*, *Dialister*, *Dichelobacter*, and *Tannerella*.<sup>2</sup>

Skepticism about the classification of what was then called ‘*B. distasonis*’ and another closely related species, *B. merdae*, arose in the mid-1990s and early 2000s, when 16S rRNA gene sequence analyses raised the idea that the two species may also have not been members of the *Bacteroides* genus. Shortly after the discovery of another species closely related to *B. distasonis* and *B. merdae*, *B. goldsteinii*, Sakamoto and Benno analyzed the

16S rRNA gene sequences of all three species, using *Tannerella forsynthesis* as an outgroup. Phylogenetic analyses showed that the three species were closely related and therefore belonged to the same genus, with *Tannerella* being the most closely related genus. This was confirmed by analyses of the 16S-23S rRNA gene internal transcribed spacer (ITS) regions, which showed that *B. distasonis* was phylogenetically distinct from species of the genus *Bacteroides*, along with other former *Bacteroides* species including *B. goldsteinii* and *B. merdae*.<sup>2</sup>

In addition to genetic differences, Sakamoto and Benno demonstrated differences in chemotaxonomic features. Ratios of anteiso-C<sub>15:0</sub> to iso-C<sub>15:0</sub> ranged from 3.1 to 10.3 in *B. distasonis*, *B. goldsteinii*, and *B. merdae* strains, while those for *T. forsynthesis* ranged from 22.8 to 95.2. Major menaquinones of *B. distasonis*, *B. goldsteinii*, and *B. merdae* were MK-9 and MK-10, while the same for *T. forsynthesis* and *Bacteroides* species were MK-10 and MK-11. Taken together, the differences observed prompted Sakamoto and Benno to propose that these species be placed in their own genus, named *Parabacteroides*, meaning “adjacent to *Bacteroides*”.<sup>2</sup>

### 3.2 Ecology

*P. distasonis* has been detected in healthy and unhealthy patients, although the loads compared to other members of the *Bacteroides* group may vary across studies depending on the methods of detection used and factors like diet, which remain largely uncharacterized. Earlier studies utilizing immunoassays and monoclonal antibodies to identify *Bacteroides* species revealed that more than 30% of bacteria in human feces were *Bacteroides* species.

As a member of the distal gut microbiome, *P. distasonis* is present in fecal matter, which allows it to pollute waters and reach other environments passively. Consequentially, this bacterium has been proposed to be used as an indicator of fecal pollution in public environments, including recreational waters. In the waters of the Ohio River in the USA, Kreader *et al.*<sup>22</sup> showed that *P. distasonis* can survive for up to two weeks if kept under 4°C; however, the bacterium can only survive for two days if kept at 24°C. Kreader *et al.*<sup>22</sup> also showed that in

filtered water or in the presence of cycloheximide (a protein synthesis inhibitor) the persistence of *P. distasonis* in water at 24°C was extended by at least 7 days.<sup>22</sup> These are remarkable observations that support the fact that such a strict anaerobe can remain dormant in adverse conditions,<sup>22</sup> as also observed in our laboratory. In another study, researchers using PCR-specific primers and Luminex® 100™ based technology (a suspension array that assays multiple analytes in a single well of a microtiter plate) detected *P. distasonis* in river samples, public water systems, and beach sand.<sup>23</sup> Innovative membrane filtration techniques have also shown that *P. distasonis* and similar *Bacteroides* species are commonly found in natural waters.<sup>24</sup>

The sources of fecal pollution in water for this abundant *Bacteroidetes* species could be traced to homeothermic hosts (including humans) with microbiome-based approaches; however, such methods often lack the resolution to classify sequenced bacteria down to the species level.<sup>25</sup> A few examples for the genus may serve to illustrate the ecological range of this species. Recent high-throughput microbiome sequencing has shown that *Parabacteroides* is one of the main genera present in the GI, ceca, and feces of ducks raised with free access to swimming waters, but not in ducks raised with no access to swimming waters.<sup>26</sup> Regarding abundance in this context, at the genus level, *Parabacteroides* was among the most abundant genera in ducks allowed to swim (*Bacteroides*, 25%; *Escherichia-Shigella*, 11%; *Peptococcus*, 7.7%; and *Parabacteroides*, 5.86%) compared to the genera in ducks with no swimming access (*Bacteroides*, 18.1%; *Erysipelatoclostridium*, 10.9%; *Ruminococcaceae\_unclassified*, 10.4%; *Lachnospiraceae\_unclassified*, 5.2%; *Coriobacteriales\_unclassified*, 5.89%; and *Faecalibacterium*, 4.2%).

The presence of this species in feces and their survival in natural waters supports our proposal that water and migratory birds could serve as an important avenue for *Parabacteroides* species dissemination between and across avian and mammalian species, possibly increasing the risk of antimicrobial resistant strains appearing in clinical settings. If human-originated isolates of *P. distasonis* can appear in chicken or other animals, then this bacterium might be able to reach humans via food and livestock, as is common

among other aerobic or anaerobic foodborne or food-dwelling microbes.<sup>27</sup>

### 3.3 Phenotyping and identification

Numerous methods exist for culturing *P. distasonis*. As a saccharolytic bacterium, *P. distasonis* is capable of metabolizing carbohydrates such as mannose and raffinose for energy production and can grow on a medium containing 20% bile.<sup>2</sup> Furthermore, the latter enables *P. distasonis* to selectively grow on *Bacteroides* bile esculin agar.<sup>28</sup>

*P. distasonis* is urease negative, but possesses genes that confer resistance to oxidative stress, such as the *KatE* gene and the *OxyR* gene. Deemed advantages of *P. distasonis* as an aerotolerant commensal stem from the unique properties of its versions of catalase and superoxide dismutase.<sup>29</sup> Catalase production enables a detoxifying role against oxidative stress mediated by hydrogen peroxide, which is often produced by inflammatory cells. The decomposition of H<sub>2</sub>O<sub>2</sub> is vitally important for the survival of bacterial cells in conditions where oxygen is present. H<sub>2</sub>O<sub>2</sub> is a major inhibitor of growth for gut bacteria that lack the ability to decompose H<sub>2</sub>O<sub>2</sub> into less reactive molecules. Catalase production is not a universal survival strategy among *Bacteroides*; *P. distasonis* and *B. fragilis* are catalase positive, while *B. eggerthii*, *B. thetaiotaomicron*, and *B. ovatus* have variable catalase production; and *B. vulgatus* and *B. uniformis* are catalase-negative.<sup>30</sup> Molecular studies have shown that *P. distasonis* produces a catalase similar to that of *B. fragilis*, but in contrast, the enzyme is twice the size (250,000).<sup>30</sup> Several variables may affect *P. distasonis*' catalase production, including the type of medium used, the presence of agar, and the addition of hemin either pre- or post-autoclaving.<sup>31</sup> Higher catalase levels occur after hemin is added post-autoclaving and with a high carbohydrate content in the selected medium.<sup>31</sup>

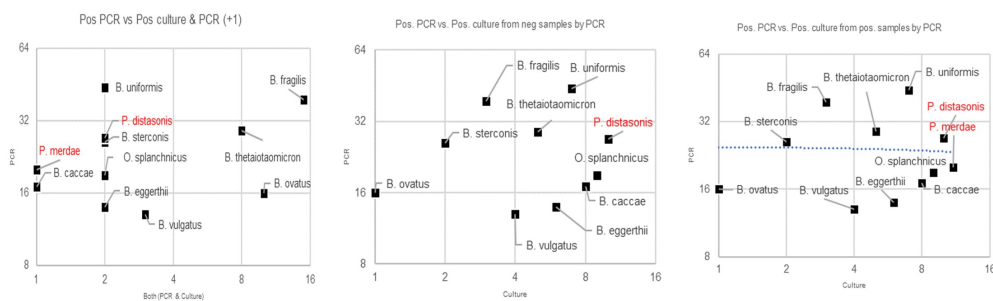
*P. distasonis* cannot hydrolyze gelatin in liquefaction tests used to identify bacterial proteolytic enzymes, unlike *Bacteroides* species such as *B. ovatus*.<sup>2</sup> Gelatin tests primarily seek to assess the presence of bacterial matrix metalloproteinases, which are also abundant in activated (gene expression upregulated) host cells. Although gelatin

hydrolysis tests may yield false negatives, tests indicate that *P. distasonis*' mechanisms of intestinal modulation are not mediated by gelatinases (*gelE* gene co-transcribed with *sprE*, regulated by the *fsrA*, *fsrB*, and *fsrC* gene family) as they are in *Enterococcus faecalis*, which is important to help the bacteria translocate across polarized T84 human colon cancer cell models.<sup>32</sup>

In addition to culturing, polymerase chain reaction (PCR) methods enable the identification (with Sanger sequencing of the 16s rRNA gene), quantification of bacterial abundance in tissues when using quantitative reverse transcriptase (qRT-PCR), and differentiation of *P. distasonis* from other closely related bacteria including *P. merdae* and *Odoribacter splanchnicus*. Relative to anaerobic culturing methods, qRT-PCR is a highly accurate and rapid alternative when identifying *Bacteroides* species and related species, including *P. distasonis*, as shown in Figure 1.<sup>33</sup> Probes designed for *P. distasonis* ATCC 8503 include the forward primer sequence 5'-TGC CTA TCA GAG GGG GAT AAC-3', the reverse primer sequence 5'-GCA AAT ATT CCC ATG CGG GAT-3', and the probe sequence 5'Fam-CGA AAG TCG GAC TAA TAC CGC ATG AAGC-3'Tam.<sup>23</sup>

Another identification method for *P. distasonis* is Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS), which is a fast and cost-effective method for accurately identifying microorganisms based on the profiling of the ionized ribosomal proteins.<sup>34</sup> Unlike PCR methods, the clinical value of MALDI-TOF depends on the quality and breadth of coverage of the databases used to identify any given sample.

In a study by Veloo *et al.*,<sup>35</sup> *P. distasonis* was the most commonly identified *Parabacteroides* species in human clinical specimens when using MALDI-TOF MS. The role of the MALDI-TOF MS system in correctly identifying emerging species that are either rare or difficult to grow *in vitro* is influenced by several factors that impair our ability to identify and correctly assign a pathogenic role to clinical specimens for such emergent species<sup>1</sup>. Using the identification dataset version 5 for MALDI-TOF MS, Veloo *et al.*, on behalf of the numerous laboratories collaborating within the European Network for the Rapid Identification of Anaerobes (ENRIA)



**Figure 1. Correlation of PCR identification vs culture isolation from samples that were detected or undetected via qRT-PCR.** Eleven *Bacteroides* and *Parabacteroides* species detected via qRT-PCR in 400 human surgical wound infection samples or closed abscesses. Target bacteria were detected from 31 samples (8%) via culture vs. 132 samples (33%) via qRT-PCR ( $p$ -value < 0.001). For each species, qRT-PCR detected higher counts than culture; this may reflect the detection of DNA of dead organisms by qRT-PCR. Plot created for this manuscript to illustrate the correlation between qRT-PCR and anaerobic culture results for *Bacteroides* species isolated from wound samples using 132 isolates.<sup>33</sup> **a)** y-axis corresponds to number of isolates detected by qRT-PCR; x-axis corresponds to number of isolates detected by both qRT-PCR and culture. **b)** y-axis corresponds to number of isolates detected by qRT-PCR; x-axis corresponds to number of isolates detected by culture. **c)** y-axis corresponds to number of isolates detected by qRT-PCR. Adapted from using data from Tong *et al.*<sup>33</sup> with permission. Available from Anaerobe and used with permission from Elsevier.

project and using 6309 human clinical anaerobic bacterial strains, reported that only four well-established species were represented in the database with most clinical infections linked to *P. distasonis* ( $n = 45/54$ ), *P. goldsteinii* ( $n = 3/54$ ), *P. johnsonii* ( $n = 1/54$ ), and *P. merdae* ( $n = 5/54$ ). The MALDI-TOF MS database is regularly updated. Currently, *P. faecis* and *P. gordonii* are also represented in the database.

Of these species, *P. distasonis* was the species with the highest MALDI-TOF MS log-score values indicating a “more reliable identification” profile.<sup>35</sup> Current efforts of optimization that exist on MALDI-TOF MS databases show that *P. distasonis* can be accurately identified by this method, with log-score values of  $\geq 2.0$  in 44 of 45 isolates.<sup>35</sup> In a similar study by Rodríguez-Sánchez *et al.*,<sup>34</sup> six *P. distasonis* isolates were identified by the MALDI-TOF MS system at the species level. This was the same number of isolates detected using 16S rRNA sequencing at the genus level, meaning that all isolates were successfully identified via the MALDI-TOF MS system. The Rapid ID 32A system, a phenotypic method, was also able to identify all six *P. distasonis* isolates at the species level.<sup>34</sup>

In another study, using both the MALDI-TOF MS system and the Rapid ID 32A system, the single *P. distasonis* isolate (abundance = 0.74%) involved was successfully detected.<sup>36</sup> Together, these studies, indicate that *P. distasonis* is a species of higher

abundance, is more readily cultivable, and is identifiable by several detection methods. This is perhaps the reason why it was the first species isolated to be assigned to the *Parabacteroides* genus. These methods prompt future studies to examine mechanisms by which this species mediates human health; however, emerging strains will need to be considered to help define the genetics behind the modulation of either effect.

## 4 Evolution, biochemical features, and metabolism

### 4.1 Evolution and symbiotic adaptation to the gut

According to phylogenetic analyses, *P. distasonis* diverged from a common ancestor shared with *Bacteroides* species, as confirmed via nucleotide sequencing of the complete 16S rRNA gene from distinct bacterial species.<sup>37</sup> A study by Xu *et al.*<sup>37</sup> explored the driving forces behind the adaptation of *Bacteroidetes* in the distal gut environment and their importance to the evolution of human gut commensals. To examine how the intestinal environment affects microbial genome evolution, Xu *et al.*<sup>37</sup> sequenced the genomes of two members of the distal human gut microbiota, *B. vulgatus* and *P. distasonis*. Through comparison with other sequenced gut and non-gut *Bacteroidetes*, and analyzing their niches and habitat adaptations, Xu *et al.* identified three general functions that could illustrate an evolutionary



uniqueness for the *Bacteroidetes* phylum: polysaccharide metabolism, environmental sensing and gene regulation, and membrane transport. These processes are important in aiding with lateral gene transfer, mobile elements, and gene amplification, all of which affect the ability of gut-dwelling *Bacteroidetes* to vary their cell surface, sense their environment, and harvest nutrients present in the distal colon.<sup>37</sup> More recently, genome-based analyses have examined the metabolic features that are typical for a wide array of *Bacteroides*, which complements the taxonomic classification of the phylum/species within.<sup>38–40</sup>

*P. distasonis* possesses a vast number of laterally transferred genes relative to similar species, which may help it thrive in the distal gut microbiome. Some genes that have been transferred allow hydrogen to be the final electron acceptor in the electron transport chain rather than oxygen. This is an important trait for successful energy utilization in the anaerobic environment of the gut. In addition, *P. distasonis* contains fewer environmental sensing and gene regulation genes compared to similar species and has a smaller number of carbon source degradation genes such as hemicellulases, pectinases, and other polysaccharides that help break down non-plant-based carbohydrates.<sup>37</sup>

In a study involving an individual that received ceftriaxone therapy, *P. distasonis* demonstrated monodominance in the gut microbiota community. Hildebrand *et al.*<sup>41</sup> observed an extreme bloom of *P. distasonis* in the gut, where it was the second most abundant commensal with a 95% relative abundance. Here, *Borkfalki ceftriaxensis* was the most common conditionally monodominant taxa (CMT) that was not invasive. The initial colonization by this species was succeeded by *P. distasonis*.<sup>41</sup> Thus, *P. distasonis* is a key member of the gut microbiota community, rendering it as a highly influential bacterium.

#### 4.2 Lipopolysaccharide and S-layer to blend in with the gut environment

*Parabacteroides distasonis*' lack of polysaccharide degradation genes is compensated for by the bacterium's unique surface layer (S-layer) composed of glycoproteins. Fletcher *et al.*<sup>42</sup> identified at least nine glycoproteins utilized by *P. distasonis* using the lectin-affinity purification technique. Intriguingly, the researchers found that seven of

the nine glycoprotein promoters identified undergo DNA inversion, predominantly in their endogenous human environment.<sup>42</sup>

In 1996, before *P. distasonis* was considered a member of the *Parabacteroides* genus, Corthier *et al.*<sup>43</sup> utilized freeze etch electron microscopy to visualize an S-layer in *P. distasonis* that was not present in most *Bacteroides* spp., including *B. fragilis* and *B. thetaiotaomicron*.<sup>43</sup> This monomolecular layer allows both for the breakdown of complex polysaccharides that cannot be broken down by human enzymes and for the acquisition and synthesis of polysaccharides to “blend in” with the surrounding intestinal tissue. This is possible through the use of an enzyme to coat the S-layer with sugar residues from the surrounding environment. It is thought that this ability to “blend in” with the surrounding gut tissue allows *P. distasonis* to avoid triggering a strong immune response from the host.<sup>42</sup> In fact, in one study, after a lipopolysaccharide (LPS) challenge, the introduction of a membrane fraction of *P. distasonis* “reduced the release of TNF, IL-6, CCL2 (MCP-1) and CCL12 (MCP-5) by macrophages.”<sup>44</sup>

This unique S-layer could form the basis of *P. distasonis*' effective pathogenic nature. Understanding the potentially pathogenic nature of *P. distasonis* is crucial for comprehending the interactions between the host or host's environment and this bacterium.<sup>42,45–49</sup>

#### 4.3 Hypothetical contribution to local anti-inflammatory methane production

It is thought that fermentation by *P. distasonis* results in the production of methane. It is unclear if direct production of methane occurs in *P. distasonis*; however, it is known that *P. distasonis* produces hydrogen, carbon dioxide, formic acid, acetic acid, carboxylic acid, and succinic acid.<sup>2</sup> Other microbes may convert the carbon dioxide and acetic acid to methane. Acetogenic bacteria might then oxidize the acids, obtaining more acetic acid and either hydrogen or formic acid. Finally, in complex gut communities, methanogens may convert acetic acid to methane.

However, there is evidence that methane may also serve a pathogenic role. Methane production has been shown to be involved in the pathogenesis

of other intestinal diseases, such as constipation-predominant irritable bowel syndrome (C-IBS), diverticulosis, and colorectal cancer.<sup>50</sup> Furthermore, methane production may hinder ileal motility, providing an explanation for its ability to induce constipation.<sup>51</sup>

Whether *P. distasonis* contributes (likely indirectly) to local production of methane is at this time speculative, but there is a need to consider that the ultimate beneficial or pathogenic role on any animal model of disease, or clinical disease, might also depend on other species present in the local microbiome.

#### 4.4 Succinic acid as proinflammatory signaling molecule

Comprehensive analysis of the end-products of fermentation have indicated that *P. distasonis* and other *Bacteroides* and *Prevotella* spp. primarily produce acetic acid and succinic acid, in contrast to other microbes that produce other acids in a less specific manner.<sup>2</sup> Succinate serves as an inflammatory signal in immune cells to induce IL-1 $\beta$  through HIF-1 $\alpha$  (transcription factor induced by hypoxia), a downstream target of succinate.<sup>52–54</sup> Succinate can stimulate reactive oxygen species (ROS),<sup>55,56</sup> and succinate accumulation in immune cells acts as an inflammatory signal for macrophages via HIF-1 $\alpha$ .<sup>52</sup> HIF-1 $\alpha$  activation attenuates Treg development, induces IL-17 production, and increases ROR $\gamma$ t transcription, favoring differentiation of T lymphocytes into pro-inflammatory T<sub>H</sub>17 cells.<sup>57</sup> Succinate is also a ligand for succinate-receptor 1 (SUCNR1; formally GPCR91) expressed by dendritic cells<sup>53</sup> and can enhance both the pro-inflammatory cytokine (TNF $\alpha$  and IL-1 $\beta$ ) production and the antigen presentation capacity of dendritic cells, thereby inducing adaptive immune responses.<sup>53,58</sup> In the colonic mucosa of rats, succinic acid leads to reduced crypt size and inhibition of epithelial cell proliferation rate.<sup>59</sup> Notably, leptin is also a well-known HIF-1 $\alpha$ -inducible modulator,<sup>60</sup> with HIF-1 $\alpha$  overexpression observed in obese adipose tissue, and reduction during weight loss.<sup>61</sup>

## 5 Antimicrobial resistance

### 5.1 Antibiotics

Since the isolation of *P. distasonis* from fecal samples, sites of infection, abscess formation, or the gut wall in IBD patients is potentially linked to clinical outcomes affected by antimicrobial resistance, the sections below provide an overview of studies examining the resistance patterns of *P. distasonis* to various classes of antibiotics.

### 5.2 Beta-lactams

*Bacteroides* spp. and *Parabacteroides* spp. are becoming increasingly more resistant to certain antibiotics<sup>62</sup> because of an increase in the number of antimicrobial resistance-related virulence genes. Within the genus *Parabacteroides*, *P. distasonis* has a wide spectrum of resistance to various beta-lactams, being particularly resistant to the penicillin class.<sup>63–67</sup> Nakano *et al.*<sup>67</sup> demonstrated that up to 99% of *P. distasonis* isolates were resistant to penicillin and the first generation cephalosporin, cephalexin, while more than 85% of the *P. distasonis* isolates could be resistant to amoxicillin and ampicillin. Resistance to the second generation of cephalosporin, cefoxitin, was less frequent; it was found in only 12% of isolates tested.<sup>67</sup> Differential resistance to cephalosporins, including cefotetan, cephalothin,<sup>63,68</sup> cephalexin,<sup>67</sup> cefoxitin,<sup>64,68</sup> cefotaxime,<sup>64</sup> cefazolin,<sup>68</sup> and cefotetan,<sup>69</sup> have also been reported, with resistances higher in earlier generations.

The overall resistance to the antibiotic classes penicillin and cephalosporin can be attributed to the presence of various genes encoding beta-lactamases. These include, but are not limited to, *cfxA*,<sup>64,66</sup> *cfiA*,<sup>67</sup> and *cepA*.<sup>66,67</sup> While almost all *P. distasonis* strains produce beta-lactamase, there are unique strains expressing beta-lactamase genes at significantly elevated rates. Some additional protection from imipenem can be also attributed to the outer membrane of *P. distasonis*.<sup>70,71</sup>

*P. distasonis* is generally susceptible to carbapenems such as meropenem and imipenem.<sup>72,73</sup> However, recently it was found that a small percentage of clinical isolates of *P. distasonis* are resistant to imipenem, ranging from 4%<sup>66</sup> to 11%.<sup>69</sup> This is clinically relevant considering the apparent current

trends of increasing antimicrobial resistance in *P. distasonis* over time, a pattern that has been reported in the *Bacteroides fragilis* group in Europe, where high-level resistance to ampicillin (MIC 64 mg/L) increased from 16% to 44.5% over 20 years.<sup>69</sup>

### 5.3 Lincosamides

Several studies have reported the various rates of clindamycin resistant isolates of *P. distasonis*: from 9%<sup>74</sup> in the 1990s to about 30% in the 2000s,<sup>67,75,76</sup> and even up to 50% in the early 2010s.<sup>76</sup> This suggests that a growing proportion of *P. distasonis* strains have reduced susceptibility to clindamycin.<sup>62</sup> It is possible that the indiscriminate use of this antibiotic in clinical settings, especially for members of the *Bacteroides* and *Parabacteroides* genera, exerts selective pressure, leading to increased resistance of *P. distasonis* to clindamycin over time.<sup>77</sup>

One of the potential underlying mechanisms for clindamycin resistance in *P. distasonis* is the presence of the resistance gene *ermF*, known to be frequently found in various anaerobes.<sup>78,79</sup> This gene encodes the ribosomal methylase that modifies peptidyl transferase in the ribosome, resulting in resistance to lincosamides, macrolides, and streptogramin drugs.<sup>80</sup> However, the data on the frequency of this gene in different *P. distasonis* isolates remain controversial – it varies from being completely absent in isolates<sup>66</sup> to being detected in 37.5% of all samples.<sup>67</sup> Further confirmation for the potential role of *ermF* in clindamycin resistance comes from a study by Kierzkowska *et al.*,<sup>77</sup> in which 88.9% (8 out of 9) of clindamycin-resistant isolates were found to harbor the *ermF* gene.<sup>77</sup>

### 5.4 Other antibiotics and antimicrobial agents

*P. distasonis* demonstrates considerable resistance to tetracycline – up to 87.5% among tested clinical isolates.<sup>68</sup> The high frequency of tetracycline resistance among *Parabacteroides* isolates has been attributed to the presence of the *tetQ* gene.<sup>68,81</sup> The conjugative transposon that *tetQ* resides on harbors *ermF*, and exposure to low concentrations of tetracycline can trigger horizontal gene transfer, thereby also triggering the transfer of other transposons present in

the genome, like Tn4555 which harbors *cfxA*. Thus, this has the potential to be horizontally transferred to other susceptible intestinal species of the *Bacteroides* and *Parabacteroides* genera.<sup>68</sup>

An enzyme found in *P. distasonis*, Pd\_dinase (short for *P. distasonis* protease) has diaminopeptidase activity. This enzyme can hydrolyze some human antimicrobial peptides normally present in the gut, such as keratin-derived antimicrobial peptides (KAMPs), human  $\beta$ -defensin 2, and human neutrophil peptide 3. If secreted into the extracellular milieu of the gut, Pd\_dinase may promote intestinal colonization by *P. distasonis* via the inactivation of the aforementioned host antimicrobial peptides.<sup>82</sup>

## 6 Resistance in clinical vs. intestinal isolates

In a study by SÓki *et al.* conducted in 2020,<sup>83</sup> the resistance of intestinal isolates of different bacterial species, including *P. distasonis*, was evaluated against several antimicrobials. The relevant MIC values were compared between clinical and intestinal isolates. Several notable observations can be made from their findings, summarized in Table 1. For example, in the clinical and intestinal isolates of all species examined, ampicillin resistance is almost 100%; moxifloxacin, cefoxitin, and clindamycin resistance is intermediate (13–44%); and amoxicillin/clavulanate, imipenem, metronidazole, and tigecycline resistance is very low (0–4%).<sup>83</sup>

However, two findings in particular stand out: first, the significantly greater resistance of *P. distasonis* to most classes of antibiotics tested compared to other *Bacteroides* species; and second, the significantly higher resistance of clinical isolates to antibiotics compared to their intestinal counterparts.<sup>83</sup> Specifically, for ampicillin, amoxicillin/clavulanic acid, cefoxitin, and in the intestinal isolates, moxifloxacin and tetracycline, resistance was significantly higher in *P. distasonis* compared to almost all other isolates. In addition, the MIC<sub>50</sub> and MIC<sub>90</sub> values for *P. distasonis* in both clinical and intestinal isolates were significantly higher than that of other isolates for cefoxitin and amoxicillin/clavulanic acid. *P. distasonis* was the most resistant or second-most resistant species in tests with ampicillin, amoxicillin/clavulanic acid, and

**Table 1.** Antimicrobial Activity against *Parabacteroides distasonis* and other species\*.

	Clinical isolates				Intestinal isolates				p-val
	Range	MIC50	MIC90	% of resistant isol.	Range	MIC50	MIC90	% of resistant isol.	
<b>Ampicillin</b>									
All isolates	1->256	32	>256	98.2	1->256	128	>256	96.6	
<i>B. fragilis</i>	1->256	32	>256	97.4	4->256	32	>256	100	
<i>P. distasonis</i>	8->256	16	>256	100	8->256	>256	>256	100	
Other species	4->256	64	>256	100	1->256	256	>256	94.1	
<b>Amox./clavulanic acid</b>									
All isolates	0.016->256	1	16	10.4	0.064-32	0.5	4	4.5	0.021
<i>B. fragilis</i>	0.016->256	1	16	8.7	0.064-16	0.5	8	4.2	
<i>P. distasonis</i>	0.5-256	2	32	21.4	0.064-32	2	32	23.1	
Other species	0.125-64	2	16	11.5	0.064-16	0.5	4	3.9	
<b>Cefoxitin</b>									
All isolates	1->256	16	128	17.2	0.5-256	16	64	14.9	
<i>B. fragilis</i>	1->256	16	256	13.7	1-32	16	32	0	0.031.
<i>P. distasonis</i>	8->256	32	>256	35.7	0.5-128	8	128	23.1	
Other species	2->256	16	>256	26.9	0.5-128	8	64	15.7	
<b>Imipenem</b>									
All isolates	0.002->32	0.5	1	0.85	0.032-324	0.5	2	2	
<i>B. fragilis</i>	0.002->32	0.25	0.5	1.2	0.064-16	0.5	16	13.6	<0.001
<i>P. distasonis</i>	0.125-2	0.5	1	0	0.25-2	1	2	0	
Other species	0.012-8	0.5	4	0	0.064-4	0.5	2	0	
<b>Clindamycin</b>									
All isolates	0.016->256	2	>256	32.4	0.064->256	4	>256	47.3	<0.001
<i>B. fragilis</i>	0.016->256	1	256	28.5	0.125->256	2	>256	20.8	
<i>P. distasonis</i>	0.016->256	1	>256	28.6	0.064->256	8	>256	53.9	
Other species	0.047->256	2	256	34.6	0.064->256	8	>256	49	
<b>Moxifloxacin</b>									
All isolates	<0.125-32	1	16	13.6	0.064-64	1	8	11.4	
<i>B. fragilis</i>	<0.125-64	0.5	8	14	0.064-4	0.5	2	0	0.032
<i>P. distasonis</i>	<0.125-2	0.5	1	0	0.25-16	0.5	16	30.8	0.048
Other species	<0.125-32	0.25	4	11.5	0.064-64	1	4	7.8	
<b>Metronidazole</b>									
All isolates	0.016-256	0.5	1	0.5	0.5-1	0.5	1	0	
<i>B. fragilis</i>	0.016-32	0.5	1	0.5	0.064-4	0.5	1	0	
<i>P. distasonis</i>	0.125-2	0.5	1	0	0.064-0.5	0.5	0.5	0	
Other species	<0.125-4	0.5	2	0	0.064-2	0.5	1	0	
<b>Tetracycline</b>									
All isolates					0.064->256	32	128	66.2	
<i>B. fragilis</i>					0.125-128	32	128	58.3	
<i>P. distasonis</i>					0.5-32	16	64	69.2	
Other species					0.125-64	32	32	58.8	

\*For the intestinal isolates, the study examined 202 *Bacteroides* and *Parabacteroides* strains (11.9%, were *B. fragilis*) collected between 2014 and 2016 in Europe and compared resistance levels between clinical and commensal isolates. Isolates were recovered from feces using *Bacteroides* Chromogenic Agar (BCA) method and tested via agar dilution for ten antibiotics. For clinical isolates, the study used published data from a 20-year survey of isolates in Europe.<sup>69</sup> These results were similar to previous European clinical *Bacteroides* antibiotic susceptibility survey, all the variations existed across countries and antibiotics.<sup>83</sup> Adapted from Table 2 from S6ki et al.<sup>83</sup> with permission. Available from Anaerobe and used with permission from Elsevier. Note that that metronidazole, imipenem, cefoxitin, and amoxicillin/clavulanic acid MIC ranges were significantly greater in clinical isolates compared to intestinal isolates. Notably, however, intestinal isolates of *P. distasonis* are significantly more resistant ( $p = 0.048$  via X2 test) to moxifloxacin than their clinical counterparts.<sup>83</sup>

cefoxitin for both the clinical and intestinal isolates, and was often significantly more resistant across antibiotics compared to the average of all isolates.<sup>83</sup>

These results highlight the possibility and importance of differential antimicrobial resistance in samples of clinical origin compared to commensal intestinal isolates. In addition, these results also indicate a need for greater study of antimicrobial resistance at the strain level, since multiple genetic factors may confer resistance to antibiotics and/or enable bacteria to induce chronic illness. Culture-based studies and genomic analyses of strains well-defined with respect to the source region (healthy vs diseased) are warranted to understand the drivers that enable some isolates or strains to become pathogenic compared to others that remain as commensals.

## 7 Diseases

### 7.1 Dichotomous role in inflammatory bowel disease

Inflammatory bowel disease (IBD) is a spectrum of life-long chronic conditions that affect the digestive tract of humans and animals in a slow, progressive manner. In humans, the prototypic forms of IBD are Crohn's disease (CD) and ulcerative colitis. Crohn's disease affects the entirety of the gastrointestinal tract, and patients have chronic, debilitating symptoms. These include abdominal pain, severe diarrhea, stools containing blood, weight loss, and fatigue. In general, IBD has aberrant exaggerated host immune and inflammatory responses to luminal antigens that reveal recurring themes in IBD pathogenesis: first, lesions in IBD predominate in areas of highest bacterial exposure; and second, manipulation of luminal

**Table 2.** Examples of studies reporting effects of *Parabacteroides distasonis* (PD) on intestinal health.

Disease	Study Model	Study Design	Clinical Effect
Familial Mediterranean Fever <sup>99</sup> <i>Inflammation</i>	Human blood serum and fecal samples. ELISA analysis of varying antibodies that correspond to different bacterial antigens in both FMF patients and healthy controls. DNA extraction was performed with Wizard Genomic DNA Purification kit and sequencing of the 16S rRNA performed with regions V1, V2, V3.	PD, along with other common gut flora, elicited an enhanced nonspecific humoral response to nonspecific antigens present on bacteria in the presence of FMF.	<i>Aggravator</i>
Colorectal cancer <sup>86</sup> <i>Cancer/ Inflammation</i>	Six-week-old male A/J mouse models treated with different chow diets laced with PD.	Increase in colonic IL-10, TGF- $\beta$ and tight junction proteins Zonula occludens and occludin expression in mouse models given the PD long term in comparison to the control diet. Results support a protective role of PD in colonic tumorigenesis.	<i>Protective</i>
Colorectal cancer <sup>100</sup> <i>Cancer/ Inflammation</i>	6-w-old male A/J mice fed low-fat (LF) diet, high-fat (HF) diet or a HF + whole freeze-dried PD diet (HF + Pd). Mice received 4 weekly injections of azoxymethane after 1 week on diet. PD analyzed with 16s rRNA gene sequencing.	PD membrane fraction (PdMB) largely suppressed production of pro-inflammatory cytokines, lowered MyD88 and pAkt abundance, and induced apoptosis in colon cancer cell lines, suggesting anti-inflammatory and anti-cancer effects.	<i>Protective</i>
Colitis <sup>84</sup> <i>Inflammation</i>	Murine Model of 2,4,6-Trinitrobenzenesulfonic Acid (TNBS)-Induced Colitis based on BALB/C ByJ mice. PD sequenced using V3-V4 16s rRNA regions.	PD reinforces the gut barrier and promotes pro-anti-inflammatory profile. This has a positive association with reducing colitis in tested mouse models. <i>In vitro</i> benefits relied heavily on the strain.	<i>Protective</i>
Colitis <sup>17</sup> <i>Inflammation</i>	DSS-induced BALB/c mice – oral treatment of PD. PD analyzed via 16s rRNA sequencing.	PD membrane components decreases the severity of gut inflammation in the non-immunocompromised mouse models that had induced acute and chronic colitis. Also, increased PD serum antibodies and decreased pro-inflammatory cytokines.	<i>Protective</i>
Colorectal carcinogenesis <sup>101</sup> <i>Cancer/ Inflammation</i>	Shotgun metagenomic sequencing analysis between feces of wild-type mice and mice with defects in TGFB signaling. Analysis of microbiota changes prior to colon tumors development. Shotgun metagenomics sequencing was performed using 150 BP, pair-ended sequences through Illumina sequencing.	PD abundance decreased when there were defects in transformation growth factor beta (TGFB) signaling pathway in mouse models. TGFB-deficient mice have more colorectal cancer and lower PD abundance.	<i>Protective</i>
Crohn's Disease and Ulcerative Colitis <sup>89</sup> <i>Inflammation</i>	WT and antibiotic-depleted intestinal microflora mouse models. Pyrosequencing was performed on the variable regions of bacterial 16s rRNA. The sequences were classified with GreenGenes and compared with using QIIME.	Low PD abundance in patients with CD and UC. Higher PD abundance in gut microbiota of the <i>Pglyrp</i> -deficient mouse models promote colitis.	<i>Aggravator</i>

content using selective antibiotics reduces inflammation in IBD patients. Thus, it remains to be determined if IBD is triggered by the presence of an imbalanced microbiota composition and if such composition can be corrected with probiotic-like enhanced anti-inflammatory bacteria like *P. distasonis*.<sup>17</sup>

BALB/c mice inoculated with a whole cell lysate of *P. distasonis* prior to the onset of DSS-induced colitis demonstrated a significant decrease in inflammation compared to the control,<sup>17</sup> thus providing some evidence supporting the anti-inflammatory role of *P. distasonis* in the intestinal microbiome. Several strains of *P. distasonis* showed anti-inflammatory effects both *in vitro* and *in vivo* and were able to restore both the epithelial barrier in a cell culture model and strengthen the gut barrier in a mouse model of 2,4,6-trinitrobenzenesulfonic acid (TNBS)-

induced colitis. Intriguingly, *P. distasonis* stimulated CD4<sup>+</sup> T cells to differentiate toward the CD4<sup>+</sup> FoxP3<sup>+</sup> IL-10<sup>+</sup> regulatory phenotype.<sup>84</sup> These results correlate with other studies illustrating the potential role of *P. distasonis* in stimulating regulatory T cell differentiation.<sup>85</sup> A study by Koh *et al.*<sup>86</sup> also corroborated similar findings in regard to *P. distasonis*' ability to restore the epithelial barrier: the expression of the tight junction proteins Zonula occludens-1 ( $p < .001$ ) and occludin ( $p < .001$ ) were significantly increased in mice fed a diet containing 0.04% freeze-dried *P. distasonis* both at the transcriptional (2-3-fold,  $p < .01$ ) and post-translational (30–50%,  $p < .05$ ) levels, regardless of when *P. distasonis* was introduced into the diet of the mice, but so long as it was introduced.<sup>86</sup> In another study, *P. distasonis* and several *Bacteroides* species were identified to attenuate *E. coli* lipopolysaccharide-induced IL-8 release

from HT-29 cells and to lack genes to synthesize hexa-acylated, proinflammatory lipid A, exhibiting anti-inflammatory properties. *P. distasonis* was also found to exert enterocyte monolayer reinforcing action, reinforcing the gut barrier. The study suggested that *P. distasonis* and the other tested *Bacteroides* species could be used as “next-generation probiotics.”<sup>87</sup>

In past literature, it is consistently reported that there is a lack of species diversity in the inflamed regions of the gut overall. Strikingly, however, *P. distasonis* was identified as a recurring bacterium in the stools of CD patients.<sup>88</sup> Notably, there are currently very few studies in humans that provide evidence for the idea that *P. distasonis* is a bacterium associated with any of the main forms of IBD, and potential evidence of the possible contribution of *P. distasonis* to IBD pathogenesis remains inconclusive; however, the results from studies in animal models still raise alarm. In one study, researchers demonstrated that in a DSS-induced colitis mouse model, *P. distasonis* had an anti-inflammatory effect on the gut; however, there is some evidence that, in mice with preexisting abnormal conditions such as SHIP or PGRP gene deficiency, *P. distasonis* seems to promote intestinal inflammation rather than attenuate it.<sup>89</sup> This may be due to *P. distasonis* ability to protect itself against human immune responses.<sup>86</sup> Furthermore, in a murine model of acute and chronic DSS-induced ulcerative colitis, *P. distasonis* abundance was significantly increased compared to healthy controls.<sup>90</sup> More evidence for the potential proinflammatory role of *P. distasonis* in CD comes from one of our own studies. Here, germ-free senescence-accelerated prone mice (SAMP) were inoculated with *P. distasonis*. In mice already with Crohn’s disease, myeloperoxidase (MPO) activity was significantly increased, causing further inflammation in the gut.<sup>91</sup>

Peptidoglycan recognition proteins (PGRPs) are known to control inflammation in the gut, partly by reducing IFN- $\gamma$  induction and NK cell migration.<sup>92</sup> Mice deficient in any of the four types of PGRPs demonstrate markedly increased severity of DSS-induced experimental colitis and a more proinflammatory gut microbiome.<sup>92</sup> Dziarski *et al.*<sup>93</sup> demonstrated that, in PGRP-deficient mice, gut levels

of *P. distasonis* were consistently elevated, indicating a possible lack of immune control for *P. distasonis* through PGRPs. Additionally, the gavage of wild type mice with *P. distasonis* enhanced DSS-induced colitis and possibly predisposed mice to IBD.<sup>89</sup>

A study by Gonzalez-Paez *et al.*<sup>93</sup> found that C11 proteases could promote host immune responses and bacterial pathogenesis, especially via activation of bacterial pathogenic toxins from the likes of *P. distasonis*. Here, *P. distasonis* was found to promote intestinal inflammation in mouse models, degrading mucosal barrier health, and thus potentially contributing to the development of IBD. Additionally, the researchers reported that there was a correlation between elevated proteolytic activity and amino acids with the dysbiosis of the distal gut microbiome in patients. *P. distasonis* was found to influence gastrointestinal homeostasis and the responding immune activity, especially in the form of enteric cysteine proteases. These proteases were hypothesized to either be tethered to a bacterial cell wall; packaged into outer membrane vesicles (OMVs) that hydrolyze substrates from the parental bacterium surface, other bacteria, or host epithelial cells; or both.<sup>93</sup>

As previously mentioned, *P. distasonis* can produce catalase to enable its detoxifying role against oxidative stress mediated by hydrogen peroxide, often produced by inflammatory cells. However, it is suspected that these oxidative agents may be inflammatory triggers for CD.<sup>94,95</sup> This is because catalases produced by various species of bacteria, including *P. distasonis*, catabolize reactive oxygen species (ROS), which may exacerbate inflammation.<sup>29</sup>

While there are few studies involving humans concerning the potential pathogenicity of *P. distasonis* in relation to IBD, those published present similarly alarming results. Nagayama *et al.*<sup>96</sup> demonstrated that *P. distasonis*, along with nine other anaerobic bacteria cultured from the small intestinal mucosa of CD patients, enhanced T<sub>H</sub>1 and T<sub>H</sub>17 cell accumulation and intestinal inflammation.<sup>96</sup> Furthermore, we have recently identified *P. distasonis* in the deep-gut wall tissues from patients that underwent surgery for the removal of chronically inflamed bowel segments,

specifically patients afflicted with CD,<sup>97</sup> supporting a probable pathologic role for this species in CD. This corroborates the observed enrichment of *Bacteroidetes* in the gut metagenome of the SAMP mouse line, which is naturally prone to CD-like ileitis.<sup>98</sup>

Taken together, these often-conflicting reports underscore the potential importance of *P. distasonis* in gut health and the need to elucidate its pathogenic effects so that clinically relevant solutions can be developed to address such effects. An overview of studies reporting an experimental or observational effect on IBD and intestinal health is presented in Table 2.

## 7.2 Protective role in colorectal cancer

To date, *P. distasonis* has only been shown to have beneficial effects on colorectal cancer. Multiple researchers have identified that levels of *P. distasonis* in stool are inversely correlated to the presence of intestinal tumors.<sup>101</sup> Koh *et al.*<sup>86</sup> determined that *P. distasonis* membrane fractions were responsible for suppressing the production of pro-inflammatory cytokines in a colon cancer cell line. Studies by other researchers have suggested that *P. distasonis* has anti-inflammatory and anti-tumor properties mediated by the reduction of signaling via TLR4, MYD88, and Akt and the stimulation of apoptosis.<sup>86</sup> These results are in agreement with observations of reduced microbiome levels of *P. distasonis* in mouse models of colorectal cancer.<sup>44</sup> In a study by Koh *et al.*<sup>86</sup> that was previously noted for revealing *P. distasonis* effect on the expression of tight junction proteins, the same mice were later treated with the carcinogen azoxymethane (AOM). The investigators stated that TLR4, IL-4, and TNF- $\alpha$  expression were 40% ( $p < .01$  using a one-way ANOVA), 58% ( $p < .05$ ), and 55% ( $p < .001$ ) lower in mice fed a chow diet containing *P. distasonis* throughout the investigation than mice that were never fed a diet containing *P. distasonis*. In mice fed a diet containing *P. distasonis* after switching from a chow diet, the IL-10 and TGF- $\beta$  expression were 217% ( $p = .05$ ) and 185% ( $p < .001$ ) higher compared to mice fed a chow diet without *P. distasonis*.<sup>86</sup> Furthermore, Gu *et al.*<sup>101</sup> found that the abundance of *P. distasonis*, in addition to that of *Bacteroides*

*vulgatus*, decreased in response to CEACAM proteins disrupting TGF- $\beta$  signaling. This alteration in the intestinal microbiome in turn promoted colorectal carcinogenesis, thus providing further evidence of *P. distasonis* potential role in preventing colorectal carcinogenesis.<sup>101</sup>

The evidence for the potential anti-inflammatory effects of *P. distasonis* in colorectal cancer is further supported by the inverse correlation between *P. distasonis* levels and IL-1 $\beta$  production in the gut.<sup>44</sup> A comparison of the fecal microbiota composition between patients with spontaneous colorectal adenocarcinomas and patients without any proliferative lesions in the colon revealed a lack of *P. distasonis* in the patients with tumors.<sup>102</sup> Collectively, these studies suggest that *P. distasonis* has anti-tumorigenic and anti-inflammatory potential in colorectal cancer patients.

## 7.3 Protective role in obesity

Further potential benefits of *P. distasonis* have been identified, particularly in relation to obesity. Xu *et al.*<sup>103</sup> found that mice with high-fat diet-induced obesity (DIO) had higher relative abundances of *P. distasonis* and *Akkermansia muciniphila* in the gut, resulting in a gut microbiota that was significantly more capable of reducing host adiposity. Here, this change in gut microbiome composition was induced by *Panax notoginseng* saponins (PNS), often used as a form of traditional Chinese medicine. This suggests a potential ability for PNS and, in turn, *P. distasonis*, to be used in the treatment of obesity.<sup>103</sup> This also raises the potential for *P. distasonis* to be important in modulating host adiposity. Furthermore, a study by Gallardo-Becerra *et al.*<sup>104</sup> found that the abundance of *P. distasonis* in the gut microbiota of Mexican children with obesity and metabolic syndrome (MetS), a multicomponent condition associated with obesity, was reduced compared to Mexican children of normal weight. This, the investigators noted, was “correlated with clinical and anthropometric parameters associated with obesity and metabolic syndrome”.<sup>104</sup> A study by Haro *et al.*<sup>105</sup> illustrated a link between the gut microbiota, diet, and patients diagnosed with MetS. The study’s assertion implied that *P. distasonis* was originally diminished in MetS

patients who were then presented with a Mediterranean diet for 2 years, partially restoring *P. distasonis* populations. The data from the MetS patients exhibited a negative relationship between waist circumference and the relative abundance of *P. distasonis* ( $R = -0.213$ ). The patients on this Mediterranean diet, which was enriched with antioxidant phenolic-compound-rich foods, saw a statistically significant increase in the abundance of *P. distasonis* ( $p = .025$  via ANOVA). These results suggest that the Mediterranean diet could potentially be utilized to correct microbial imbalances especially in reference to *P. distasonis*, providing further evidence that *P. distasonis* may be involved in mitigating obesity.<sup>105</sup>

Recently, Wang *et al.*<sup>106</sup> demonstrated that *P. distasonis* alleviated obesity, hyperglycemia, and hepatic steatosis in *ob/ob* and high-fat diet mice via the production of secondary bile acids and a previously mentioned byproduct of fermentation: succinate. Here, succinate was found to bind to fructose-1,6-bisphosphatase, a rate-limiting enzyme involved in intestinal gluconeogenesis (IGN), decreasing hyperglycemia in *ob/ob* mice. Furthermore, treatment with live *P. distasonis* dramatically altered the bile acid profiles of the mice, increasing the levels of lithocholic acid (LCA) and ursodeoxycholic acid (UDCA), in turn reducing hyperlipidemia by activating the FXR pathway and, as a result, repairing gut barrier integrity, highlighting additional suspected benefits of *P. distasonis* in relation to obesity and gut barrier integrity.<sup>106</sup> Of interest, the abundance of *P. goldsteinii* in feces has been reported to also have an inverse (protective) correlation with obesity in rats.<sup>107</sup>

#### 7.4 Dichotomous role in diabetes

As with IBD, *P. distasonis* has been shown to have both beneficial and detrimental effects on diabetes, complicating its definition as a beneficial commensal or pathogenic bacterium. However, research on *P. distasonis*' effects on diabetes is currently very limited. Cai *et al.*<sup>108</sup> found that supplementing extract of propolis (EEP) in high-fat diet mice increased the abundance of *P. distasonis*, which was identified as an 'anti-obesity and anti-inflammatory bacterium,' in line with associated metabolic parameters of insulin resistance.<sup>106</sup>

Based on these findings, one may conclude that *P. distasonis* could play a role in decreasing insulin resistance and preventing diabetes.

However, some evidence that *P. distasonis* may be involved in the pathogenesis of diabetes has begun to emerge. Hasain *et al.*,<sup>109</sup> citing several metagenomics studies, concluded that *P. distasonis*, which has been shown to be enriched in women with gestational diabetes mellitus (GDM), could serve as a gut microbiota signature in women with GDM.<sup>109</sup> This suggests that *P. distasonis* may play a role in the pathogenesis of certain types of diabetes rather than prevent it, as Cai *et al.*'s<sup>106</sup> findings suggest, underscoring the importance of further investigation into *P. distasonis*' effects on diabetes.

#### 7.5 Gut microbiome and dichotomous role on autoimmune diseases

Several studies have suggested that *P. distasonis* may play a role in various forms of autoimmunity. For example, in a recent study addressing the potential functional relationship between gut bacteria and T cell responses in multiple sclerosis (MS), *P. distasonis* levels were shown to be lower in human MS patients compared to healthy controls.<sup>110</sup> *P. distasonis* was shown to drive T cell differentiation toward an increased percentage of anti-inflammatory CD25<sup>+</sup> T cells relative to the total CD3<sup>+</sup> CD4<sup>+</sup> T cell population. There was also an abundance of CD25<sup>+</sup> IL-10<sup>+</sup> FoxP3<sup>-</sup> Tr1 cells, which are strongly associated with the immunoregulatory phenotype.<sup>111</sup> Interestingly, these results are corroborated by the elevated proportion of FoxP3<sup>+</sup> T regulatory cells in the overall population of CD4<sup>+</sup> T cells found in the colonic lamina propria of C57BL/6 J mice monocolonized with *P. distasonis*.<sup>85</sup> Transplantation of the gut microbiome from the MS patients into germ-free mice augmented the severity of experimental autoimmune encephalomyelitis symptoms in comparison to their counterparts that received the microbiome transplant from healthy human donors.<sup>110</sup>

*P. distasonis* abundance was significantly elevated in fecal samples from patients with ankylosing spondylitis (AS), a chronic inflammatory disease affecting the spine and the sacroiliac joint. This suggests a possible role for *P. distasonis* in the



development of more diverse autoimmune responses.<sup>112,113</sup> *In vitro* experiments showed that *P. distasonis*, along with other AS-enriched species including *Bacteroides coprophilus*, *Eubacterium siraeum*, *Acidaminococcus fermentans*, and *Prevotella copri*, increased the amount of IFN- $\gamma$ -producing cells through a bacterial peptide of these species, mimicking type II collagen and likely serving as “triggers of autoimmunity by molecular mimicry”.<sup>105,110,114</sup>

In psoriatic patients, *P. distasonis* presence was found to be significantly decreased relative to non-psoriatic patients.<sup>115</sup> In mouse models of imiquimod-induced skin inflammation (IISI), animals treated with the antibiotic metronidazole showed a significantly higher abundance of *P. distasonis* in their intestines and demonstrated reduced severity of skin inflammation via downregulation of the T<sub>H</sub> 17 immune response.<sup>116</sup> These findings suggest that the intestinal microbiota may play an important role in the regulation of IISI.

Notably, Moreno-Arrones *et al.*<sup>117</sup> found that patients with alopecia areata – an autoimmune disease mediated by T cells – had higher abundances of *P. distasonis* in their gut microbiota (LDA score > 2). In conjunction with *Clostridiales vadin* BB60 group, *P. distasonis* could correctly predict alopecia areata status in 80% of patients, indicating that *P. distasonis* could be involved in the pathophysiology of alopecia areata.<sup>117</sup> However, whether these findings indicate a causative relationship between the abundance of *P. distasonis* in the gut and alopecia areata and if the bacterium presence serves as a biomarker for the disease remains to be elucidated. At the genus level, elevated systemic antibodies toward commensal gut *P. distasonis* and *P. merdae* have been reported to be consistently increased in Familial Mediterranean fever (FMF, which is an autoinflammatory condition characterized by acute, self-limiting episodes of fever and serositis and chronic subclinical inflammation in remission), irrespective of disease activity (remission, 2180  $\pm$  1150 and 2508  $\pm$  1241; vs. flare, 2383  $\pm$  1207 and 2393  $\pm$  1069) compared to controls (658  $\pm$  161 and 995  $\pm$  363, for *P. distasonis* and *P. merdae*, respectively).<sup>99</sup> The relevance of these findings are not well understood because in IBD, increased IgA antibody concentrations have been found to work against other commensal microbes, including *Lactobacillus casei*.<sup>118</sup>

## 7.6 Dichotomous role in cardiovascular disease

*P. distasonis* has also been implicated in the pathogenesis of cardiovascular disease (CVD). However, as with its impact on diabetes, research on *P. distasonis* impact on CVD is limited. A study focused on exploring the relationship between the gut microbiota and CVD in patients with cardiac valve calcifications and coronary artery disease found *P. distasonis* and other bacterial species to be potential pathogens contributing to CVD.<sup>114</sup>

Conversely, a study on the role of the intestinal microbial flora in vascular inflammation in rats found a potential anti-inflammatory role for *P. distasonis*, contributing to potentially beneficial effects on CVD. Here, the relative abundance of *P. distasonis* was inversely correlated to the neointimal hyperplasia and composite intima+media area after a carotid artery angioplasty.<sup>119</sup> Thus, the role of *P. distasonis* in the pathogenesis of CVD is yet to be determined and remains another point of controversy surrounding this bacterium. It is clear that more research will be needed to determine *P. distasonis* role in relation to CVD.

## 7.7 Established pathogen in intestinal and non-intestinal abscess formation

Abscesses are a prime hotspot for numerous infectious bacteria to manifest and thrive. Clinical studies have reported finding culturable *P. distasonis* isolates in abscesses. Clinical studies and case reports have implied a possible role for *P. distasonis* in abscess formation in various tissues, including the spleen,<sup>120,121</sup> liver,<sup>122</sup> and wounds.<sup>33</sup> For example, Gunalan *et al.*<sup>120</sup> reported a case of splenic abscess in a 40-year-old man presenting to the hospital with fever, left-side abdominal pain, altered sensorium, and vomiting. After the patient received antimicrobial therapy and underwent a splenectomy, it was discovered that pus aspirated from the splenic abscess grew *P. distasonis*. Gunalan *et al.*<sup>120</sup> noted that this is one of only a few recorded cases of *P. distasonis* causing splenic abscess in humans; nonetheless, such a finding is alarming and supportive of a pathogenic role of *P. distasonis* in human infections.<sup>120</sup> Furthermore, CD4<sup>+</sup> T-cells were shown to play a key role in the formation of *P. distasonis*-induced intra-abdominal abscesses in rodent models.<sup>123</sup>

The mechanism behind *P. distasonis* pathology regarding abscess formation is still under investigation. One study examined the abscedative and intra-abdominal sepsis role of *P. distasonis* infections using rats that were intraperitoneally infected with different bacterial pathogens, namely, *Staphylococcus aureus*, *Bacteroides fragilis*, and a combination of *Enterococcus faecium* and *P. distasonis*.<sup>123</sup> The study aimed to define the mechanisms by which *i*) T-cells mediate abscess induction secondary to intra-abdominal sepsis, *ii*) the contribution of T-cell activation and *iii*) the role of co-stimulation of antigen-presenting cells via CD28-B7 pathways. Researchers found that T cells activated with zwitterionic bacterial polysaccharides *in vitro* required CD28-B7 co-stimulation to induce abscesses when adoptively transferred to the peritoneal cavity of naïve rats, promoting abscess formation. Although not exclusively specific to *P. distasonis* pathogenesis, the study demonstrated that blockade of T-cell activation via the CD28-B7 with CTLA4Ig prevented abscess formation, while an alarming 82.4% (n = 28) of 34 induced abscess yielded *P. distasonis*.<sup>123</sup>

### 7.8 Cervical cancer, ketogenic diet and glutamate and gamma-aminobutyric acid

It has become apparent that *P. distasonis* might have a modulatory effect on the predisposition to or protection against numerous other types of diseases. For example, elevated levels of *P. distasonis* were positively associated with the progression of cervical cancer.<sup>124</sup> However, the number of patients in this particular study was rather small, so these results should be interpreted with caution. More possible positive roles for *P. distasonis* and *P. merdae* were described in another recent study. Both of these *Parabacteroides* species were shown to promote the beneficial anti-seizure effects of the ketogenic diet. Presence of these bacterial species strongly correlated with protection against seizures, potentially via increasing levels of glutamate and gamma-aminobutyric acid (GABA) in the hippocampus.<sup>125</sup> Reduced levels of GABA are well-known to exacerbate seizures.<sup>126</sup> Furthermore, a whole metagenome sequence analysis demonstrated a lower abundance of *P. distasonis* in

children with autism spectrum disorder (ASD) compared to their neurotypical counterparts. Here, metagenomic analysis revealed decreases in the expression of genes linked to the production of melatonin, butyric acid, and GABA.<sup>127</sup> A summary of these and additional studies reporting an experimental or observational effect on non-intestinal diseases is presented in Table 3.

## 8 Genomics, phages, and genetic engineering

Among the *Parabacteroides* species associated with the human gut, *P. distasonis* has the smallest genome (<5Mb, vs >6.5Mb) and the smallest repertoire of genes that are members of the environmental sensing and gene regulation categories. *P. distasonis* type strain ATCC 8503 possesses a 4,811,369-bp genome, 3,867 protein-coding genes, and shares 1,416 sets of orthologous protein-coding genes with other gut *Bacteroidetes*. Figure 2 illustrates the genomic relatedness of the *Parabacteroides* genus with that of other relatively new reclassified genera within the *Bacteroidetes* phylum. Additionally, *P. distasonis* has the smallest number of genes associated with carbon source degradation; however, *P. distasonis* has two classes of carbohydrate-processing enzymes that are more abundant in its proteome than in the proteomes of other gut *Bacteroidetes*.<sup>37</sup> According to the PATRIC database, all *P. distasonis* sequences strains correspond to single chromosome isolates, with only one phage reported in the system (*Parabacteroides* phage YZ-2015a, NCBI Taxon ID 1,655,644)<sup>131</sup>.

Recently, a comparative genomic study of Microviridae, a family of bacteria-infecting ssDNA viruses (deemed as a poorly characterized bacteriophage group, even though it includes phage PhiX174, which is one of the main models in virology for genomic and capsid structure studies) across 17 peatlands showed that two new distinct prophages were identified in the genomes of *P. merdae* and *P. distasonis* representing a potential new subfamily of Microviridae that matches the protein similarity of the viron capsid protein VP1, in viromes of French wetlands.<sup>132</sup>

Elegantly, Quaiser *et al.*<sup>132</sup> showed through Blast searches that the major capsid protein VP1 sequences from the assembled viral wetland

**Table 3.** Examples of studies reporting effects of *Parabacteroides distasonis* (PD) on non-intestinal health.

Disease of Interest	Study Model	Study Effect/Type of Association	Effect/PMID
Necrotizing fasciitis (NF) <sup>128</sup> <i>Muscular</i>	Patient of study diagnosed with HIV. Fournier's Gangrene Polymicrobial mixture isolated from a patient's tissue culture. Computerized tomographic imaging	Opportunistic/polymicrobial mixture found in perineal and scrotal abscess included PD.	<i>Aggravator</i>
Oral Health and Patients with Acrylic partial dentures <sup>129</sup> <i>Digestive</i>	Patients lacking teeth and using prosthetic treatments. Microbial culture using Schaedler K3 solid medium with 5% sheep blood at 37°C after use of active toothpaste containing propolis and tee tree oil-containing hygienic agent versus control group.	PD isolated from some patients before and on day 7 of trial using tested toothpaste. Authors stated that PD was "eliminated" after use of active toothpaste.	N/A
Obesity, Hyperlipidemia, hepatic steatosis, Intestinal Gluconeogenesis <sup>106</sup> <i>Digestive/Endocrine</i>	Obese mouse models modulating gut microbiota. In vivo assays that validate beneficial effects of PD. The bacterial 16s rRNA regions V3 and V4 were sequenced using Illumina HiSeq PE250. The primers F341 and R806 were used.	PD associated with reduced weight gain, decrease of hyperglycemia, and hepatic steatosis in obese and high-fat (HDF)-fed mice. PD is lower in patients that are obese. Improved glucose homeostasis and obesity-related abnormalities.	<i>Protective</i>
Gestational diabetes mellitus and gestational diabetes <sup>109</sup>	Human, fecal samples	GDM is associated with metabolic disorder phenotypes (obesity, low-grade inflammation, insulin resistance). PD has high abundance in women with GDM. Suggested as part of gut microbiota signature for GDM.	<i>Positive Correlation (Aggravator?)</i>
Amyotrophic lateral sclerosis (ALS) <sup>130</sup> <i>Nervous System</i>	ALS-prone Sod1 transgenic (Sod1-Tg) mouse models. 16s rDNA sequencing was performed on region V4 using a Illumina MiSeq kit with 2 × 250 BP pair-ended sequencing.	PD reportedly exacerbates ALS symptoms whereas other bacteria such as <i>Akkermansia muciniphila</i> (AM) improves ALS symptoms.	<i>Aggravator</i>
Multiple Sclerosis <sup>110</sup> <i>Inflammation/Neurological</i>	Germ-free mouse models.	PD was of less abundance in multiple sclerosis patients. However when introduced to mouse model. PD stimulated anti-inflammatory IL-10-expressing human CD4+ CD25 + T cells and IL-10+ FoxP3+ Tregs in mouse models.	<i>Protective</i>
Ankylosing spondylitis <sup>113</sup> <i>Inflammation/Autoimmune disorder</i>	Fecal microbial metagenomic analysis of patients.	PD along with other microbiota found in Ankylosing spondylitis (AS) patients. May be a trigger of autoimmunity via molecular mimicry.	<i>Aggravator</i>
Alopecia Areata <sup>117</sup> <i>Autoimmune disorder</i>	16S rRNA sequencing of stool samples.	Alopecia areata is T-cell mediated autoimmune disease and gut microbiota has been identified as key modulator of this disease. PD could be used as potential diagnostic tools due to its enriched presence in stool samples.	<i>Aggravator</i>
Autism spectrum disorders <sup>127</sup> <i>Developmental Disorder</i>	Metagenomic analysis of fecal specimens of children.	There were decreases in the average abundance of gut microbiota in children with autism spectrum disorder (ASD), including PD. Gut microbiota can be a neurometabolic signature for ASD transcriptional and metabolomic activity.	N/A

genomes showed similarities to VP1 proteins encoded in the *P. merdae* and *P. distasonis* genomes.<sup>2</sup> The *Parabacteroides* VP1 genes show that genes encoding for homolog VP2 and VP4 genes (other capsular phage proteins) were juxtaposed next to the bacterial VP1 genes, supporting the presence of a prophage Microviridae in both *Parabacteroides* species. Comparing their organization, the identified prophage regions flanking the VP1 gene (5 kbp) were extracted from the genomes and considered circular for analysis, using the VP1 gene as an arbitrary start. Synteny analysis showed, remarkably, that the prophages have the same gene order in such bacteria (*P. distasonis*: VP1-ORF1-ORF2-

ORF3-VP2-VP4, and *P. merdae*: VP1-ORF1-VP2-VP4) suggesting that both have a common functional prophage ancestor. The gene coding for the arbitrarily assigned protein ORF1 downstream of VP1 in both bacteria were specific to each prophage and did not match to genes from other Microviridae or in NCBI non-redundant databases. This strengthens the hypothesis that these prophages represent a distinct subfamily of Microviridae, suggesting the relevance of this genus and species in water sources previously described.

*P. distasonis* can make deacetylated products available for itself and other components of the microbiota, devoting a greater proportion of its



genome to protein degradation than other *Bacteroidetes* such as *B. thetaiotaomicron*.<sup>37</sup> This supports the postulate that *in vitro* tests that quantify the proteolytic activity of this and other potentially pathogenic microbes are not predictive of the genomic potential that exists among gut *Bacteroidetes*. For example, gelatin hydrolysis and collagenase tests may suggest a lack of proteolytic activity in *P. distasonis*, but, as previously mentioned, we have recovered this species from microcavitating (fistulizing) lesions in the gut wall of Crohn's disease resected bowels, supporting the potential for proinflammatory activity in IBD.<sup>97</sup> Additional tests and growth assays will be performed to confirm this observation.

As of January 12, 2021, there are 133 genomes for *P. distasonis* registered in the bioinformatics resource database PATRIC ver. 3.6.8, which provides integrated data and analysis tools to support biomedical research on infectious diseases, including data on genome sequencing efforts.<sup>131</sup> At least seven of these genome sequencing efforts are complete assemblies of strains derived from sources described "feces," "clinical isolates," a "peritonitis case," "sphagnum-peat soil," and "intramural gut wall lesions from Crohn's disease patients."<sup>131</sup> Most other registered genomes originate from isolates sourced from feces, while at least seven genomes originate from unspecified regions of the human gut and two additional genomes originate from unspecified parts of the rat gut. At least five genomes originate from sources specified as "tissues." Numerous isolates originate from mice. Unfortunately, however, most isolates have non-specific descriptions with respect to the source.<sup>131</sup>

Other identified *P. distasonis* strains of interest include *P. distasonis* ATCC 82G9, *P. distasonis* NBRC 113,806, *P. distasonis* ATCC 8503, *P. distasonis* CavFT-Har46, and *P. distasonis* FDAARGOS\_615. The complete genome sequence of *P. distasonis* CavFT-Har46 was completed by our team and is of great clinical interest as this strain was isolated from a gut wall-cavitating microlesion in a patient with severe Crohn's Disease. We have identified that this strain exhibits an 80% match to other *P. distasonis* strains, including strains *P. distasonis* ATCC 8503 and 82G9, both isolated from the feces of patients.<sup>97</sup>

Due to horizontal gene transfer of antimicrobial resistance genes, *Bacteroides* and *Parabacteroides* isolates from European and American patients now show tetracycline and erythromycin resistance, complicating genetic selection processes. Furthermore, while genetic manipulation via CRISPR-Cas9 can be done on *Bacteroidales*, off-target mutations, inefficient transformation, and the need for strain-specific modifications prevent CRISPR-Cas9 from being an effective method for molecular-level genetic manipulation.<sup>133</sup> Recently, however, a new avenue for the genetic manipulation of *P. merdae* and diverse *Bacteroides* isolates from the human gut microbiota has been identified by García-Bayona & Comstock. This avenue consists of placing a selection cassette based on the dietary fiber inulin (the selection of which *P. merdae* is thought to be amenable to) into pNBU2, a "pir-dependent suicide vector," resulting in facilitated transconjugation in *P. merdae*. This signifies that this combination, dubbed "pLGB28," could be utilized in the genetic engineering of *P. distasonis*, thus providing a facilitated method of genetic manipulation for the species.<sup>133</sup>

## 9 Conclusions and future directions

*P. distasonis* is a unique bacterium that is involved in many of the biochemical processes of numerous human diseases. While there are apparent associations between *P. distasonis*, related IBDs, and numerous other diseases, there is a lack of an established consensus on *P. distasonis*' role in modulating the human gut microbiota and, more importantly, the pathogenicity of the bacterium. Going forward, the directive for new studies is to understand and identify the mechanisms of *P. distasonis*, its pathogenesis, its antimicrobial resistance, and its commensal relationship with the gut mucosal wall. Additionally, it is imperative to understand its impact on our intestinal microbiota.

Ultimately, it may be that *P. distasonis* does not cleanly fit into either the beneficial commensal bacterium or pathogenic bacterium categories: perhaps *P. distasonis*, and potentially, other species of bacteria, straddle both definitions. If this were the case, it could have major implications for research on the gut microbiome, including prompting further

investigation into other bacteria thought to be beneficial commensals for potential pathogenicity. However, more research is needed to determine whether *P. distasonis* truly straddles both the pathogenic and beneficial commensal definitions.

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## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Author’s Contribution

A.R.-P identified the need for and conceived the manuscript. All authors contributed to the review, discussion of the findings, and wrote the manuscript. All authors discussed and edited the manuscript and approved its final version.

## Contribution to the field

This is a review of the controversial and intriguing roles of *Parabacteroides distasonis* (PD) on human and animal health. This is a re-classified bacterium, originally called *Bacteroides distasonis*, and a part of the *Bacteroidetes* phylum. This well-established and extensively described bacterium was identified and isolated from patients with clinical infections as well as from microscopic cavitating lesions in the gut wall of a patient with Crohn’s disease, a major prototype of inflammatory bowel disease (IBD). These observations contrast those made by other

investigators who have reported potential probiotic effects of *P. distasonis* for a multitude of diseases, including IBD.

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