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MINIREVIEW

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Opportunistic diseases in marine eukaryotes: Could Bacteroidota be the next threat to ocean life?

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Abstract

Bacteria within the phylum Bacteroidota (Bacteroidetes) are known to cause devastating and widespread disease outbreaks in marine eukaryotic hosts. However, with few pathogens described in detail, their prevalence and virulence strategies remain largely unknown. Here, we systematically reviewed the literature to evaluate the current understanding of Bacteroidota that cause disease in marine hosts. Isolates affiliated with the genera Tenacibaculum and Aquimarina (Flavobacteriaceae) were the most widely reported and characterized pathogens. Although cultured isolates were predominantly Flavobacteriia, culture-independent studies also found classes Bacteroidia, Cytophagia and Sphingobacteriia associated with disease. We found that pathogenic marine Bacteroidota largely conformed to an opportunistic lifestyle but could also act as secondary pathogens or were involved in polymicrobial diseases. Many diseases were also associated with an environmental stressor, especially those affecting coral, macroalgae and fish. Key virulence traits included the production of adhesins and host tissue-degrading enzymes. Overall, the nature of disease involving Bacteroidota pathogens appears to be an outcome of complex host-pathogenenvironment interactions; however, our understanding of virulence remains limited by the lack of functional characterization studies. This is concerning as Bacteroidota have the potential to emerge as a serious threat to marine ecosystems and aquaculture industries, driven by global changes in ocean conditions.

INTRODUCTION

Bacteria belonging to the phylum Bacteroidota are broadly distributed across a diverse range of ecological niches on earth, including host-associated microbiomes, soil, polar, freshwater and marine habitats (Thomas et al., 2011). Originally referred to as the Cytophaga-Flavobacterium-Bacteroides group, this phylum has been the subject of extensive taxonomic revision in recent years (García-López et al., 2019; Hahnke et al., 2016), including a recent revision of the phylum name from Bacteroidetes to Bacteroidota (Oren & Garrity, 2021). Since the phylum was first described by Krieg et al. (2010), the number of species has more than doubled, and is now comprised of six classes with Flavobacteriia, Cytophagia and Bacteroidia having the most described species (García-López et al., 2019, Hahnke et al., 2016). Bacteroidota have traditionally been classified as chemoheterotrophic Gram-negative bacteria that are non-motile (except by gliding), non-spore forming and can be either aerobic or anaerobic (Krieg et al., 2010). Although genomic approaches have improved our understanding of their core functions and phylogeny, the diversity and complexity of this phylum mean that it remains relatively uncharacterised (García-López

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et al., 2019; Hahnke et al., 2016; Munoz et al., 2016; Munoz et al., 2020).

In the oceans, Bacteroidota constitute a high proportion of the bacterial biomass and are largely affiliated with the order Flavobacteriales (El-Swais et al., 2015; Glöckner et al., 1999; Kirchman et al., 2010; Wietz et al., 2010; Yilmaz et al., 2016; Zhang et al., 2019a). Marine Bacteroidota are most widely recognized for their important role in biogeochemical cycling due to the diverse array of carbohydrate-active enzymes (CAZymes) and peptidases typically encoded within their genomes (Fernández-Gomez et al., 2013; Gavriilidou et al., 2020). This attribute provides Bacteroidota with the ability to degrade an assortment of complex plant- and animal-derived polymers (such as agar, cellulose and chitin) and hence they are generally considered to be primary degraders of organic matter (Fernández-Gomez et al., 2013; Thomas et al., 2011). Bacteroidota also employ several unique strategies to further enhance the efficiency of their carbohydrate metabolism including the phylum-specific type 9 secretion system (T9SS). The T9SS is highly effective in the secretion of CAZymes and other extracellular proteins while additionally mediating surface-associated gliding motility (Lasica et al., 2017; McBride, 2019). Together, these traits ultimately confer a key competitive advantage allowing Bacteroidota members to successfully colonize and thrive in diverse ecological niches (Munoz et al., 2020).

As a result of their versatile metabolic profile, Bacteroidota are often found in association with nutrientrich eukaryotic organisms where they have evolved complex symbiotic relationships. In some instances, Bacteroidota encompass mutualistic or commensal functions such as promoting larval settlement and development in sea sponges (Li et al., 2021; Webster et al., 2013) and correct morphogenesis of green algae (Marshall et al., 2006; Matsuo et al., 2003; Nakanishi et al., 1996). Bacteroidota are also consistently identified in the gut microbiomes of omnivorous fishes and mammals where they may facilitate the breakdown of ingested polysaccharides (Nelson et al., 2015; Smriga et al., 2010; Sullam et al., 2012).

Conversely, there are reports of Bacteroidota that have adopted a pathogenic lifestyle, leading to severe negative health outcomes for the host. Arguably, the most widely documented marine pathogens within Bacteroidota belong to the genus Tenacibaculum within the family Flavobacteriaceae (Nowlan et al., 2020). Multiple species of Tenacibaculum have been implicated as aetiological agents of tenacibaculosis disease which affects a range of commercially valuable fish species globally (Avendaño-Herrera et al., 2006; Fernández-Alvarez & Santos, 2018; Nowlan et al., 2020). Although considerably less is known about other pathogenic marine Bacteroidota, the advent of culture-independent techniques such as 16S rRNA gene amplicon sequencing has provided evidence for the involvement of a range

of Bacteroidota across numerous marine diseases. For example, studies in corals found that Flavobacteriaceae are abundant in individuals affected by white band disease and reduced in healthy hosts (Gignoux-Wolfsohn & Vollmer, 2015). Similar patterns have also been seen in diseased sponges (Webster et al., 2008), sea stars (Lloyd & Pespeni, 2018), macroalgae (Zozaya-Valdes et al., 2015) and lobsters (Meres et al., 2012; Ooi et al., 2020). An increasing number of pathogenic species are also being isolated, characterized and implicated as aetiological agents of disease. Moreover, some of these pathogens have been hypothesised to be opportunistic in nature due to their presence on healthy tissue.

Overall, the body of research concerning the prevalence, virulence strategies and pathogenicity of marine Bacteroidota remains remarkably limited, especially when compared to the scope of research on other marine pathogens. Hence, this study aims to review the available literature to: (i) determine the prevalence and range of bacterial pathogens belonging to Bacteroidota that cause disease in marine eukaryotic hosts, (ii) examine their virulence strategies and external fachost-pathogen tors influencing dynamics and (iii) identify limitations in our current understanding and avenues for future research.

LITERATURE SEARCHING AND FILTERING

Peer-reviewed primary literature was retrieved using PubMed, Scopus and Web of Science on 10 May 2022. The following search string was applied: [(Bacteroid* OR Chitinophag* OR Cytophag* OR Flavobacter* OR Saprospir* OR Sphingobacter*) AND (marine OR ocean OR seawater) AND (disease* OR pathogen* OR virulen*)], with Scopus records limited to title/abstract/ keywords.

The literature search yielded 590 (PubMed), 446 (Scopus) and 955 (Web of Science) publications. Search results were exported to EndNote citation management program and duplicates were removed resulting in 1258 unique records. Unique publications were manually screened for inclusion. A total of 46 additional studies identified during the screening process (i.e. via citations within articles) were also included.

Studies were included if they:

- i. involved the isolation of a novel Bacteroidota species or strain that was directly associated with a disease phenotype or mortality outcome in a marine eukaryotic host,
- ii. used culture-independent methods to identify a positive association of Bacteroidota with a disease phenotype in a marine host,
- iii. characterized virulence traits of known marine Bacteroidota pathogens,
- iv. were published in English.

Studies were excluded if they:

- i. were unrelated to marine systems or hosts
- ii. concerned the host response to infection,
- solely focused on disease management or iii.
- iv. were not directly linked to a disease phenotype or mortality outcome.

Studies related to Flavobacterium psychrophilum infections in diadromous fish (including salmonids and eel) were not included in this review as these studies primarily concerned freshwater systems and have been reviewed elsewhere (Loch & Faisal. 2015: Starliper, 2011; Wahli & Madsen, 2018). Other Bacteroidota pathogens affecting diadromous fishes were included if the studies were specifically performed within a marine context.

PREVALENCE AND HOST RANGE OF PATHOGENIC MARINE BACTEROIDOTA

Members of the phylum Bacteroidota have been implicated as the causal agent of disease across a range of marine eukaryotic hosts [Figure 1(A)]. In this study, we identified 155 reports of disease where Bacteroidota were positively involved, with 43% of these studies utilizing culture-independent techniques and 56% applying culture-dependent methods, and one study using both approaches (Table S1).

Putative pathogenic Bacteroidota have been cultured from a range of marine eukaryotic hosts, primarily from fish and macroalgae, but also from arthropods, corals, echinoderms, mammals, molluscs and reptiles [-Figure 1(A) and Table S2]. Here, most of these putative pathogens belong to the family Flavobacteriaceae. Few isolates were also assigned to Cytophagaceae; however, these classifications were derived from biochemical traits prior to the taxonomic revision of Bacteroidota and have not been verified (Dungan et al., 1989; Largo et al., 1995a). Pathogens belonging to the genus Tenacibaculum were the most well-characterized, which is not unexpected due to the severe nature of tenacibaculosis disease that results in high mortality rates in fish with substantial economic losses (Avendaño-Herrera et al., 2006). The genus Aquimarina was also represented in the literature with numerous studies covering infection dynamics, phenotypic characterization and genome analysis across pathogens of crustacean and macroalgal hosts (Table 1). No specific reports concerning the virulence strategies of other pathogenic genera including Lacinutrix, Kordia, Chryseobacterium and Gaetbulibacter were identified (Table 1). Most of these pathogens were only recently identified and so more research is needed to characterize the mechanisms they use to cause damage to marine plants and animals.



FIGURE 1 (A) Number of studies related to Bacteroidota in disease by host type. (B) Number of reports related to pathogenic Bacteroidota over time. Dark bars represent the number of culturedependent studies and light bars represent the number of cultureindependent studies

In cases where putative Bacteroidota pathogens were cultured, only 27 isolates had their pathogenicity confirmed using infection experiments (Table 1). This number does not include studies in T. maritimum for which pathogenicity has been demonstrated in a range of fish species. Many reports identified here described the isolation of bacteria from diseased hosts and subsequent biochemical characterization was only pursued for taxonomic purposes. Therefore, further investigation is needed to understand their ecological importance in relation to disease in eukaryotic hosts.

A large number of studies published over the last decade utilized culture-independent methods to implicate Bacteroidota in disease [Figure 1(B)]. A high proportion of these studies applied 16S rRNA gene sequencing techniques, including DGGE, cloning and next-generation sequencing technologies, with only

TABLE 1 List of pathogenic marine Bacter	oidota isolates where pathogenicity has been cor	nfirmed using an infection experiment fulfilli	ng, or partially fulfilling, Koch's postulates
Pathogen name ^a	Host organism(s)	Disease name/phenotype	Reference
Aquimarina hainanensis	Giant mud crab, Gazami crab, Brine shrimp	Tissue necrosis, mortality events	Dan and Hamasaki (2015), Midorikawa et al. (2020)
Aquimarina latercula	Gracilaria lemaneformis (Red algae)	Mid-thallus bleaching	Liu et al. (2019)
Aquimarina sp. AD1	Delisea pulchra (Red algae)	Mid-thallus bleaching	Kumar et al. (2016), Hudson et al. (2019)
Aquimarina sp. BL5	Delisea pulchra (Red algae)	Mid-thallus bleaching	Kumar et al. (2016), Hudson et al. (2019)
Aquimarina sp. 132.4	American lobster	Epizootic, enzootic and impoundment shell disease	Chistoserdov et al. (2005), Chistoserdov et al. (2012), Quinn et al. (2012)
Aquimarina sp. TRL1	Omate spiny lobster, Slipper lobster	White leg disease	Ooi et al. (2020)
Chryseobacterium scophthalmum	Turbot	Gill hyperplasia, haemorrhagic septicaemia	Mudarris and Austin (1989), Mudarris et al. (1994)
<i>Cytophaga</i> sp. P25	Kappaphycus alvarenzii, Eucheuma denticulatum (Red algae)	Ice-ice whitening	Largo et al. (1995a), Largo et al. (1995b)
Cytophaga/Flavobacterium like	Chondrus crispus (Red algae)	Green spot/rot disease	Craigie and Correa (1996)
Flavobacterium columnare FK401	Ring plate coral	Black band disease	Rahmi et al. (2020)
Flavobacterium sp.	<i>Gracilaria verrucosa</i> (Red algae)	Ice-ice whitening	Zainuddin et al. (2019)
Flavobacterium sp. LAD-1	Porphyra yezoensis (Red algae)	Anaaki disease	Sunairi et al. (1995)
Flavobacterium-Cytophaga group bacteria	<i>Gracilaria conferta</i> (Red algae)	Apical necrosis	Weinberger et al. (1997)
Gaetbulibacter saemankumensis	Porphyra yezoensis (Red algae)	Suminori disease	Mine et al. (2009)
Ichthyobacterium seriolicida	Japanese amberjack	Bacterial haemolytic jaundice	Takano et al. (2016), Matsuyama et al. (2018)
Kordia algicida	Agarophyton vermiculophyllum (Red algae)	Tip bleaching	Saha and Weinberger (2019)
Lacinutrix venerupis	European sea bass, Gilt-head bream	Fin erosion, haemorrhaging, mortality	López et al. (2017)
Tenacibaculum dicentrarchi	Atlantic salmon	Tail rot, frayed fins, gill damage, mortality events	Avendaño-Herrera et al. (2016), Klakegg et al. (2019)
Tenacibaculum disolor	Senegalese sole	Tenacibaculosis	Piñeiro-Vidal et al. (2007), Piñeiro-Vidal et al. (2008)
Tenacibaculum finnmarkense	Atlantic salmon	Tenacibaculosis	Småge et al. (2016), Småge et al. (2018)
Tenacibaculum maritimum	Multiple fish hosts	Tenacibaculosis	Wakabayashi et al. (1986), Avendaño-Herrera et al. (2006), Nowlan et al. (2020)
Tenacibaculum maritimum-like	Whiteleg shrimp	Lesions, mortality events	Mouriño et al. (2008), Avendaño-Herrera (2009)
Tenacibaculum ovalyticus	Atlantic halibut	Larval mortality	Bergh et al. (1992), Hansen et al. (1992), Skiftesvik and Bergh (1993)
Tenacibaculum piscium	Atlantic salmon	Winter ulcers	Olsen et al. (2011), Olsen et al. (2020)
Tenacibaculum soleae	Wedge sole, Senegalese sole, Brill, European sea bass	Tenacibaculosis	López et al. (2010) Castro et al. (2014)
Tenacibaculum soleae	Pacific oyster	Lesions	Burioli et al. (2018)
Tenacibaculum sp. F-2	Sea urchin	Spotting disease	Tajima et al. (1997a), Tajima et al. (1997b), Taniguchi et al. (2006)
Tenacibaculum sp. Pbs-1	Akoya pearl oyster	Black spot shell disease	Sakatoku et al. (2018)
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Pathogen name: taxonomically validated names, isolate name or isolate best BLAST match as reported by authors.

seven studies characterizing putative gene functions using meta-genomic, meta-transcriptomic and metaproteomic techniques. Culture-independent studies generally identified a broader taxonomic range of Bacteroidota, including Bacteroidia, Sphingobacteriia and Cytophagia, in addition to Flavobacteriia, also represented in disease phenotypes [Figure 2(A)]. Although limited information is available surrounding pathogenic marine members of Bacteroidia, Sphingobacteriia and Cytophagia, these findings suggest that they may play a yet unknown role in marine diseases. Studies that employed culture-independent methodologies also examined a greater number of ecologically relevant host species, particularly corals and sponges, in contrast with culture-dependent studies which had a greater focus towards species farmed in aquaculture systems [Figure 1(A)].

HOST-PATHOGEN DYNAMICS

We found most marine Bacteroidota that were linked to disease could be classified as opportunistic pathogens as they were also associated with healthy hosts and typically only caused disease symptoms following an environmental perturbation [Figure 2(B) and Table S2]. For example, *Tenacibaculum maritimum* was categorized as an opportunistic pathogen where the onset of disease is associated with increased temperature and salinity, and reduced water quality (Avendaño-Herrera et al., 2006). Many other putative pathogens, especially those identified by culture-independent methods, were not explicitly identified as opportunistic but were found to normally reside on the host surface and were enriched on diseased tissue.

Overall, diseases reported in corals and macroalgae were most frequently linked to an environmental stressor, such as changes in salinity, light, aeration and nutrient availability, with temperature being the most reported stress [Figure 2(B) and Table S2]. In macroalgal hosts, diseases caused by Bacteroidota appear to predominantly affect Rhodophyta (red algae) with fewer cases of disease reported in brown and green algae (Table 1). This includes ice-ice whitening in Gracilaria verrucosa and Kappaphycus alvarezii, and bleaching diseases in Delisea pulchra, Gracilaria lemaneformis and Agarophyton vermiculophyllum. In many cases of macroalgal disease, natural disease outbreaks typically occur in warmer seawater temperatures (Largo et al., 1995b; Zainuddin et al., 2019). Thermal stress is broadly considered to have negative effects on macroalgal physiology by eliciting damage to cellular structures which in turn leads to a downregulation in nonessential metabolic processes (Xu et al., 2014) including reduced chemical defences (Campbell et al., 2011). Reduced host immune functioning may present an opportunity for commensal Bacteroidota to take advantage and exploit the polysaccharide-rich resources of the host resulting in tissue damage. Most coral diseases identified here were similarly associated with increased seawater temperatures, suggesting that commensal Bacteroidota may function advantageously under these environmental conditions.

In other cases, such as shell diseases in lobsters, black spot shell disease in the Akoya pearl oyster, and Suminori disease in *Porphyra yezoensis*, the onset of disease was aided by pre-existing surface damage (Table S2). For example, infection experiments in epizootic shell disease of lobsters showed that disease caused by the chitinolytic pathogen *Aquimarina* sp. I32.4 only occurred when the dermal layer was compromised to expose the underlying chitin layer (Quinn et al., 2012). Damage originating from microeukaryotes could also contribute to disease outcomes



FIGURE 2 (A) Number of times each class level taxa within the phylum Bacteroidota was reported in a disease phenotype using cultureindependent methods, as a proportion of total culture-independent studies. (B) Number of times a culture-independent study reported an environmental condition associated with a disease phenotype, as a proportion of total culture-independent reports. In both graphs, bars are coloured to provide a breakdown of the host from which a particular taxon was identified (A), or the host in which the environmental condition was associated with (B). Note: Bars do not add up to 100% due to multiple taxa or environmental conditions being reported

involving Bacteroidota, such as the gastropod grazing of a sea urchin (Becker et al., 2007) and amoeba and lice infections in salmon (Bowman & Nowak, 2004; Llewellyn et al., 2017; Slinger et al., 2020). Trauma to the external layer of eukaryotic hosts, either from biotic or abiotic means, can compromise the innate defences of the host, providing a route of entry for opportunistic bacteria to proliferate. This is a concern for commercially reared eukaryotes where intensive farming practices, such as high stocking density and handling processes, can result in trauma and provide favourable conditions for parasites to thrive (Bowman & Nowak, 2004; Chistoserdov et al., 2012; Llewellyn et al., 2017; Ogawa & Yokoyama, 1998; Smolowitz et al., 2014).

We also identified the involvement of Bacteroidota in polymicrobial diseases. Black band disease in coral is a well-established model for polymicrobial disease in marine systems, consisting of a complex microbial consortium of cyanobacteria, heterotrophic and sulfurcycling bacteria (Frias-Lopez et al., 2004). Of the publications employing culture-independent methods identified in this study, 10 identified the positive involvement of Bacteroidota in coral black band disease. One study additionally observed black band disease on coral following inoculation with a strain identified as Flavobacterium columnare FK401 (Rahmi et al., 2020). Epizootic shell disease in lobster is also thought to be polymicrobial, where Aquimarina sp. I32.4 colonization is followed by a succession of other microorganisms collectively contributing to the disease phenotype (Chistoserdov et al., 2012; Meres, 2016; Meres et al., 2012; Quinn et al., 2012).

It is also possible that many Bacteroidota were isolated from diseased tissue because they act as secondary pathogens or saprophytic colonizers which proliferate on decaying tissue to exploit the available nutrients. Although no studies reviewed here specifically identified Bacteroidota as secondary pathogens, several studies did detect the involvement of Bacteroidota in diseases with a known aetiological agent. For example, the aetiology of 'Syndrome 93' in white leg shrimp (Costa et al., 1998; Saulnier et al., 2000), skin ulceration disease in sea cucumber (Zhang et al., 2019b) and white spot disease in crustaceans (Ding et al., 2017) were attributed to an infection with Vibrio penaeicida, Vibrio splendidus and white spot syndrome virus, respectively. Yet, in each case, Bacteroidota were found to be enriched on the diseased tissue. As specialist degraders of organic matter, Bacteroidota may have an affinity for decaying tissue where they can utilize complex polysaccharides that other bacteria are not capable of metabolizing (Larsbrink & Mckee, 2020; Thomas et al., 2011). However, even in situations where Bacteroidota do not directly initiate disease, they can play a synergistic role in the progression of disease by exacerbating damage

and escalating mortality rates. This concern is becoming increasingly apparent in freshwater aquaculture, where secondary infections with *Flavobacterium* spp. led to higher mortality rates in fish affected by viral primary pathogens (Adamek et al., 2013; Adamek et al., 2018; Boonthai et al., 2018; Kim et al., 2018; Ma et al., 2019). In marine systems, it is possible that secondary or co-infections with Bacteroidota can confer a threat even in cases with known aetiology and present further challenges for diagnostic, treatment and disease mitigation strategies.

Taken together, it is evident that diseases involving Bacteroidota pathogens in the oceans are complex. Rather than acting exclusively as primary pathogens that follow the 'one pathogen-one disease' paradigm, disease caused by Bacteroidota results from dynamic and multifactorial interactions between the host, pathogen, other biotic agents and the environment. Thus, knowing how and when a host is susceptible to opportunistic pathogens will provide a valuable insight into how diseases caused by Bacteroidota emerge. However, our understanding of opportunistic pathogens is also dependent upon recognizing which commensal Bacteroidota have the capacity to cause disease and the virulence mechanisms they use to inflict damage.

VIRULENCE MECHANISMS OF PATHOGENIC MARINE BACTEROIDOTA

Surface attachment and motility

Attachment to host tissue is widely accepted as a prerequisite in the infection process of all pathogens. Marine Bacteroidota typically encode a high number of adhesins in their genomes, and have a general tendency to exist attached to particulate matter in the oceans (DeLong et al., 1993; Fernández-Gomez et al., 2013; Kirchman et al., 2010), where they can move by surface-mediated gliding motility. Studies of pathogenic marine Bacteroidota have attributed adhesins as potential virulence traits, including the sea urchin pathogen Tenacibaculum sp. F-2 where attachment to the host, via carbohydrate-binding receptors, was directly linked to disease outcomes (Taniguchi et al., 2006). The genome of the fish pathogen T. maritimum was found to encode 17 adhesins, as well as genes for the biosynthesis of exopolysaccharides and carbohydrate-binding motifs, which were proposed as virulence traits (Pérez-Pascual et al., 2017a). In addition, surface attachment appears to be regulated by external conditions that are hypothesised to induce changes in surface-exposed proteins. For example, the host attachment of Tenacibaculum sp. F-2 is positively regulated by temperature, coinciding with the onset of disease occurring in the summer months (Tajima

et al., 1997b; Taniguchi et al., 2006). Earlier studies in *T. maritimum* also found surface attachment could be enhanced by hydrophobic surface chemistry, bacterial growth stage and nutrient availability (Burchard et al., 1990; Sorongon et al., 1991). These studies collectively signify that the environment can influence the attachment of Bacteroidota to hosts, and further studies should focus on identifying specific adhesion proteins and host receptors that influence attachment under different environmental conditions in order to develop a greater understanding of host–microbe interactions.

Following attachment. Bacteroidota can utilize surface-associated gliding motility which is mediated by the T9SS. Both the T9SS and gliding apparatus are often implicated in the virulence of pathogenic marine Bacteroidota. especially those characterized via genome sequencing. The role of gliding motility in virulence is clear in pathogens such as F. columnare and F. psychrophilum, where mutants deficient in gliding motility-associated genes lacked adhesion properties, and exhibited reduced biofilm, proteolytic and virulence characteristics (Pérez-Pascual et al., 2017b; Thunes et al., 2021). However, as gliding motility and the T9SS are common and widespread in Bacteroidota (McBride, 2019; McBride & Zhu, 2013), these functions are unlikely to be inherent virulence traits. Rather, such observations highlight how some Bacteroidota can efficiently utilize common adaptive traits to facilitate pathogenesis.

Tissue invasion and destruction

Traits centred around the acquisition of iron, polysaccharides and peptides from eukaryotic hosts were strongly associated with virulence phenotypes and often designated as key virulence traits (Table S2). This is not surprising as marine eukaryotes represent a rich source of nutrients for bacteria, and the evolutionary drive for microbes to adopt pathogenic characteristics in order to access these nutrients has long been recognized (Persson et al., 2009; Rohmer et al., 2011). Indeed, the unique nutrient uptake strategies exhibited by Bacteroidota, such as the secretion of CAZyme and peptidase effector proteins via the T9SS, are likely to confer damage to the extracellular matrix of plants and animals, facilitating colonization and access to nutrients. This also correlates with the pathology that is commonly observed, which typically presents as surface lesions, rotting and necrosis (Table 1).

Iron uptake strategies are recognized as general virulence traits in most pathogens and have been identified in multiple pathogens within the phylum Bacteroidota. This includes lobster pathogens *Aquimarina* sp. 132.4 (Ranson et al., 2018) and *Aquimarina* sp. TRL1 (Ooi et al., 2020), and the macroalgal pathogens *Aquimarina* sp. AD1 and *Aquimarina* sp. BL5

(Hudson et al., 2019) which encode multiple iron uptake systems. The iron uptake strategies of *T. maritimum* have been studied in the most detail, including the production of multiple siderophores and the ability to use iron from a variety of sources (including transferrin, hemin, haemoglobin and ferric ammonic citrate) (Avendaño-Herrera et al., 2005; Pérez-Pascual et al., 2017a).

Some studies also identified pathogens to either encode or produce a repertoire of enzymes that specifically target and degrade host macromolecules. For example, T. maritimum encodes sphingomyelinase and ceramidase which are predicted to degrade host cell membranes, as well as a range of proteases that can elicit destruction of the host tissue (Nowlan et al., 2020; Pérez-Pascual et al., 2017a; Rahman et al., 2014). In particular, chondroitin AC lyases produced by T. maritimum are predicted to function as major virulence factors by targeting the chondroitin sulfate component of fish cartilage and connective tissue (Pérez-Pascual et al., 2017a; Rahman et al., 2014). This observation was initially supported by studies in the freshwater fish pathogen F. columnare, where the activity of chondroitin sulfate lyases was correlated with virulence (Stringer-Roth et al., 2002; Suomalainen et al., 2006). However, the contribution of this enzyme to the virulence phenotype was later attributed, in part, to the competitive advantage it conferred on the pathogen, rather than being an essential virulence trait (Li et al., 2015).

Interestingly, pathogens belonging to the genus Aquimarina have a strong tendency to affect hosts rich in complex polysaccharides and encode an enzymatic repertoire orientated towards the carbohydrate makeup of their host. The algal pathogens Aquimarina sp. AD1, Aquimarina sp. BL5 and Aquimarina latercula possess CAZymes, including agarases and carrageenases, which may act on the agar and carrageenan matrix of the algal cell wall (Hudson et al., 2019; Liu et al., 2019; Nedashkovskaya et al., 2005). Likewise, pathogens of crustaceans including Aquimarina hainanensis and Aquimarina sp. TRL1 exhibited strong chitinolytic activity that potentially degrades the chitin-rich shell of their crustacean hosts (Chistoserdov et al., 2005: Midorikawa et al., 2020; Ooi et al., 2020; Ranson et al., 2018). However, in other cases, the involvement of host cell wall-degrading enzymes remains ambiguous, as is the case for the chitinases that are linked to the virulence of the lobster pathogen Aquimarina sp. I32.4 despite the destruction of the chitin shell not being apparent in the pathology of epizootic shell disease (Smolowitz et al., 2005). There are also cases of non-pathogenic Bacteroidota displaying similar phenotypic properties as pathogenic strains. This has been investigated in the algal symbiont Aquimarina sp. AD10 which displays a similar agarolytic and carrageenolytic phenotype to the pathogens Aquimarina sp. AD1 and

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Aquimarina sp. BL5, yet virulence experiments have demonstrated that it is not pathogenic (Hudson et al., 2019; Kumar et al., 2016).

Thus, while host tissue-degrading enzymes are associated with virulence phenotypes, their contribution to the disease outcome is complex, and their presence alone is not a determinant of pathogenesis. A reason behind these discrepancies may be due to differences in expression, regulation and catalytic activity of these enzymes that instead determine the virulence potential of Bacteroidota. This has been demonstrated in the plant pathogen F. johnsoniae where pathogenic isolates were observed to secrete more pectate lyase than non-pathogenic strains (Liao & Wells, 1986). Although enzyme analysis and mutagenesis studies are limited for marine Bacteroidota, studies that have applied more functional approaches have achieved a deeper insight into the specific factors contributing to virulence. For example, a comparative insight of algal pathogens identified specific enzymes produced by Aquimarina sp. AD1 and Aquimarina sp. BL5, notably the genes encoding for unique alpha-agarases (GH96) that were absent in Aquimarina sp. AD10 that may instead play a deterministic role in virulence (Hudson et al., 2019). A phenotypic comparison between pathogenic and nonpathogenic strains of T. maritimum identified deficiencies in gliding motility in the non-pathogen that was hypothesised to additionally disrupt the secretion of adhesins and host tissue-degrading enzymes, rendering it avirulent (Rahman et al., 2014). In addition, gene regulation strategies, such as quorum sensing (QS) have been studied in T. maritimum and may provide a benefit to pathogens by allowing for the coordinated expression of traits that confer virulence in response to specific environmental conditions (Romero et al., 2010). QS has also been explored in white band disease of coral, where the addition of QS autoinducers correlated with an increase in the abundance of disease-associated Flavobacteriaceae. Moreover, the addition of a QS inhibitor reduced the abundance of Flavobacteriaceae and inhibited the onset of disease. suggesting a role for QS in the virulence of Flavobacteriaceae pathogens (Certner & Vollmer, 2015; Certner & Vollmer, 2018). However, QS has not been widely studied in Bacteroidota and so its specific contribution to virulence is not fully understood.

Host evasion and pathogen proliferation

Evading host immune responses is critical for pathogens to establish infection and persist on host tissue. However, only *T. maritimum* has been noted for encoding known evasion strategies, including the production of a capsule and a sphingomyelinase haemolytic factor that aids phagosome escape (Nowlan et al., 2020; Pérez-Pascual et al., 2017a). The genomes of *T. maritimum* and some *Aquimarina* pathogens also encode for

multiple superoxide dismutase and catalase enzymes, which were proposed as virulence factors since they provide resistance to host oxidative defences (Ooi et al., 2020; Pérez-Pascual et al., 2017a). Beyond this, host evasion strategies of marine Bacteroidota are not widely reported in the literature. However, as commensal bacteria that normally reside in the host microbiome, Bacteroidota may not need to directly hide from host defences, as they generally appear to cause disease only when the host defences are impaired. Opportunistic Bacteroidota may instead need to encode traits that offer a competitive advantage over other members of the host-associated microbiome to successfully proliferate and cause disease. This has been suggested for the macroalgal pathogens Aquimarina sp. AD1 and Aquimarina sp. BL5 which encode for non-ribosomal peptide synthase (NRPS) and NRPS type 1 polyketide synthase clusters, respectively. These secondary metabolite clusters were found to exhibit homology to genes involved in the synthesis of antimicrobial peptides (Hudson et al., 2019). Likewise, the genome of the lobster pathogen Aquimarina sp. 132.4 encodes multiple secondary metabolite clusters (Ranson et al., 2018). Other pathogens also encode numerous antibiotic resistance genes, such as the lobster pathogen Aquimarina sp. TRL1, potentially allowing it to resist antimicrobial metabolites produced by other microorganisms (Ooi et al., 2020). These traits may allow Bacteroidota pathogens to resist and suppress bacteria within the microbiota that are beneficial to the host and therefore promote dysbiosis and disease.

Collectively, the virulence of marine Bacteroidota arises from a suite of virulence traits that ultimately confer a competitive advantage allowing for successful colonization and proliferation (Figure 3). Moreover, these traits can generally be considered as 'dual use' virulence traits, where they typically function to provide an adaptive advantage, but can also function as virulence traits under certain conditions to cause disease (Casadevall et al., 2003). Utilizing these 'dual use' virulence traits may have resulted in many aspects of their pathogenesis being overlooked in studies that aim to characterize the virulence of Bacteroidota pathogens. Therefore, an improved understanding of the role of these traits in virulence may benefit from a greater focus on gene expression, regulation and enzyme activity as well as the effect of environmental conditions on these factors.

CURRENT LIMITATIONS AND OPPORTUNITIES FOR FUTURE RESEARCH

Despite what has already been inferred through genomics and phenotypic observations, our collective understanding of pathogenicity in marine Bacteroidota is considerably limited. Of the pathogens that have



FIGURE 3 Mechanisms of pathogenesis in marine Bacteroidota. (A) Bacteroidota with the capacity to act as pathogens on marine eukaryotic hosts need to encode an appropriate suite of virulence traits. This may include 'dual use' traits (Casadevall et al., 2003) such as CAZymes or peptidases that can specifically target host polymers, the T9SS which can aid in the secretion of these enzymes, adhesins that are specific to host tissue, or the ability to resist oxidative stress. These pathogens may also produce quorum sensing (QS) signals to regulate these traits and antimicrobials to suppress other competitors, which collectively confer a competitive advantage over other microorganisms, including Bacteroidota that do not encode these traits. (B) Using an example of a macroalgal disease, an environmental stress (such as changes in salinity, temperature, light intensity, pollution or the presence of biotic grazers) can weaken host immunity and select for microorganisms that function advantageously under these conditions. Bacteroidota are normally present in the host-associated microbiota (1) but following the onset of an environmental stress and reduced host immune functioning, Bacteroidota equipped with the necessary virulence traits can rapidly proliferate to degrade and metabolize host macromolecules (2). As infection progresses, secondary Bacteroidota pathogens may colonize and further exploit the available nutrients (3). Tissue damage resulting from enzymatic degradation results in a disease phenotype on the host

been isolated, few studies have been able to demonstrate their mode of pathogenicity or functionally characterize their virulence traits. This is concerning as an understanding of virulence is critical to our knowledge of disease, predicting outbreaks and improving mitigation, diagnostics, and treatment strategies, particularly in the face of emerging pathogens.

The most striking deficiency in our understanding of virulence in Bacteroidota is the lack of studies analysing gene function, with no studies identified here having produced genetic mutants. This contrasts with studies in Proteobacteria, including marine isolates of Pseudomonas and Vibrio, where mutagenesis methods are routinely applied, and the function of key genes has been validated and extensively characterized. Although genetic mutants have been successfully created in some non-marine Bacteroidota, such as Cytophaga hutchinsonii (Zhu & McBride, 2014), F. johnsoniae (Rhodes et al., 2010), F. psychrophilum (Gomez et al., 2015) and F. columnare (Li et al., 2015), further work is needed to develop genetic tools that are suitable for marine strains. Thus, whilst greater focus should be given to creating applicable genetic tools, in their absence, studies will need to utilize more phenotypic analysis, enzyme characterization, gene expression and metabolic interaction approaches to allow for a functional understanding of their virulence.

Culture-independent studies have provided a valuable insight into the role of Bacteroidota, particularly in cases of polymicrobial, dysbiotic diseases or where secondary/saprophytic pathogens are involved by allowing for community-level changes to be studied. These studies can further benefit from a shift towards metagenomics and transcriptomics to provide a more functionally driven understanding of these complex diseases. Lastly, as environmental conditions appear to greatly influence virulence phenotypes and disease outcomes, studies assessing virulence should include relevant environmental factors in their experimental design.

CONCLUDING REMARKS

Members of the phylum Bacteroidota thrive in the marine environment owing to their highly competitive nature, unique adaptive traits and ability to form dynamic relationships with eukaryotic hosts. It appears that pathogenic marine Bacteroidota are inherently opportunistic, with virulence arising from a competitive evolutionary drive to exploit host nutrients, often following a perturbation that weakens host immunity (Figure 3). As increasing environmental stressors from climate change, urbanization and pollution put pressure

on our marine ecosystems, pathogenic and commensal Bacteroidota equipped with the appropriate suite of virulence traits may therefore have the potential to emerge as a serious threat to ocean life in the future. With the prevalence of disease already increasing in the marine environment, many of which have unresolved aetiology, more investigation is needed to develop a comprehensive understanding of the molecular mechanisms Bacteroidota use to cause disease. With this knowledge, it may be possible to mitigate the emerging threat of marine Bacteroidota, protecting food security and ecologically important marine species alike.

AUTHOR CONTRIBUTIONS

The study was conceptualized by J.H. and S.E. J.H. wrote the first draft of the manuscript. Both authors contributed to and approved the final version of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The full list of publications reviewed in this study is provided in Table S1.

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REFERENCES

- Adamek, M., Syakuri, H., Harris, S., Rakus, K., Brogden, G., Matras, M. et al. (2013) Cyprinid herpesvirus 3 infection disrupts the skin barrier of common carp (*Cyprinus carpio L.*). Veterinary Microbiology, 162, 456–470.
- Adamek, M., Teitge, F., Jung-Schroers, V., Heling, M., Gela, D., Piackova, V. et al. (2018) Flavobacteria as secondary pathogens in carp suffering from koi sleepy disease. *Journal of Fish Diseases*, 41, 1631–1642.
- Avendaño-Herrera, R. (2009) Identification of Flexibacter maritimus or Tenacibaculum maritimum from post-larvae of Litopenaeus vannamei? Comment on Mouriño et al.(2008). Brazilian Journal of Biology, 69, 225–226.
- Avendaño-Herrera, R., Toranzo, A.E., Romalde, J.L., Lemos, M.L. & Magariños, B. (2005) Iron uptake mechanisms in the fish pathogen *Tenacibaculum maritimum*. *Applied and Environmental Microbiology*, 71, 6947–6953.

- Avendaño-Herrera, R., Toranzo, A.E. & Magariños, B. (2006) Tenacibaculosis infection in marine fish caused by *Tenacibaculum maritimum*: a review. *Diseases of Aquatic Organisms*, 71, 255–266.
- Avendaño-Herrera, R., Irgang, R., Sandoval, C., Moreno-Lira, P., Houel, A., Duchaud, E. et al. (2016) Isolation, characterization and virulence potential of *Tenacibaculum dicentrarchi* in salmonid cultures in Chile. *Transboundary and Emerging Diseases*, 63, 121–126.
- Becker, P., Gillan, D.C. & Eeckhaut, I. (2007) Microbiological study of the body wall lesions of the echinoid *Tripneustes gratilla*. *Diseases of Aquatic Organisms*, 77, 73–82.
- Bergh, Ø., Hansen, G.H. & Taxt, R.E. (1992) Experimental infection of eggs and yolk sac larvae of halibut, *Hippoglossus hippoglossus* L. *Journal of Fish Diseases*, 15, 379–391.
- Boonthai, T., Loch, T.P., Yamashita, C.J., Smith, G.D., Winters, A.D., Kiupel, M. et al. (2018) Laboratory investigation into the role of largemouth bass virus (Ranavirus, Iridoviridae) in smallmouth bass mortality events in Pennsylvania rivers. *BMC Veterinary Research*, 14, 1–15.
- Bowman, J.P. & Nowak, B. (2004) Salmonid gill bacteria and their relationship to amoebic gill disease. *Journal of Fish Diseases*, 27, 483–492.
- Burchard, R.P., Rittschof, D. & Bonaventura, J. (1990) Adhesion and motility of gliding bacteria on substrata with different surface free energies. *Applied and Environmental Microbiology*, 56, 2529– 2534.
- Burioli, E.A., Varello, K., Trancart, S., Bozzetta, E., Gorla, A., Prearo, M. et al. (2018) First description of a mortality event in adult Pacific oysters in Italy associated with infection by a *Tenacibaculum soleae* strain. *Journal of Fish Diseases*, 41, 215–221.
- Campbell, A.H., Harder, T., Nielsen, S., Kjelleberg, S. & Steinberg, P. D. (2011) Climate change and disease: bleaching of a chemically defended seaweed. *Global Change Biology*, 17, 2958–2970.
- Casadevall, A., Steenbergen, J.N. & Nosanchuk, J.D. (2003) 'Ready made' virulence and 'dual use' virulence factors in pathogenic environmental fungi—the *Cryptococcus neoformans* paradigm. *Current Opinion in Microbiology*, 6, 332–337.
- Castro, N., Balboa, S., Núñez, S., Toranzo, A.E. & Magariños, B. (2014) First isolation and characterization of *Tenacibaculum* soleae from sea bass *Dicentrarchus labrax*. *Fish Pathology*, 49, 16–22.
- Certner, R.H. & Vollmer, S.V. (2015) Evidence for autoinduction and quorum sensing in white band disease-causing microbes on *Acropora cervicornis*. *Scientific Reports*, 5, 9.
- Certner, R.H. & Vollmer, S.V. (2018) Inhibiting bacterial quorum sensing arrests coral disease development and disease-associated microbes. *Environmental Microbiology*, 20, 645–657.
- Chistoserdov, A.Y., Smolowitz, R., Mirasol, F. & Hsu, A. (2005) Culture-dependent characterization of the microbial community associated with epizootic shell disease lesions in American lobster, *Homarus americanus*. *Journal of Shellfish Research*, 24, 741–747.
- Chistoserdov, A.Y., Quinn, R.A., Gubbala, S.L. & Smolowitz, R. (2012) Bacterial communities associated with lesions of shell disease in the American lobster, *Homarus americanus* Milne-Edwards. *Journal of Shellfish Research*, 31, 449–462.
- Costa, R., Mermoud, I., Koblavi, S., Morlet, B., Haffner, P., Berthe, F. et al. (1998) Isolation and characterization of bacteria associated with a *Penaeus stylirostris* disease (syndrome 93) in New Caledonia. *Aquaculture*, 164, 297–309.
- Craigie, J.S. & Correa, J.A. (1996) Etiology of infectious diseases in cultivated *Chondrus crispus* (Gigartinales, Rhodophyta). *Hydrobiologia*, 327, 97–104.
- Dan, S. & Hamasaki, K. (2015) Evaluation of the effects of probiotics in controlling bacterial necrosis symptoms in larvae of the mud crab *Scylla serrata* during mass seed production. *Aquaculture International*, 23, 277–296.

- DeLong, E.F., Franks, D.G. & Alldredge, A.L. (1993) Phylogenetic diversity of aggregate-attached vs. free-living marine bacterial assemblages. *Limnology and Oceanography*, 38, 924–934.
- Ding, Z.F., Cao, M.J., Zhu, X.S., Xu, G.H. & Wang, R.L. (2017) Changes in the gut microbiome of the Chinese mitten crab (*Erio-cheir sinensis*) in response to white spot syndrome virus (WSSV) infection. *Journal of Fish Diseases*, 40, 1561–1571.
- Dungan, C.F., Elston, R.A. & Schiewe, M.H. (1989) Evidence for colonization and destruction of hinge ligaments in cultured juvenile Pacific oysters (*Crassostrea gigas*) by cytophaga-like bacteria. *Applied and Environmental Microbiology*, 55, 1128–1135.
- El-Swais, H., Dunn, K.A., Bielawski, J.P., Li, W.K. & Walsh, D.A. (2015) Seasonal assemblages and short-lived blooms in coastal north-west Atlantic Ocean bacterioplankton. *Environmental Microbiology*, 17, 3642–3661.
- Fernández-Álvarez, C. & Santos, Y. (2018) Identification and typing of fish pathogenic species of the genus *Tenacibaculum*. Applied Microbiology and Biotechnology, 102, 9973–9989.
- Fernández-Gomez, B., Richter, M., Schüler, M., Pinhassi, J., Acinas, S.G., González, J.M. et al. (2013) Ecology of marine Bacteroidetes: a comparative genomics approach. *The ISME Journal*, 7, 1026–1037.
- Frias-Lopez, J., Klaus, J.S., Bonheyo, G.T. & Fouke, B.W. (2004) Bacterial community associated with black band disease in corals. *Applied and Environmental Microbiology*, 70, 5955– 5962.
- García-López, M., Meier-Kolthoff, J.P., Tindall, B.J., Gronow, S., Woyke, T., Kyrpides, N.C. et al. (2019) Analysis of 1,000 typestrain genomes improves taxonomic classification of Bacteroidetes. *Frontiers in Microbiology*, 10, 2083.
- Gavriilidou, A., Gutleben, J., Versluis, D., Forgiarini, F., Van Passel, M.W., Ingham, C.J. et al. (2020) Comparative genomic analysis of Flavobacteriaceae: insights into carbohydrate metabolism, gliding motility and secondary metabolite biosynthesis. *BMC Genomics*, 21, 1–21.
- Gignoux-Wolfsohn, S.A. & Vollmer, S.V. (2015) Identification of candidate coral pathogens on white band disease-infected staghorn coral. *PLoS One*, 10, e0134416.
- Glöckner, F.O., Fuchs, B.M. & Amann, R. (1999) Bacterioplankton compositions of lakes and oceans: a first comparison based on fluorescence in situ hybridization. *Applied and Environmental Microbiology*, 65, 3721–3726.
- Gomez, E., Alvarez, B., Duchaud, E. & Guijarro, J.A. (2015) Development of a markerless deletion system for the fish-pathogenic bacterium *Flavobacterium psychrophilum*. *PLoS One*, 10, e0117969.
- Hahnke, R.L., Meier-Kolthoff, J.P., García-López, M., Mukherjee, S., Huntemann, M., Ivanova, N.N. et al. (2016) Genome-based taxonomic classification of Bacteroidetes. *Frontiers in Microbiol*ogy, 7, 2003.
- Hansen, G.H., Bergh, O., Michaelsen, J. & Knappskog, D. (1992) Flexibacter ovolyticus sp. nov, a pathogen of eggs and larvae of Atlantic halibut, Hippoglossus hippoglossus L. International Journal of Systematic Bacteriology, 42, 451–458.
- Hudson, J., Kumar, V. & Egan, S. (2019) Comparative genome analysis provides novel insight into the interaction of *Aquimarina* sp. AD1, BL5 and AD10 with their macroalgal host. *Marine Genomics*, 46, 8–15.
- Kim, S.W., Jun, J.W., Giri, S.S., Chi, C., Yun, S., Kim, H.J. et al. (2018) First report of carp oedema virus infection of koi (*Cyprinus carpio* haematopterus) in the Republic of Korea. *Transboundary and Emerging Diseases*, 65, 315–320.
- Kirchman, D.L., Cottrell, M.T. & Lovejoy, C. (2010) The structure of bacterial communities in the western Arctic Ocean as revealed by pyrosequencing of 16S rRNA genes. *Environmental Microbiology*, 12, 1132–1143.
- Klakegg, Ø., Abayneh, T., Fauske, A.K., Fülberth, M. & Sørum, H. (2019) An outbreak of acute disease and mortality in Atlantic

salmon (Salmo salar) post-smolts in Norway caused by Tenacibaculum dicentrarchi. Journal of Fish Diseases, 42, 789–807.

- Krieg, N.R., Ludwig, W., Euzéby, J. & Whitman, W.B. (2010) *Phylum XIV. Bacteroidetes phyl. nov. Bergey's manual of systematic bacteriology*. New York, NY: Springer.
- Kumar, V., Zozaya-Valdes, E., Kjelleberg, S., Thomas, T. & Egan, S. (2016) Multiple opportunistic pathogens can cause a bleaching disease in the red seaweed *Delisea pulchra. Environmental Microbiology*, 18, 3962–3975.
- Largo, D.B., Fukami, K. & Nishijima, T. (1995a) Occasional pathogenic bacteria promoting ice-ice disease in the carrageenanproducing red algae *Kappaphycus alvarezii* and *Eucheuma denticulatum* (Solieriaceae, Gigartinales, Rhodophyta). Journal of Applied Phycology, 7, 545–554.
- Largo, D.B., Fukami, K., Nishijima, T. & Ohno, M. (1995b) Laboratoryinduced development of the ice-ice disease of the farmed red algae Kappaphycus alvarezii and Eucheuma denticulatum (Solieriaceae, Gigartinales, Rhodophyta). Journal of Applied Phycology, 7, 539–543.
- Larsbrink, J. & Mckee, L.S. (2020) Bacteroidetes bacteria in the soil: glycan acquisition, enzyme secretion, and gliding motility. *Advances in Applied Microbiology*, 110, 63–98.
- Lasica, A.M., Ksiazek, M., Madej, M. & Potempa, J. (2017) The type IX secretion system (T9SS): highlights and recent insights into its structure and function. *Frontiers in Cellular and Infection Microbiology*, 7, 215.
- Li, N., Qin, T., Zhang, X.L., Huang, B., Liu, Z.X., Xie, H.X. et al. (2015) Gene deletion strategy to examine the involvement of the two chondroitin lyases in *Flavobacterium columnare* virulence. *Applied and Environmental Microbiology*, 81, 7394–7402.
- Li, M., Wang, K., Jia, C., Liu, T., Yang, S., Ou, H. et al. (2021) Bacteroidetes bacteria, important players in the marine sponge larval development process. *iScience*, 24, 102662.
- Liao, C.H. & Wells, J.M. (1986) Properties of *Cytophaga johnsonae* strains causing spoilage of fresh produce at food markets. *Applied and Environmental Microbiology*, 52, 1261–1265.
- Liu, X., Chen, Y., Zhong, M., Chen, W., Lin, Q. & Du, H. (2019) Isolation and pathogenicity identification of bacterial pathogens in bleached disease and their physiological effects on the red macroalga *Gracilaria lemaneiformis*. Aquatic Botany, 153, 1–7.
- Llewellyn, M.S., Leadbeater, S., Garcia, C., Sylvain, F.E., Custodio, M., Ang, K.P. et al. (2017) Parasitism perturbs the mucosal microbiome of Atlantic Salmon. *Scientific Reports*, 7, 10.
- Lloyd, M.M. & Pespeni, M.H. (2018) Microbiome shifts with onset and progression of sea star wasting disease revealed through time course sampling. *Scientific Reports*, 8, 1–12.
- Loch, T.P. & Faisal, M. (2015) Emerging flavobacterial infections in fish: a review. *Journal of Advanced Research*, 6, 283–300.
- López, J., Piñeiro-Vidal, M., García-Lamas, N., De La Herran, R., Navas, J., Hachero-Cruzado, I. et al. (2010) First isolation of *Tenacibaculum soleae* from diseased cultured wedge sole, *Dicologoglossa cuneata* (Moreau), and brill, *Scophthalmus rhombus* (L.). *Journal of Fish Diseases*, 33, 273–278.
- López, J., Alcantara, R., Lorenzo, L. & Navas, J. (2017) Isolation of Lacinutrix venerupis strains associated with disease outbreaks in sea bream *Sparus aurata* and European sea bass *Dicentrarchus labrax*. *Diseases of Aquatic Organisms*, 124, 85–90.
- Ma, J., Bruce, T.J., Oliver, L.P. & Cain, K.D. (2019) Co-infection of rainbow trout (*Oncorhynchus mykiss*) with infectious hematopoietic necrosis virus and *Flavobacterium psychrophilum*. *Journal* of Fish Diseases, 42, 1065–1076.
- Marshall, K., Joint, I., Callow, M.E. & Callow, J.A. (2006) Effect of marine bacterial isolates on the growth and morphology of axenic plantlets of the green alga *Ulva linza*. *Microbial Ecology*, 52, 302–310.
- Matsuo, Y., Suzuki, M., Kasai, H., Shizuri, Y. & Harayama, S. (2003) Isolation and phylogenetic characterization of bacteria capable

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of inducing differentiation in the green alga Monostroma oxyspermum. Environmental Microbiology, 5, 25–35.

- Matsuyama, T., Fukuda, Y., Takano, T., Sakai, T. & Nakayasu, C. (2018) Antibody-mediated bacterial killing of *Ichthyobacterium* seriolicida in Japanese amberjack. *Veterinary Immunology and Immunopathology*, 203, 73–77.
- McBride, M.J. (2019) Bacteroidetes gliding motility and the type IX secretion system. *Microbiology Spectrum*, 7.1.5.
- McBride, M.J. & Zhu, Y. (2013) Gliding motility and Por secretion system genes are widespread among members of the phylum Bacteroidetes. *Journal of Bacteriology*, 195, 270–278.
- Meres, N.J. (2016) Surface biofilm interactions in epizootic shell disease of the American lobster (*Homarus americanus*). In: Dhanasekaran, D. (Ed.) *Microbial biofilms-importance and applications*. London: IntechOpen.
- Meres, N.J., Ajuzie, C.C., Sikaroodi, M., Vemulapalli, M., Shields, J. D. & Gillevet, P.M. (2012) Dysbiosis in epizootic shell disease of the American lobster (*Homarus americanus*). *Journal of Shellfish Research*, 31, 463–472.
- Midorikawa, Y., Shimizu, T., Sanda, T., Hamasaki, K., Dan, S., Lal, M.T.B.M. et al. (2020) Characterization of Aquimarina hainanensis isolated from diseased mud crab Scylla serrata larvae in a hatchery. Journal of Fish Diseases, 43, 541–549.
- Mine, T., Tanaka, S., Kawamura, Y., Kobayashi, G. & Kanda, K. (2009) Diversity of incidence factors in Suminori disease during laver cultivation. *Aquaculture Science*, 57, 601–608.
- Mouriño, J., Vinatea, L., Buglione-Neto, C., Ramirez, C., Vieira, F., Pedrotti, F. et al. (2008) Characterization and experimental infection of *Flexibacter maritimus* (Wakabayashi et al. 1986) in hatcheries of post-larvae of *Litopenaeus vannamei* Boone, 1931. *Brazilian Journal of Biology*, 68, 173–177.
- Mudarris, M. & Austin, B. (1989) Systemic disease in turbot Scophthalmus maximus caused by a previously unrecognised Cytophaga-like bacterium. Diseases of Aquatic Organisms, 6, 161–166.
- Mudarris, M., Austin, B., Segers, P., Vancanneyt, M., Hoste, B. & Bernardet, J.F. (1994) *Flavobacterium scophthalmum* sp. nov, a pathogen of turbot (*Scophthalmus maximus* I). *International Journal of Systematic Bacteriology*, 44, 447–453.
- Munoz, R., Rosselló-Móra, R. & Amann, R. (2016) Revised phylogeny of Bacteroidetes and proposal of sixteen new taxa and two new combinations including Rhodothermaeota phyl. nov. Systematic and Applied Microbiology, 39, 281–296.
- Munoz, R., Teeling, H., Amann, R. & Rossello-Mora, R. (2020) Ancestry and adaptive radiation of Bacteroidetes as assessed by comparative genomics. *Systematic and Applied Microbiol*ogy, 43, 126065.
- Nakanishi, K., Nishijima, M., Nishimura, M., Kuwano, K. & Saga, N. (1996) Bacteria that induce morphogenesis in Ulva Pertusa (chlorophyta) grown under axenic conditions. Journal of Phycology, 32, 479–482.
- Nedashkovskaya, O.I., Kim, S.B., Lysenko, A.M., Frolova, G.M., Mikhailov, V.V., Lee, K.H. et al. (2005) Description of Aquimarina muelleri gen. nov., sp. nov., and proposal of the reclassification of [Cytophaga] latercula Lewin 1969 as Stanierella latercula gen. nov., comb. nov. International Journal of Systematic and Evolutionary Microbiology, 55, 225–229.
- Nelson, T.M., Apprill, A., Mann, J., Rogers, T.L. & Brown, M.V. (2015) The marine mammal microbiome: current knowledge and future directions. *Microbiology Australia*, 36, 8–13.
- Nowlan, J.P., Lumsden, J.S. & Russell, S. (2020) Advancements in characterizing *Tenacibaculum* infections in Canada. *Pathogens*, 9, 1029.
- Ogawa, K. & Yokoyama, H. (1998) Parasitic diseases of cultured marine fish in Japan. *Fish Pathology*, 33, 303–309.
- Olsen, A.B., Nilsen, H., Sandlund, N., Mikkelsen, H., Sørum, H. & Colquhoun, D.J. (2011) *Tenacibaculum* sp. associated with

winter ulcers in sea-reared Atlantic salmon Salmo salar. Diseases of Aquatic Organisms, 94, 189–199.

- Olsen, A.B., Spilsberg, B., Nilsen, H.K., Lagesen, K., Gulla, S., Avendaño-Herrera, R. et al. (2020) *Tenacibaculum piscium* sp. nov., isolated from skin ulcers of sea-farmed fish, and description of *Tenacibaculum finnmarkense* sp. nov. with subdivision into genomovars finnmarkense and ulcerans. *International Journal of Systematic and Evolutionary Microbiology*, 70, 6079– 6090.
- Ooi, M.C., Goulden, E.F., Trotter, A.J., Smith, G.G. & Bridle, A.R. (2020) Aquimarina sp. associated with a cuticular disease of cultured larval palinurid and scyllarid lobsters. *Frontiers in Microbiology*, 11, 2395.
- Oren, A. & Garrity, G.M. (2021) Valid publication of the names of forty-two phyla of prokaryotes. *International Journal of Systematic and Evolutionary Microbiology*, 71, 005056.
- Pérez-Pascual, D., Lunazzi, A., Magdelenat, G., Rouy, Z., Roulet, A., Lopez-Roques, C. et al. (2017a) The complete genome sequence of the fish pathogen *Tenacibaculum maritimum* provides insights into virulence mechanisms. *Frontiers in Microbiol*ogy, 8, 1542.
- Pérez-Pascual, D., Rochat, T., Kerouault, B., Gómez, E., Neulat-Ripoll, F., Henry, C. et al. (2017b) More than gliding: involvement of GldD and GldG in the virulence of *Flavobacterium psychrophilum. Frontiers in Microbiology*, 8, 2168.
- Persson, O.P., Pinhassi, J., Riemann, L., Marklund, B.I., Rhen, M., Normark, S. et al. (2009) High abundance of virulence gene homologues in marine bacteria. *Environmental Microbiology*, 11, 1348–1357.
- Piñeiro-Vidal, M., Centeno-Sestelo, G., Riaza, A. & Santos, Y. (2007) Isolation of pathogenic *Tenacibaculum maritimum*-related organisms from diseased turbot and sole cultured in the northwest of Spain. *Bulletin European Association of Fish Pathologists*, 27, 29.
- Piñeiro-Vidal, M., Riaza, A. & Santos, Y. (2008) Tenacibaculum discolor sp. nov. and Tenacibaculum gallaicum sp. nov., isolated from sole (Solea senegalensis) and turbot (Psetta maxima) culture systems. International Journal of Systematic and Evolutionary Microbiology, 58, 21–25.
- Quinn, R.A., Metzler, A., Smolowitz, R.M., Tlusty, M. & Chistoserdov, A.Y. (2012) Exposures of *Homarus americanus* shell to three bacteria isolated from naturally occurring epizootic shell disease lesions. *Journal of Shellfish Research*, 31, 485–493.
- Rahman, T., Suga, K., Kanai, K. & Sugihara, Y. (2014) Biological and serological characterization of a non-gliding strain of *Tenacibaculum maritimum* isolated from a diseased puffer fish *Takifugu rubripes. Fish Pathology*, 49, 121–129.
- Rahmi, Jompa, J., Tahir, A., Malina, A.C. & Rantetondok, A. (2020) In vitro analysis of pathogenic bacteria causing black band disease on *Pachyseris Speciosa* (Dana, 1846). AACL Bioflux, 13, 1865– 1876.
- Ranson, H.J., Laporte, J., Spinard, E., Chistoserdov, A.Y., Gomez-Chiarri, M., Nelson, D.R. et al. (2018) Draft genome sequence of the putative marine pathogen *Aquimarina* sp. strain I32. 4. *Genome Announcements*, 6, e00313-18.
- Rhodes, R.G., Samarasam, M.N., Shrivastava, A., Van Baaren, J.M., Pochiraju, S., Bollampalli, S. et al. (2010) *Flavobacterium johnsoniae* gldN and gldO are partially redundant genes required for gliding motility and surface localization of SprB. *Journal of Bacteriology*, 192, 1201–1211.
- Rohmer, L., Hocquet, D. & Miller, S.I. (2011) Are pathogenic bacteria just looking for food? Metabolism and microbial pathogenesis. *Trends in Microbiology*, 19, 341–348.
- Romero, M., Avendaño-Herrera, R., Magariños, B., Cámara, M. & Otero, A. (2010) Acylhomoserine lactone production and degradation by the fish pathogen *Tenacibaculum maritimum*, a

member of the Cytophaga–Flavobacterium–Bacteroides (CFB) group. FEMS Microbiology Letters, 304, 131–139.

- Saha, M. & Weinberger, F. (2019) Microbial "gardening" by a seaweed holobiont: surface metabolites attract protective and deter pathogenic epibacterial settlement. *Journal of Ecology*, 107, 2255–2265.
- Sakatoku, A., Fujimura, T., Ito, M., Takashima, S. & Isshiki, T. (2018) Newly isolated bacterium *Tenacibaculum* sp strain Pbs-1 from diseased pearl oysters is associated with black-spot shell disease. *Aquaculture*, 493, 61–67.
- Saulnier, D., Avarre, J.C., Le Moullac, G., Ansquer, D., Levy, P. & Vonau, V. (2000) Rapid and sensitive PCR detection of *Vibrio penaeicida*, the putative etiological agent of syndrome 93 in New Caledonia. *Diseases of Aquatic Organisms*, 40, 109–115.
- Skiftesvik, A.B. & Bergh, Ø. (1993) Changes in behaviour of Atlantic halibut (*Hippoglossus hippoglossus*) and turbot (*Scophthalmus maximus*) yolk-sac larvae induced by bacterial infections. Canadian Journal of Fisheries and Aquatic Sciences, 50, 2552– 2557.
- Slinger, J., Adams, M.B. & Wynne, J.W. (2020) Bacteriomic profiling of branchial lesions induced by *Neoparamoeba perurans* challenge reveals commensal dysbiosis and an association with *Tenacibaculum dicentrarchi* in AGD-affected Atlantic Salmon (Salmo salar L.). *Microorganisms*, 8, 1189.
- Småge, S.B., Brevik, Ø.J., Duesund, H., Ottem, K.F., Watanabe, K. & Nylund, A. (2016) *Tenacibaculum finnmarkense* sp. nov., a fish pathogenic bacterium of the family Flavobacteriaceae isolated from Atlantic salmon. *Antonie Van Leeuwenhoek*, 109, 273–285.
- Småge, S.B., Frisch, K., Vold, V., Duesund, H., Brevik, O.J., Olsen, R.H. et al. (2018) Induction of tenacibaculosis in Atlantic salmon smolts using *Tenacibaculum finnmarkense* and the evaluation of a whole cell inactivated vaccine. *Aquaculture*, 495, 858–864.
- Smolowitz, R., Chistoserdov, A.Y. & Hsu, A. (2005) A description of the pathology of epizootic shell disease in the American lobster, *Homarus americanus*, H. Milne Edwards 1837. *Journal of Shellfish Research*, 24, 749–756.
- Smolowitz, R., Quinn, R., Cawthorn, R., Summerfield, R. & Chistoserdov, A. (2014) Pathology of two forms of shell disease of the American lobster *Homarus americanus* Milne Edwards in Atlantic Canada. *Journal of Fish Diseases*, 37, 577–581.
- Smriga, S., Sandin, S.A. & Azam, F. (2010) Abundance, diversity, and activity of microbial assemblages associated with coral reef fish guts and feces. *FEMS Microbiology Ecology*, 73, 31–42.
- Sorongon, M.L., Bloodgood, R.A. & Burchard, R.P. (1991) Hydrophobicity, adhesion, and surface-exposed proteins of gliding bacteria. *Applied and Environmental Microbiology*, 57, 3193–3199.
- Starliper, C.E. (2011) Bacterial coldwater disease of fishes caused by Flavobacterium psychrophilum. Journal of Advanced Research, 2, 97–108.
- Stringer-Roth, K., Yunghans, W. & Caslake, L. (2002) Differences in chondroitin AC lyase activity of *Flavobacterium columnare* isolates. *Journal of Fish Diseases*, 25, 687–691.
- Sullam, K.E., Essinger, S.D., Lozupone, C.A., O'connor, M.P., Rosen, G.L., Knight, R. et al. (2012) Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis. *Molecular Ecology*, 21, 3363–3378.
- Sunairi, M., Tsuchiya, H., Tsuchiya, T., Omura, Y., Koyanagi, Y., Ozawa, M. et al. (1995) Isolation of a bacterium that causes anaaki disease of the red algae *Porphyra yezoensis*. *Journal of Applied Microbiology*, 79, 225–229.
- Suomalainen, L.R., Tiirola, M. & Valtonen, E. (2006) Chondroitin AC lyase activity is related to virulence of fish pathogenic *Flavobacterium columnare. Journal of Fish Diseases*, 29, 757–763.
- Tajima, K., Hirano, T., Nakano, K. & Ezura, Y. (1997a) Taxonomical study on the causative bacterium of spotting disease of sea urchin *Strongylocentrotus intermedius*. *Fisheries Science*, 63, 897–900.

- Tajima, K., Hirano, T., Shimizu, M. & Ezura, Y. (1997b) Isolation and pathogenicity of the causative bacterium of spotting disease of sea urchin *Strongylocentrotus intermedius*. *Fisheries Science*, 63, 249–252.
- Takano, T., Matsuyama, T., Sakai, T., Nakamura, Y., Kamaishi, T., Nakayasu, C. et al. (2016) *Ichthyobacterium seriolicida* gen. nov., sp. nov., a member of the phylum 'Bacteroidetes', isolated from yellowtail fish (*Seriola quinqueradiata*) affected by bacterial haemolytic jaundice, and proposal of a new family, Ichthyobacteriaceae fam. nov. *International Journal of Systematic and Evolutionary Microbiology*, 66, 580–586.
- Taniguchi, R., Sawabe, T. & Tajima, K. (2006) Adhesion of *Tenaciba-culum* sp. to short-spined sea urchin *Strongylocentroutus inter-medius* and control of spotting disease by inhibiting adhesion using carbohydrate. *Fish Pathology*, 41, 13–17.
- Thomas, F., Hehemann, J.-H., Rebuffet, E., Czjzek, M. & Michel, G. (2011) Environmental and gut bacteroidetes: the food connection. *Frontiers in Microbiology*, 2, 93.
- Thunes, N.C., Conrad, R.A., Mohammed, H.H., Zhu, Y., Barbier, P., Evenhuis, J.P. et al. (2021) Type IX secretion system effectors and virulence of the model *Flavobacterium columnare* strain MS-FC-4. *Applied and Environmental Microbiology*, 88, 01705-21.
- Wahli, T. & Madsen, L. (2018) Flavobacteria, a never ending threat for fish: a review. *Current Clinical Microbiology Reports*, 5, 26–37.
- Wakabayashi, H., Hikida, M. & Masumura, K. (1986) Flexibacter maritimus sp. nov., a pathogen of marine fishes. *International Journal* of Systematic and Evolutionary Microbiology, 36, 396–398.
- Webster, N.S., Xavier, J.R., Freckelton, M., Motti, C.A. & Cobb, R. (2008) Shifts in microbial and chemical patterns within the marine sponge *Aplysina aerophoba* during a disease outbreak. *Environmental Microbiology*, 10, 3366–3376.
- Webster, N.S., Luter, H.M., Soo, R.M., Botté, E.S., Simister, R.L., Abdo, D. et al. (2013) Same, same but different: symbiotic bacterial associations in GBR sponges. *Frontiers in Microbiology*, 3, 444.
- Weinberger, F., Hoppe, H.-G. & Friedlander, M. (1997) Bacterial induction and inhibition of a fast mecrotic response in *Gracilaria conferta* (Rhodophyta). *Journal of Applied Phycology*, 9, 277–285.
- Wietz, M., Gram, L., Jørgensen, B. & Schramm, A. (2010) Latitudinal patterns in the abundance of major marine bacterioplankton groups. *Aquatic Microbial Ecology*, 61, 179–189.
- Xu, Y., Chen, C., Ji, D., Hang, N. & Xie, C. (2014) Proteomic profile analysis of *Pyropia haitanensis* in response to high-temperature stress. *Journal of Applied Phycology*, 26, 607–618.
- Yilmaz, P., Yarza, P., Rapp, J.Z. & Glöckner, F.O. (2016) Expanding the world of marine bacterial and archaeal clades. *Frontiers in Microbiology*, 6, 1524.
- Zainuddin, E.N., Anshary, H., Huyyirnah, H., Hiola, R. & Baxa, D.V. (2019) Antibacterial activity of *Caulerpa racemosa* against pathogenic bacteria promoting "ice-ice" disease in the red alga *Gracilaria verrucosa*. *Journal of Applied Phycology*, 31, 3201– 3212.
- Zhang, H., Yoshizawa, S., Sun, Y., Huang, Y., Chu, X., González, J. M. et al. (2019a) Repeated evolutionary transitions of flavobacteria from marine to non-marine habitats. *Environmental Microbiology*, 21, 648–666.
- Zhang, Z., Zhang, W.W., Hu, Z.G., Li, C.H., Shao, Y.N., Zhao, X.L. et al. (2019b) Environmental factors promote pathogen-induced skin ulceration syndrome outbreak by readjusting the hindgut microbiome of *Apostichopus japonicus*. *Aquaculture*, 507, 155–163.
- Zhu, Y. & McBride, M.J. (2014) Deletion of the Cytophaga hutchinsonii type IX secretion system gene sprP results in defects in gliding motility and cellulose utilization. Applied Microbiology and Biotechnology, 98, 763–775.

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Zozaya-Valdes, E., Egan, S. & Thomas, T. (2015) A comprehensive analysis of the microbial communities of healthy and diseased marine macroalgae and the detection of known and potential bacterial pathogens. *Frontiers in Microbiology*, 6, 146.

SUPPORTING INFORMATION

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