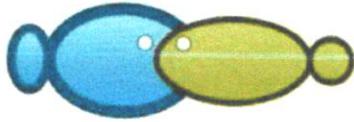


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In vitro analysis of pathogenic bacteria causing black band disease on *Pachyseris speciosa* (Dana, 1846)

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Abstract. The aim of this study was to isolate black band disease (BBD) samples from hard coral (*Pachyseris speciosa*) and to identify the main pathogenic bacteria stimulating BBD. The research was conducted from October 2015 to March 2016. Samples (fragments of diseased corals) were collected from Pulau Barrang Lompo, Pulau Badi, Pulau Bonetambung, Pulau Sarappo, and Pulau Kapoposang, Spermonde Archipelago, South Sulawesi Province. Isolates of BBD bacteria taken from these diseased samples were cultured in the Microbiology Laboratory, Faculty of Medicine, Hasanuddin University; histological analysis of diseased and healthy corals was conducted in the Pathology Laboratory, Maros Veterinary Centre, and BBD bacterial pathogenicity was tested on healthy corals at the Barrang Lompo Island Marine Station Hatchery, Faculty of Fisheries and Marine Sciences, Hasanuddin University. The bacterial density in infected coral tissue reached 10^9 CFU mL⁻¹. The pathogenicity test found that the time before BBD symptoms appeared was different for each bacterium tested. BBD symptoms appeared most rapidly after infection with *Desulfovibrio salexigens* DSM 2638 (7 hours), followed by *Flavobacterium columnare* FK 401 (10 hours), *Bacillus farraginis* R-8039 (22 hours), and *Shewanella piezotolerans* WP3 (27 hours). Histology showed differences between healthy and infected coral tissue; the epidermis and gastrodermis were clearly visible in healthy corals but these structures were no longer well defined in corals infected with BBD.

Key Words: *Flavobacterium columnare*, *Bacillus farraginis*, *Pachyseris speciosa*, black band disease, bacterial pathogenicity.

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Introduction. Coral disease is considered a severe threat to coral reefs all over the world (Sutherland et al 2004; Woodley et al 2008; Precht et al 2016) and occurrences have been reported widely in recent decades (Harvell et al 1999; Green & Bruckner 2000; Harvell et al 2002; Rosenberg & Ben-Haim 2002; Sutherland et al 2004; Raymundo et al 2005; Jones et al 2012; Miller & Richardson 2014; Johan et al 2015; Ponti et al 2016), including the Spermonde Archipelago, in South Sulawesi, Indonesia (Muller et al 2012). Some coral reefs have already been severely damaged by disease, and the prevalence of coral disease is predicted to increase due to direct and indirect anthropogenic impacts (Jones et al 2012; Miller & Richardson 2014; Thurber et al 2014; Woodley et al 2015; McDevitt-Irwin et al 2017; Ziegler et al 2017; Wang et al 2018).

Numerous diseases corals have been described (Woodley et al 2015), including black band disease (BBD) (Antonius 1981; Ruzler & Santavy 1983; Rutzler et al 1983), microbial infection (Ducklow & Mitchell 1979) and brown band disease (BrBD) (Willis et al 2004). Some coral diseases are caused by bacteria and fungi, others by unknown biological agents (Woodley et al 2015), and any disease can impair coral health (Richardson 1998; Richardson et al 2001; Knowlton & Rohwer 2003; Raymundo et al 2008). The specific pathogens or organisms that cause the disease are often not fully understood or clearly identified (Sutherland et al 2004; Woodley et al 2015; Precht et al 2016). In particular, the etiology of BBD in corals is still not fully understood (Raymundo & Weil 2015; Den Uyl et al 2016; Meyer et al 2017; Sato et al 2016).

BBD in corals can be identified by the occurrence of black band/bands that move and cause damage to coral tissues, while macroscopic observation of corals infected with BrBD shows brown bands on healthy tissues and white tissues, enabling the two diseases to be distinguished in the field (Woodley et al 2015). In sub-tropical waters the disease is more active in the warmer temperatures during the summer season (Richardson & Kuta 2003), and BBD is one of the coral diseases predicted to become more widespread and severe as the oceans warm due to anthropogenic climate change (Miller & Richardson 2014; Precht et al 2016). Although BBD has been widely studied, the causative agent of this disease has proven difficult to identify (Frias-Lopez et al 2002; Zvuloni et al 2009; Sato et al 2010; Yang et al 2014; Miller & Richardson 2014). Observations made in aquaria report an average advance BBD rate of 12.5 mm day⁻¹ in *Acropora millepora* coral colonies, and a linear advance of 5 cm during 4 days of observation (Dinsdale 1994). Likewise, observations made on Caribbean coral reefs showed BBD advancing by 6.2 mm day⁻¹ (Kuta & Richardson 1997), within the range of tissue loss (0.3 to 9 mm day⁻¹) reported in colonies of *Acropora* infected with BrBD (Davies & Evison 1991), although in some cases BBD can advance even more rapidly (Nash 2003). Mean rates of BBD advance in a *Montipora* species assemblage on the Great Barrier Reef in Australia varied seasonally, from less than 1.7 mm day⁻¹ to 3.7 mm day⁻¹, and were significantly and positively correlated with temperature (Sato et al 2009).

The mechanism of bacterial pathogenicity related to the causes of BBD in corals has not been widely reported, particularly in the Indo-Pacific (Raymundo & Weil 2015). Therefore, this study aimed to examine the level of pathogenicity of bacteria isolated from BBD in *Pachyseris speciosa* in order to identify specific bacteria which could be prime candidates as the main triggers for BBD.

Material and Method. This research was conducted from October 2015 to March 2016. Samples of *P. speciosa* (fragments of healthy and diseased coral colonies) were collected from Pulau Barrang Lompo, Pulau Badi, Pulau Bonetambung, Pulau Sarappo, and Pulau Kapoposang, Spermonde Archipelago, South Sulawesi Province. BBD bacteria taken from the diseased samples were cultured in the Laboratory of Microbiology, Faculty of Medicine, Hasanuddin University in order to obtain isolates of bacterial species within the BBD bacterial mats. Histological analysis took place in the Laboratory of Pathology, Maros Veterinary Research Centre, and tests of BBD bacterial pathogenicity on healthy coral took place at the Barrang Lompo Island Marine Station Hatchery, Faculty of Fisheries and Marine Sciences, Hasanuddin University.

Bacterial culture and dilution. Bacterial mats were scraped from the black band region of the BBD infected *P. speciosa* colony samples. Bacterial isolates were obtained and identified following Rahmi et al (2016). The bacteria obtained from this 1st phase were cultured by growing a loop of each bacterial isolate in seawater complete (SWC) solid media that contained 5 g of bacto-peptone, 5 g of yeast extract, 3 mL of glycerol, 250 mL of distilled water, 750 mL of sterilized seawater and 20 g of bacto-agar. The media inoculated with the bacteria were then incubated at 28°C for 24 hours. The concentration of bacteria in the isolates was calculated using the MacFarland method (Sussman et al 2008). This involved taking a 1 mL sample of the isolate and mixing it with 1 mL of BrCl₂ 1% and H₂SO₄ 1% in a range of proportions (from 0.1:9.9 to 1.0:9.0) and measuring the absorbance at 625 µm with a spectrophotometer (Hitachi UV-2100PC) to determine the density (CFU mL⁻¹) in the isolate. Based on the results of the MacFarland test, the media was diluted to give a bacterial population density of 10⁶ CFU mL⁻¹ in each of the trial aquaria media.

Coral acclimation. The healthy coral samples were acclimated for 5 days before testing the pathogenicity of the isolated bacteria, based on the protocol by Kushamaro et al (1997). The purpose of the acclimation was to enable the corals to adapt to the new environmental conditions before being challenged by pathogens as well as to ensure that there was no contamination with microorganisms before the start of the treatments. Coral husbandry and trials were conducted in aquaria equipped with aeration and water

circulation³². The acclimation and pathogenicity challenge trials were carried out *in vitro* in aquaria (45 cm x 30 cm x 30 cm) with a media (seawater) volume of 40 litres.

Bacterial pathogenicity test. After obtaining isolates with a density of 10^6 CFU mL⁻¹ (Sussman et al 2008) for each type of bacteria isolated from BBD infected corals, these isolates were used to inoculate acclimated healthy corals. The challenge trials were carried out on 3-5 healthy coral fragments per aquarium with 3 replicates (aquaria) per treatment. The bacterial isolates were released into the trial media (seawater) in each aquarium to give a bacterial concentration of 10^6 CFU mL⁻¹. The challenged corals were then observed during a soaking period of 144 hours, considered sufficient to ensure the infection had become successfully established.

⁴⁷ Water quality parameters were monitored during the trial. In particular, temperature, salinity, and dissolved oxygen were maintained in the normal or ideal range (temperature 27-29°C, salinity 30-32 ppt, and dissolved oxygen 4.8-6.2 mg L⁻¹). Colour change,³³ sign of coral disease, was observed using a Nikon *Collfix* P7100 underwater camera and the width (cm) of the band was measured using callipers (precision 0.1 mm).

Biological parameters and observation of coral infection. The biological parameters observed included changes in coral morphology, colour, and the presence of bleached coral fragments. Biological parameters were observed every 3 hours after initiating the pathogenicity trial through soaking as described above. The establishment of infection by BBD in the trialled corals was determined based on morphological changes in the coral (Figure 1). The data obtained were analysed descriptively.

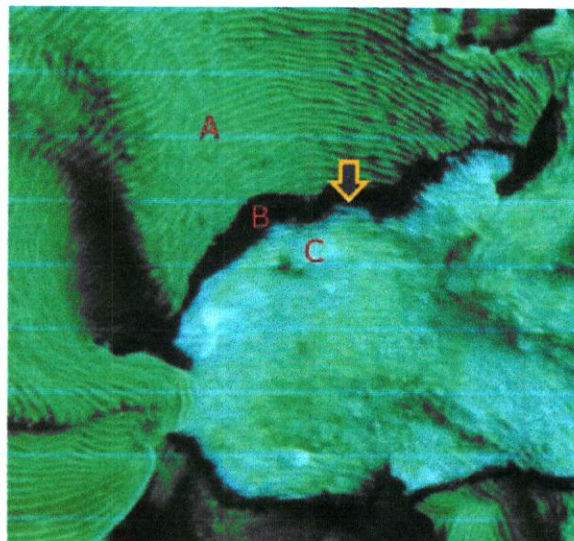


Figure 1. Morphology of healthy coral tissue (A); coral tissue infected with black band disease (B); and dead coral (C).

Preservation of samples and tissues. Healthy, diseased (BBD) and dead samples of the coral *P. speciosa* (1-2 cm²) were collected and preserved with 4% paraformaldehyde (weight/volume) in sterile phosphate buffered saline (PBS) solution (pH 7.4) for 12 hours (St. John et al 2002), then decalcified with 10% buffered neutral formalin (BNF) (pH 8) (St. John et al 2000) as a standard procedure for embedding in paraffin. The tissue samples were processed through washing as follows: washed once in with 70% alcohol and then in 90% alcohol followed by washing three times: in absolute ethanol for 1 hour and then 2 hours, and then in xylol for 1.5 hours. The samples were then washed with liquid paraffin (70°C) three times for 1.5 hours and preserved in paraffin.

Histological examination. The coral (*P. speciosa*) tissue samples taken for histopathological observation (healthy, BBD infected and dead coral tissues) were stained using hematoxylin and Mayers Eosin (with phloxine B) solutions. The embedded samples were sliced (thickness) and prepared for observation under an Olympus microscope CX23, at 400X and 100X magnification. The tissue preparates were photographed using a Nikon DXM-1200 camera. The histology of healthy coral tissue and tissue infected with BBD was described from direct observations and photographs.

Results and Discussion

Bacterial culture. Bacterial concentration based on the MacFarland standard showed that the concentration of each isolate was different (Table 1). MacFarland standards are used as a reference to adjust the turbidity of bacterial suspension. The concentration of most bacterial isolates was 10^9 CFU mL⁻¹. These results indicate that there was an increase in the density of bacteria in the coral tissues.

The increase of bacteria isolate concentration tested

Table 1

Bacteria isolate	Concentration of bacteria (CFU mL ⁻¹)
<i>Halomonas</i> sp. strain K0116	1.4×10^9
<i>Psychromonas</i> sp. strain CNPT3	1.03×10^9
<i>Thiobacillus denitrificans</i> strain NCIMB 9548	3.6×10^9
<i>Pseudoalteromonas</i> sp. strain SQN1	2.9×10^9
<i>Flavobacterium columnare</i> strain FK401	2.12×10^9
<i>Shewanella piezotolerans</i> strain WP3	1.05×10^9
<i>Bacillus farraginis</i> strain R-8039	5.2×10^9
<i>Desulfovibrio salexigens</i> strain DSM 2638	3.4×10^9

Pathogenicity test. The results of the pathogenicity test showed that the initial time to the appearance of BBD symptoms was different for each type of bacterium tested. The bacterium which caused symptoms of BBD most rapidly after initial infection was *D. salexigens* DSM 2638 (7 hours), followed by *F. columnare* FK 401 (10 hours), *B. farraginis* R-8039 (22 hours) and *S. piezotolerans* WP3 (27 hours) after infection (Figure 2).

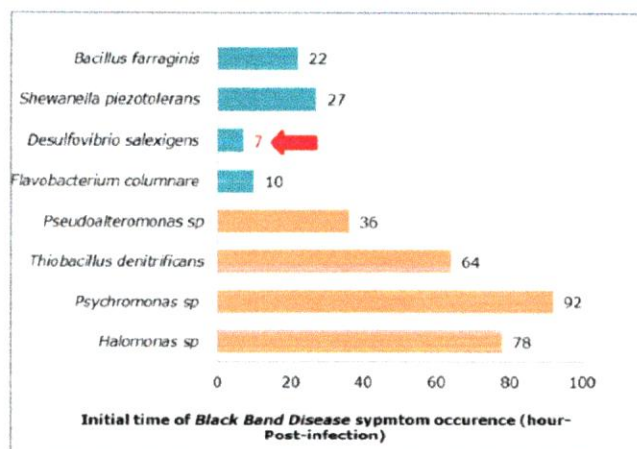


Figure 2. Post-infection time until initial appearance of black band disease (BBD) symptoms on *Pachyseris speciosa* fragments challenged with normal bacterial isolates and in the BBD band. Bacteria isolated from healthy coral fragments (orange bars) and other bacteria isolated from the BBD band of infected fragments (blue bands).

The isolate caused symptoms of BBD to emerge just 7 hours post infection, *D. salexigens* DSM 2638 (DSM = Deutsche Sammlung von Microorganism Baurnschweig, Germany) is closely related to *D. salexigens* strain M34401. This group of obligate anaerobic reducing bacteria does not require oxygen and indeed oxygen is toxic to them; instead, they are able to use sulphate as a terminal electron acceptor in anaerobic respiration (Richardson & Kuta 2003). This combination of anoxia and sulphate can be deadly to coral tissues because sulphate production is a major component of the pathogenicity of BBD (Richardson et al 1997; Richardson & Kuta 2003; Meyer et al 2017).

Bacteria of the genus *Desulfovibrio* have been reported in BBD-infected tissues based on 16 S rRNA analyses (Cooney et al 2002; Frias-Lopez et al 2004; Barneah et al 2007). Frias-Lopez et al (2002) reported that the dominant bacteria in corals infected with BBD in Curacao Dominica were: *Desulfovibrio salexigens* M34401 in *Montastrea annularis*; *D. alaskensis* NCIMBI13491 in *Montastrea cavernosa*; *D. zosteriae* Y18049 in *Diploria strigosa*; and *Desulfovibrio* sp. strain TBP-1 (Viehman et al 2006). *Desulfovibrio* sp. strain TBP-1 is known to be more pathogenic than *D. salexigens* M34401 which infected *Montastrea annularis* coral (Ramos-Flores 1983). Viehman et al (2006) also remarked that previous molecular studies had found several species of the genus *Desulfovibrio* in coral tissues infected with BBD, with the species or strain present varying between corals.

The rate of advance of the BBD infection was 2.46 cm day⁻¹ in *P. speciosa* infected with *D. salexigens*, higher than for the other bacterial isolates (Figure 3). This indicates that *D. salexigens* is a prime candidate as a BBD trigger bacteria for BBD in *P. speciosa*. This bacterium has previously been reported as associated with BBD (Cooney et al 2002), in particular in *M. annularis* (Frias-Lopez et al 2002). In addition to the anoxic conditions and high concentration of sulphite, both of which can affect nearby coral tissues and result in necrosis (Viehman et al 2006), *D. salexigens* is reported to be an opportunistic pathogen (Richardson et al 1997).

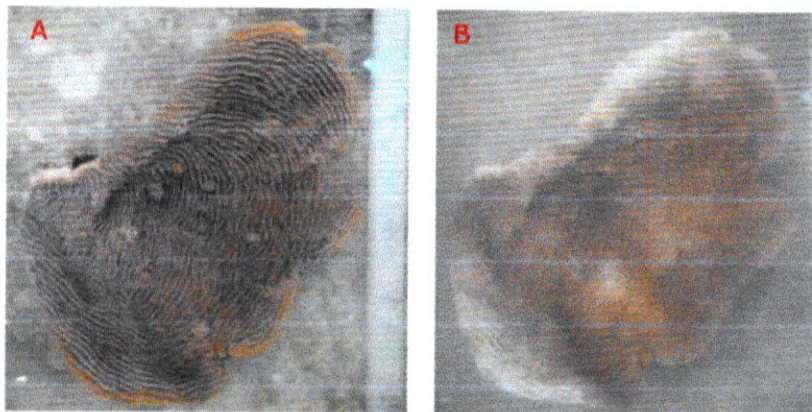


Figure 3. Appearance of black band disease (BBD) infection on *Pachyseris speciosa* fragments challenged with *Desulfovibrio salexigens* DSM 2638 bacterial isolates (A = before the challenge test; B = 24 hours after the challenge test).

In this study, *F. columnare* FK401 and *B. farraginis* R-8039 were the second and third most pathogenic BBD causing bacteria, followed by *S. piezotolerans* WP3. The *F. columnare* strain FK401 had a 401 kDa protein mass with FK being the gene code in the GenBank database. Increased nutrient levels are thought to increase the severity of coral disease (Thurber et al 2014). It has been suggested that *F. columnare* strains are related to nutrient levels in the aquatic environment, which can act as a trigger for gene expression of virulence, contributing to interactions between the environment, the host and opportunistic pathogens (Penttinen et al 2016). Clinical symptoms of columnaris disease are easy to recognize and differ for each strain, degree of disease, wound type

and location, and virulence, which vary according to the strain of *F. columnaris* bacteria present in an infection (McCarthy 1975). For example, Austin & Austin (2016) suggest that *F. columnaris* attacks the gills of small fish in marine waters, especially the gill lamella and also the tail fin; it can also cause a "columnar" disease to which eels are particularly susceptible (Hawke & Thune 1978). In the Caribbean *F. columnaris* has also been reported as a major pathogen in white-band disease in the corals *Acropora cervicornis* and *A. palmata* (Gignoux-Wolfsohn & Vollmer 2015). Other bacteria in this genus are also reported to infect fish; for example in Chile *F. chilense* and *F. araucanum* can cause disease in salmon (Kampfer et al 2012), while *F. tructae* and *F. piscis* are reported as pathogens affecting cultivated rainbow trout (Zamora et al 2014).

B. farraginis R-8039 is a bacterial strain that is more resistant than most to extreme temperatures, up to 100°C (Barrow & Feltham 1993). Furthermore, not only is the R-8038 strain of *Bacillus farraginis* a particularly virulent mutation of this bacterium; the spores of this bacteria are also pathogenic.

S. piezotolerans WP3 is a bacterium that is thought to originate from the deep sea and, like *B. farraginis* R-8038, this strain is able to withstand more extreme temperatures compared to other bacterial strains (Jian et al 2016). Furthermore, the WP3 bacterial strain is a microorganism that is thought to have an unusual adaptive strategy enabling it to live in deep ocean waters.

Some researchers consider the bacterium *Phormidium corallyticum* to be the main cause of BBD; however further research is needed to prove this hypothesis (Cooney et al 2002). The results of this study indicate that BBD is caused by the association of several microorganisms dominated by Cyanobacteria, as suggested by Séré et al (2016), rather than a single species as proposed by Rutzler & Santavy (1983). The Cyanobacteria are a diverse group including filamentous heterotrophic bacteria such as *P. corallyticum* (Garrett & Ducklow 1975), marine fungi (Ramos-Flores 1983), sulphite-oxidising bacteria (e.g. *Beggiatoa*) and sulphate reducing bacteria such as the genus *Desulfovibrio* (Frias-Lopez et al 2004). Other potential BBD pathogens include communities of various types of bacteria including *Clostridium* spp., *Arcobacter* spp., *Campylobacter* spp., *Cytophaga fermentans*, *Cytophaga columnaris* and *Trichodesmium tenue* (Frias-Lopez et al 2002). Frias-Lopez et al (2002) found that the brain-massive coral microbial communities were dominated by green sulphur bacteria, α -proteobacteria, Firmicutes and Planctomycetes. Furthermore, they reported that microbial diversity and community composition was different for each type of host coral.

The capacity of bacteria to cause disease depends on their pathogenicity, with three classes: disease-causing agents, opportunistic pathogens, and non-pathogenic bacteria. Disease-causing agents are pathogenic bacteria that will almost always cause a disease, e.g. *Salmonella* spp. (Ducklow & Mitchell 1979). An opportunistic pathogen is a bacterium that is capable of becoming pathogenic when the host's defence mechanism is weakened; e.g. *Escherichia coli* that infect the urinary tract when the host's defence system is compromised (Green & Bruckner 2000). Non-pathogens are bacteria that have never been found to be pathogenic. Because of their adaptability to potentially lethal effects, some pathogens can be used in therapies such as chemotherapy, immunotherapy, and overcoming resistance mechanisms; furthermore, Morita (1975) suggested that soil bacteria that were originally non-pathogenic (*Serratia marcescens*) had mutated to become opportunistic pathogens that could cause pneumonia, urinary tract infections, and bacteraemia in immune-compromised hosts.

Virulence is a measure of the pathogenicity of an organism, whether it is a disease-causing agent or an opportunistic pathogen. Virulence levels are directly proportional to the organism's ability to cause disease and are influenced by the number of bacteria, the entrance to the host body, the host defence mechanism, and bacterial virulence factors. Experimental virulence is measured by determining the number of bacteria that cause death, illness, or lesion within the specified time after introduction (Hu et al 2008).

Psychromonas sp. CNPT3 is classified as a virulent strain of marine pathogenic bacteria (Persson et al 2009), while *Halomonas* sp. K0116 is classified as a biofilm-forming strain of bacteria that can secrete polysaccharides so as to increase bacterial

pathogenicity (Miller & Bassler 2001). Buller (2004) reported that *Halomonas* sp. can cause mortality in black pomfret and cause carapace softening in lobsters. The bacterial isolates with the longest time before BBD symptoms occurred were *Psychromonas* sp. CNPT3 and *Halomonas* sp. K0116 (78 and 92 hours post infection, respectively). Generally, symptomatic changes began to occur initially around the broken edge of the coral fragments, indicating opportunistic pathogenicity. A heightened vulnerability to coral disease has also been observed in transplanted coral fragments (Casey et al 2015).

Psychromonas sp. CNPT3 and *Halomonas* sp. K0116 were also isolated from healthy coral fragments which did not show symptoms of BB. Additionally, both bacteria were present in the bacterial communities of *Acropora* sp. colonies. *Psychromonas* sp. are able to live in environments with high levels of salt concentration and to adapt to low temperatures (Morita 1975), while *Psychromonas* sp. strain CNPT3 was found in several small crustaceans in the deep sea (Syanos et al 1979). *Halomonas* sp. has been found in *Acropora* sp. coral tissues (Ojima et al 2012) and the strain *Halomonas* sp. K0116 was isolated from seawater in the Indian Ocean by Salunkhe et al (2011). *Halomonas* sp. belong to the γ -proteobacteria class, which were first found in peat soil in a salty environment; they are halophilic, Gram-negative bacteria and can live at temperatures of 20-45°C and within a pH range of 6-9 (Martinez-Canovas et al 2004).

The rate of BBD progression in *P. speciosa* fragments after exposure varied between pathogens (Figure 4). The BBD progression rate was highest in corals infected with *F. columnare* (3.69 cm day⁻¹), followed by *D. salexigens* (2.46 cm day⁻¹). Dinsdale (1994) observed in the aquarium that the average BBD progression rate was 12.5 mm day⁻¹ in *Acropora millepora* coral colonies with a linear advance of the band of nearly 5 cm over 4 days of observation. Observations made on Caribbean coral reefs report progression rates of around 6.2 mm day⁻¹ (Kuta & Richardson 1997).

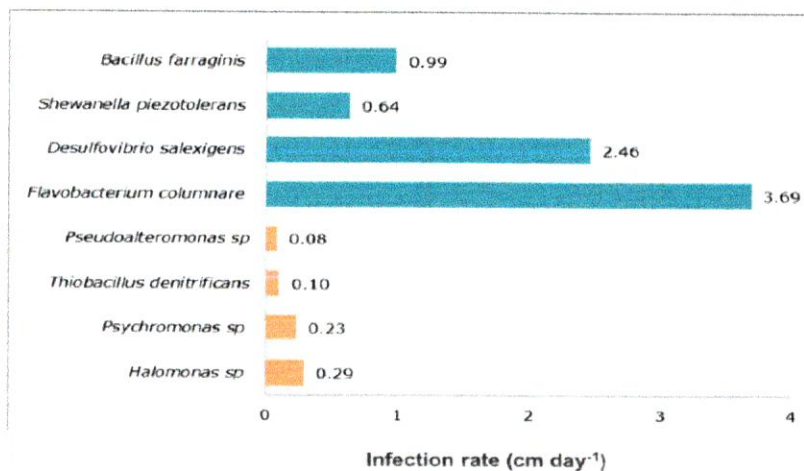


Figure 4. Rate of black band disease (BBD) progression (band advance in cm day⁻¹) in *Pachyseris speciosa* coral fragments after exposure to bacteria thought to cause BBD. Bacteria were isolated from healthy coral fragments (orange bars) or corals infected with BBD (blue bars).

The higher progression rate of BBD on *P. speciosa* corals exposed to infection with *F. columnare* compared to those exposed to *D. salexigens* is thought to be due to the production of the chondroitin sulphatase enzyme or hyaluronidase by these bacteria. The activity of this enzyme can help accelerate host tissue destruction by the bacteria. According to Hashioka et al (1992), hyaluronidase activity is one of the most important mechanisms through which bacteria can cause the destruction of host tissues; furthermore, the production of collagenase, chondroitin sulphatase or hyaluronidase play an important role in the pathogenicity of bacterial disease.

The histology of black band disease. The morphological and histological conditions of the *P. speciosa* fragments challenged with bacteria showed morphological differences among different treatments. Examples of healthy corals, corals with black band disease, and dead corals illustrate these differences (Figure 5). In healthy coral tissues, the epidermis and gastro dermis are clearly visible; on the contrary, in BBD infected coral samples, these structures are no longer clear and well-defined.

Coral fragments with bands of BBD show signs of widening vacuoles appearing in the gastrodermis. In Figure 5A (dead coral) hypertrophied nuclei can be seen; the abnormal enlargement is due to swelling triggered by the presence of foreign particles or intracellular microorganisms. In Figure 5B (specimen taken from the band of a coral with BBD) necrosis and apoptosis or programmed cell death (PCD) can be seen; necrosis is triggered by external factors that often affect cells in the tissue, whereas PCD is triggered by intracellular signals that activate the expression of specific genes at the single cell level (Dunn et al 2002). This is in line with the findings of Barneah et al (2007) who observed necrosis in cells and mesoglea released from tissue in *Favia fava* infected with BBD. The histological examination shows differences between tissue fragments from healthy *P. speciosa* tissue, where no widening of vacuoles was observed, compared to tissues from corals infected during the pathogenicity trials, both from the band of the advancing BBD or taken from recently dead tissue behind the band (Figure 5).

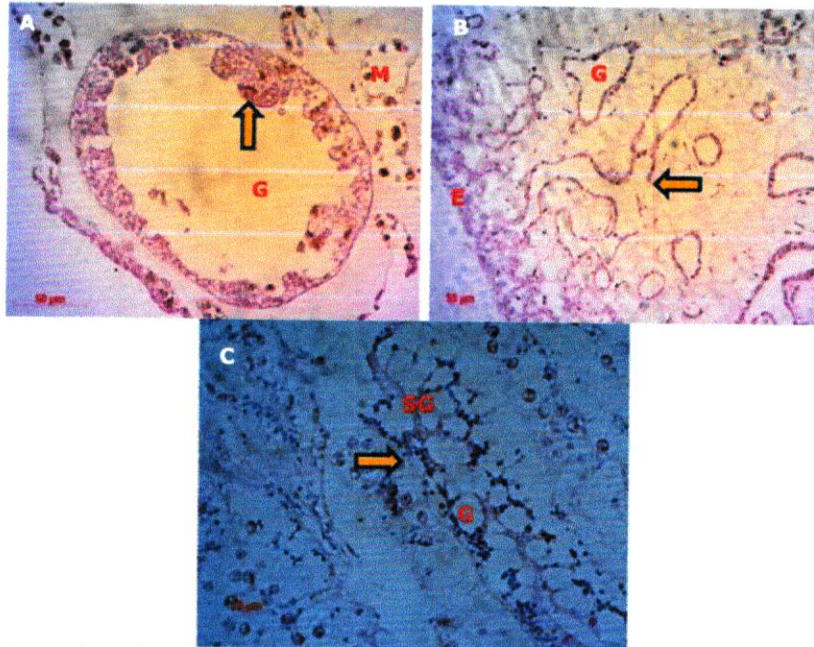


Figure 5. Histology of *Pachyseris speciosa*: A. sample from a coral which had died from black band disease (BBD) infection (hematoxylin & Eosin staining, magnification 400X); B. sample from the active band on a coral infected with BBD (hematoxylin & Eosin staining, magnification 100X); and C. sample of a healthy coral (hematoxylin & Eosin staining, magnification 100X). Legend: G = gastro dermis; E = epidermis; M = mesoglea (M); and SG = gastro vascular canal.

Conclusions. The *in vitro* pathogenicity test challenging healthy *Pachyseris speciosa* coral fragments with bacteria isolated from black band disease (BBD) infected corals showed that, after the initial infection, the most rapid appearance of BBD symptoms and fastest progression of BBD was caused by *Desulfovibrio salexigens* DSM 2638. Although less virulent than *D. salexigens*, symptoms also occurred after challenge with three other isolated bacteria; in descending order of virulence, as indicated by the rapidity of BBD appearance and progression, *Flavobacterium columnare* FK 401, *Bacillus farraginis* R-

8039 and *Shewanella piezotolerans* WP3. The interaction between pathogenic bacteria and environmental factors warrants further research in the context of BBD and the causal mechanisms leading to outbreaks of this disease in various coral species. Studies at the cellular and molecular level are needed to improve our understanding of the effects of pathogen-environment interactions, to guide measures to avoid or limit coral disease, and to improve our understanding of the ways in which disease and climate change may synergistically impact coral communities.

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