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PROBIOTIC POTENTIAL OF CORAL MICROBIOTA: A NATURAL DEFENCE AGAINST WHITE SYNDROME

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ABSTRACT

Healthy coral-associated bacteria produce antimicrobial compounds that can inhibit disease-causing pathogens. This biocontrol potential is particularly relevant in combating White Syndrome (WS), a deadly coral disease. This study aimed to identify beneficial bacteria from healthy corals that could serve as WS control agents. Healthy and WS-infected coral samples were collected from Sawopudo waters, Southeast Sulawesi, Indonesia. Bacteria were isolated using spread and streak plate techniques, and their antibacterial activity was assessed *in vitro* via agar plug assays. Isolates with strong inhibition zones were identified by 16S rRNA gene sequencing. The most promising strains were tested *in vivo* by inoculating WS-infected corals with candidate bacteria. Of six isolates with notable antimicrobial activity, the most active showed high similarity to *Bacillus tequilensis*, *Micrococcus luteus*, *Cytobacillus firmus*, *Staphylococcus arlettae*, and *Priestia aryabhattai* (similarity 99.43%–99.93%). *Cytobacillus firmus* demonstrated the strongest *in vivo* efficacy, forming a 16.6 mm inhibition zone and restoring up to 20% of infected coral tissue. These results underscore the potential of probiotic bacteria as a sustainable strategy to mitigate WS and protect coral reef ecosystems.

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INTRODUCTION

Coral diseases are primarily caused by pathogenic bacteria in marine environments, including those interacting within coral tissues. Extensive research has been conducted on the causative agents of various coral diseases, and studies continue to evolve in this field. This study explores microbiological approaches to identify beneficial bacteria as a means of mitigating coral diseases.

White Syndrome (WS) is one of the most lethal diseases, widely infecting coral organisms worldwide (Thome et al., 2021; Work et al., 2012). The primary cause of this disease remains under investigation. Previous studies have shown that the pathogenic bacteria responsible for WS, isolated from Indo-Pacific waters, belong to the Family *γ*-Proteobacteria Vibrionaceae, specifically *Vibrio coralliilyticus* (Sussman et al., 2008). Meanwhile, another study identified *Alteromonas* sp. strain McT4-15 (GenBank genome sequence JAJALG000000000) as one of the pathogenic bacteria responsible for Stony Coral Tissue Loss Disease (SCTLD) (Ushijima et al., 2023). The susceptibility of corals to bacterial infections is partly determined by their immune system. Coral-associated bacteria produce antibacterial compounds that inhibit pathogenic microbes (Carter, 2013).

Antibacterials are substances that inhibit or kill bacteria by disrupting their metabolic processes, and have been widely applied in studies as agents for controlling pathogenic bacterial growth in corals (Madigan et al., 2015; Tanjung et al., 2020). Previous studies have shown that this inhibitory mechanism can occur through competition between healthy coral-associated bacteria and pathogenic bacteria for space and nutrients essential for growth (Iqbal et al., 2014; Pamungkas et al., 2014).

The treatment of coral diseases using antibiotics has been previously attempted by Sweet et al. (2014) and Walker et al. (2021). One of the tested methods involved the use of the antibiotic *metronidazole* on corals infected with White Band Disease (WBD) through laboratory experiments. The study results indicated that this antibiotic was ineffective in eliminating the pathogenic agent causing coral disease but was only able to reduce the symptoms of WBD (Sweet et al., 2014). A follow-up study by Walker et al. (2021) examined the effectiveness of an antibiotic treatment using a mixture of *amoxicillin trihydrate* and silicon. This antibiotic, in the form of a paste, was applied directly to corals infected with Stony Coral Tissue Loss Disease (SCTLD). The findings showed that this method successfully cured up to 90% of the infected corals, allowing them to recover. However, this study had certain limitations, particularly regarding the potential environmental risks of using synthetic antibiotics, especially in conservation areas (Walker et al., 2021). The application of such antibiotics may disrupt the balance of the coral holobiont, which plays a crucial role in maintaining the health of coral reef ecosystems in natural marine environments.

Therefore, in view of these potential ecological risks, alternative approaches are being explored. One promising strategy is the use of probiotics derived from coral-associated bacteria, which has been shown to be more targeted, effective, and environmentally friendly in supporting coral resilience against disease. The discovery of the bacterium *Pseudoalteromonas* sp. McH1-7 has demonstrated broad-spectrum antibacterial activity against Stony Coral Tissue Loss Disease (SCTLD), a highly lethal coral disease (Ushijima et al., 2023). Furthermore, this bacterial strain has been proven to treat up to 68.2% of infected corals and prevent disease transmission by 100% in laboratory experiments.

The association of microbiota with corals involves understanding microbial communities that reside within and around corals. Symbiotic relationships between corals and bacteria form the basis for exploring their potential as disease-control agents. This study aims to identify the diversity of healthy coral-associated bacteria and their potential as biological control agents against White Syndrome (WS) in Southeast Sulawesi.

MATERIALS AND METHODS

Isolation and purification of bacteria

The isolation and purification process of healthy coral-associated bacteria began with sampling coral from the waters of Sawopudo, Konawe Regency, Southeast Sulawesi, Indonesia. Healthy corals were defined as coral biota that are not infected by any disease. Coral sampling in the field was conducted using scuba diving equipment. Coral fragments measuring 2–3 cm in diameter (Thome et al., 2021) were collected using a hammer and chisel from three different coral colonies as replicates. The collected coral lifeforms included mushroom, digitata, foliose and branching. The coral fragments were then placed in sample plastic bags (Ziplock) filled with seawater and stored in a cool box containing ice to prevent stress on the coral biota and to maintain the viability of coral-associated bacteria. The bacterial isolation process had to be carried out immediately upon arrival at the laboratory, within a maximum of 24 hours.

Before bacterial isolation, all coral samples were rinsed with sterile seawater to remove any attached debris on the coral surface (Mhuantong et al., 2019). Seawater sterilization was performed using an autoclave, heated at 121 °C for 15 minutes. The bacterial isolation process began with the collection of a 1 cm^2 coral tissue sample, which was then carefully ground using a sterile mortar and pestle under aseptic conditions. Next, 1 g of the sample was added to 5 mL of sterile seawater to create a 10^0 dilution. Additionally, a series of seven serial dilutions (10^{-1} to 10^{-7}) was performed. The bacterial inoculation stage used the spread plate method (Amalia et al., 2021) and was applied to the last five dilution series (10^{-3} to 10^{-7}). In each dilution, 100 µL of the sample was taken

using a micropipette and spread onto a solid Zobel agar medium (comprising peptone, yeast, and agar) in petri dishes. The samples were then evenly distributed using a spreader and incubated at room temperature for at least 48 hours. The petri dishes were sealed with parafilm and wrapped in plastic to prevent contamination by other microorganisms. Inoculation in this study was conducted in duplicate. Observations of bacterial colonies included morphological characteristics such as shape, color, surface texture, and colony edges before proceeding with purification.

The purification stage aims to obtain a pure culture or single bacterial colony. The method used is the streak method (Amalia et al., 2021). Each bacterial colony that grew from different isolates during the isolation stage was re-cultured using the quadrant zig-zag streak technique (Badan Karantina Tumbuhan, 2008). This process was repeated until a single (pure) colony was obtained, characterized by uniform shape and colour consistent with the original colony.

In vitro antibacterial assay using healthy coral-associated bacteria against bacteria that cause white syndrome disease

The pathogenic bacteria causing White Syndrome (WS) used in this study are *Pristia flexa* and *Alteromonas macleodii*. (Palupi et al., 2025). The antibacterial assay was conducted *in vitro* using the agar plug method (Ningsih et al., 2021; Sari et al., 2021; Wijaya et al., 2022). In this method, a single loop of a pure bacterial isolate from healthy coral associations was taken and inoculated onto a solid Zobel medium in a petri dish, followed by incubation at room temperature for seven days. On the second day, WS pathogenic bacteria (*P. flexa* and *A. macleodii*) were re-inoculated by taking a single loop, culturing them in liquid Zobel medium, and shaking the culture for 24 hours using a shaker. The 24-hour-old pathogenic bacteria were then assessed for density using McFarland standard 0.5 turbidity (Owoseni et al., 2023). Subsequently, the pathogenic bacteria were evenly inoculated onto a solid medium in petri dishes using a sterile cotton swab and incubated at room temperature for three days.

The agar plug assay was performed by cutting a pure culture of healthy coral-associated bacteria that had grown for seven days using a sterile blue tip. The agar plug containing the isolate was then placed onto a medium previously inoculated with WS pathogenic bacteria. This step was repeated twice. The test media were then incubated at room temperature and observed every 24 hours. Antibacterial activity was indicated by the formation of a clear zone around the agar plug of healthy coral bacteria. Documentation and measurement of the clear zone diameter were subsequently conducted.

The potential of healthy coral-associated bacteria as agents for controlling white syndrome disease

The clear zones formed in the agar plug assay with large diameters were further tested *in vivo*. Healthy coral-associated bacterial isolates with potential antibacterial activity against WS-causing pathogens were inoculated onto WS-infected coral samples under laboratory conditions. Prior to this, WS-infected coral samples were collected from the waters of Sawopudo, Southeast Sulawesi, Indonesia, using scuba diving equipment. WS-infected corals were characterized by tissue loss lesions, appearing as white patches with linear, irregular, or annular (circular) patterns spreading across the coral tissue (Raymundo et al., 2008). During transportation, coral samples were stored in Ziplock plastic bags filled with seawater and then placed in a cool box with ice to maintain stable coral conditions.

Upon arrival at the laboratory, coral samples were rinsed with sterile seawater and placed in an aquarium for the acclimatization process. This process lasted approximately three days or until the coral organisms stopped producing mucus. After acclimatization, WS-infected coral samples were separated into five small aquariums for treatment testing. Four aquariums were used for the inoculation of healthy coral-associated bacterial isolates, while one aquarium served as a control. The water medium used during acclimatization and inoculation was sterile seawater. The seawater in the large aquarium functioned as a reservoir, continuously filtered using a protein skimmer and UV light throughout the experiment.

The antibacterial inoculation process on WS-infected corals followed the procedure described by Ushijima et al. (2023). Healthy coral-associated bacterial isolates were re-cultured on solid Zobel medium and streaked using the quadrant method. The isolates were then incubated at room temperature for 24 hours. Subsequently, 5 mL of liquid medium containing yeast and peptone was prepared. A total of 2–3 bacterial colonies were collected and introduced into the liquid medium, followed by incubation in an orbital shaker for 15–24 hours with continuous shaking. After the incubation period, the bacterial isolates were diluted at a 1:100 ratio, and their density was adjusted to match the 0.5 McFarland standard (Owoseni et al., 2023). Then, 50 mL of the bacterial culture was collected and centrifuged at 8000 rpm for 5 minutes until a cell pellet was formed.

Before inoculation, the water volume in the aquarium containing WS-infected corals was reduced by 50%, and aeration was turned off. The inoculation process began by collecting the cell pellet of the healthy coral-associated bacterial isolate and mixing it with 1–2 mL of aquarium water in a test tube. The antibacterial isolate was gently pipetted onto the infected coral fragments. The inoculation was performed by briefly lifting the test coral fragments from the aquarium before treatment and returning them immediately afterwards. After two hours,

the aquarium water was replenished, and aeration was resumed. This inoculation process was repeated every two days for a total of five applications. Each inoculation followed the same bacterial preparation procedure as previously described.

Observations were conducted to assess the degree of coral tissue recovery and the progression of WS through photographic documentation for approximately one month. Healthy coral-associated bacterial isolates that demonstrated potential in repairing damaged coral tissue were further identified using biomolecular (DNA) analysis through Polymerase Chain Reaction (PCR) and sequencing to determine their species similarity.

DNA analysis

The bacterial isolate samples underwent a series of genomic DNA extraction processes, which were analyzed at the Central Laboratory of Life Sciences, Universitas Borneo Tarakan, East Kalimantan, Indonesia. The extracted DNA was amplified for the 16S rRNA gene using the Polymerase Chain Reaction (PCR) method. Gene amplification was performed using universal primers, namely 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Owoseni et al., 2023; Pauter et al., 2022). The PCR reaction, with a total volume of 35 µL, consisted of 17.5 µL of PCR mix, 2.0 µL of each primer, 2.0 µL of DNA template, and 13.5 µL of nuclease-free water (ddH_2O) (Sumarlin, Gaffar, & Edward, 2024). A positive control was conducted using the gDNA template of *Lysinibacillus capsici*, while a negative control was performed without a DNA template.

The temperature treatment in the PCR procedure followed a specific thermal cycling profile with different temperature and duration settings. The process began with an initial denaturation at 94 °C for 4 minutes, followed by denaturation at 95 °C for 20 seconds, annealing at 51 °C for 30 seconds, and extension at 72 °C for 30 seconds. This cycle was carefully repeated for 30 cycles and concluded with a final cooling phase until the temperature reached 4 °C.

After the PCR process, the amplified products were purified using the QIA quick PCR Purification Kit (Qiagen) and subsequently sequenced commercially by 1st Base in Singapore. The sequencing output was verified using SeqScanner v.2 to obtain a single forward (F) and reverse (R) primer sequence, which were then assembled using DNA Baser software (BioSoft, 2013). The alignment of the 16S rRNA gene sequence was confirmed using CLUSTAL_W, integrated into MEGA XI software. A phylogenetic tree was then constructed based on the 16S rRNA sequences using the MEGA program (Tamura et al., 2021).

RESULTS AND DISCUSSION

The isolation and purification process resulted in 16 pure bacterial isolates (coded S.KS.01 to S.KS.16) from healthy coral-associated bacteria collected from the waters of Sawopudo, Southeast Sulawesi, Indonesia. Morphological identification based on color, margin, and surface characteristics showed that 11 isolates had a circular shape, 4 isolates had an irregular shape, and 1 isolate had a spindle shape. The antibacterial assay was conducted against *Priestia flexa* and *Alteromonas macleodii*, which are pathogenic bacteria responsible for white syndrome (WS). Based on the antibacterial test using the agar plug method, three out of the 16 coral-associated bacterial isolates produced active compounds that inhibited *P. flexa*, with inhibition zones ranging from 4 to 27 mm—categorized as medium to strong. Meanwhile, six isolates inhibited *A. macleodii*, showing inhibition zones of 5 to 19 mm, which are categorized as strong (Mani et al., 2012) (Fig. 1). Bacterial isolates with large inhibition zone diameters were further analyzed using 16S rRNA gene sequencing to determine their closely related species (Table 1). 16S rRNA sequencing was employed to confirm the taxonomic identity of the bacterial isolates, allowing for the accurate assignment of species based on sequence similarity. Among the isolates shown in Figure 1 and Table 1, *Bacillus tequilensis* and *Cytobacillus firmus* exhibited the largest inhibition zones, indicating a particularly strong potential for controlling White Syndrome (WS) pathogens.

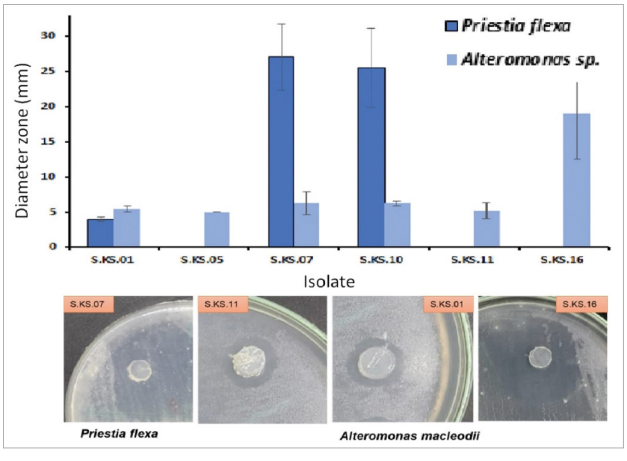

Fig 1. The inhibition zone diameters for each isolate against the pathogenic bacteria *P. flexa* and *A. macleodii*

Bacillus tequilensis has various potential applications in agriculture, industry, and healthcare. Several studies have demonstrated its ability to inhibit pathogenic bacterial growth, prevent fungal infections, and function as an antimicrobial and probiotic agent (Hidayati et al., 2021; Kwon et al., 2022; Wang et al., 2023).

Table 1. The sequence-based identification of antipathogenic bacterial isolates against WS disease

No	Sample ID	Species	Per Ident (%)	Reference ID
1	S.KS.01	*Bacillus tequilensis*	99.79	PQ357343.1
2	S.KS.05	*Micrococcus luteus*	99.43	ON869248.1
3	S.KS.07	*Cytobacillus firmus*	99.93	MN160321.1
4	S.KS.10	*Bacillus tequilensis*	99.93	PQ357343.1
5	S.KS.11	*Staphylococcus arlettae*	99.79	MK748242.1
6	S.KS.16	*Priestia aryabhattai*	99.86	MT078622.1

For instance, *B. tequilensis* has been shown to inhibit the growth of the pathogenic fungus *Colletotrichum acutatum* in chili plants (Kwon et al., 2022) and *Fusarium solani* in plant roots (Wang et al., 2023). Additionally, this bacterium exhibits potential as a thrombolytic agent for treating blood clot disorders, derived from the intestinal fermentation of sea cucumbers (Hidayati et al., 2021).

Priestia aryabhattai is a Gram-positive bacterium belonging to the Class Bacilli and the Genus *Priestia*. This bacterium has potential as a biocontrol agent due to its antagonistic properties against pathogenic bacteria and fungi, particularly in plants (Esikova et al., 2021; Ortega-Urquieta et al., 2022). *P. aryabhattai* can survive in extreme conditions, including polluted environments, heavy metal presence, and drought conditions. Consequently, it also holds promise for pollutant biodegradation. Furthermore, *P. aryabhattai* exhibits potential in disease treatment and infection prevention.

Micrococcus luteus is a Gram-positive bacterium commonly found in soil, marine sediments, water, plants, and human skin tissues. Several studies have revealed its antimicrobial potential due to its carotenoid content (Chaturwedi et al., 2024). Additionally, *M. luteus* can enhance the activity of defense enzymes, such as *superoxide dismutase* (SOD) and *peroxidase* (POX), and contribute to the accumulation of *L-proline*, total *phenolics*, and pigments, including chlorophyll and *carotenoids* (Patel et al., 2020). This bacterium also has potential as a producer of extracellular *polysaccharide-degrading enzymes* (EPE), which have been cultured from marine sediments of Panjang Island, Jepara, Central Java (Bondar et al., 2023). The enzymes produced include *carrageenase, alginate lyase, amylase* and *agarase*.

Staphylococcus arlettae is a Gram-positive bacterium commonly found in both terrestrial and marine environments, including estuaries, various mammalian species, and fermented foods. This bacterium is commensal in nature. A study by Lavecchia et al. (2019) reported that *S. arlettae* exhibits resistance to several antibiotics, including *ampicillin, cefoxitin, ceftaroline, ciprofloxacin, clindamycin, daptomycin, penicillin G* and *oxacillin* (Adade et al., 2024). This antibiotic resistance makes *S. arlettae* a bacterium of concern, particularly concerning disease treatment and recovery processes.

The potential of healthy coral bacteria as agents for controlling white syndrome disease

The bacterial isolates from healthy coral tissues demonstrated varying levels of antibacterial activity, with some strains exhibiting significant inhibition against the pathogens causing white syndrome (WS), *P. flexa* and *A. macleodii*. Figure 2 and Table 2 illustrate the stages of treatment for WS-infected corals through *in vivo* inoculation of isolates S.KS.01, S.KS.07, S.KS.10, and S.KS.16. By the final observation day (Day-34), isolate S.KS.07 was found to be the most effective in inhibiting pathogen infection and promoting tissue recovery in WS-infected corals (Figures 2e-h).

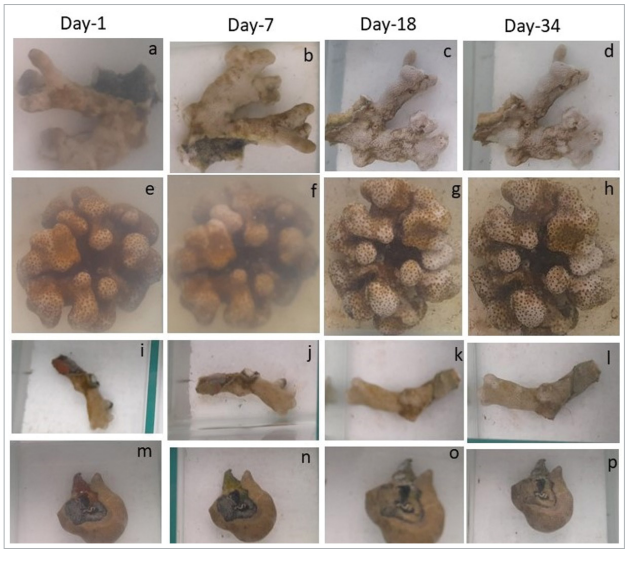

Fig 2. Direct *in vivo* treatment of WAS disease against healthy coral bacterial isolates. a-d representative photos of coral treatment with isolate S.KS.10; e-h coral treatment with isolate S.KS.07; i-l coral treatment with isolate S.KS.01; and m-p coral treatment with isolate S.KS.16. Photos represent days 1, 7, 18, and 34.

Table 2. Description of *in vivo* identification of test coral samples

Day	Isolat Code				Control
	S.KS.01	S.KS.07	S.KS.10	S.KS.16	
1	The condition of the coral is still alive, with several places showing WS disease lesions	The condition of the coral is still alive, with several places showing WS disease lesions	The condition of the coral is still alive, with several places showing WS disease lesions	The condition of the coral is still alive, with several places showing WS disease lesions	The condition of the coral is still alive, with several places showing WS disease lesions
3	The infection is spreading	Corals produce a thin white layer in the water. There is an increase in WS infection, but coral polyps are still visible	The infection is spreading	The infection is spreading	The infection is spreading
7	Some of the polyps have died (they look white) and the coral color looks faded	Coral polyps increasingly appear to be emitting tentacles (corals experience additional stress)	The coral is still 80% alive, but the colour is fading	The coral is still 90% alive, but the colour is fading	The color of the coral is fading and it looks white
12	Approximately 50% of corals die	The coral still emits a thin white layer into the aquarium water. Damage to coral tissue by 20%	Some polyps have died (look white)	Some polyps have died (look white)	Corals experience 100% death
15	Approximately 80% of corals die	Coral can still survive, even though it has experienced more bleaching than before. Coral mortality is around 40%	The colour of the coral is fading, but the coral is still alive by approximately 60%	The colour of the coral is fading, but the coral is still alive by approximately 70%	Corals experience 100% death
19	Approximately 90% of corals die	The colour of the coral gradually returns from faded brown to a slightly darker brown. Coral polyps increased by 10%	The colour of the coral appears faded, but the coral is still alive. Coral tissue death is approximately 50%	The colour of the coral appears faded, but the coral is still alive. Coral tissue death is approximately 40%	Corals experience 100% death
36	Approximately 95% of corals die	Coral begins to recover with the increase in coral polyps (brown spots) by 20%	The coral color appears faded, but it is still alive. Coral death is 70%	The coral color appears faded, but it is still alive. Coral death is 60%	Corals experience 100% death

Notes: Controls were corals infected with WS without being treated with antibacterial inoculation

Isolate S.KS.07 was the most effective in promoting tissue recovery and halting disease progression, restoring up to 20% of the infected coral tissue (Table 2). This suggests a promising role for isolate S.KS.07 as a natural biocontrol agent against white syndrome. In comparison, the test corals exhibited a mortality rate of 60–95%, whereas the control corals reached 100% mortality (Table 2). This indicates that antibacterial isolates other than S.KS.07 were unable to restore WS-infected coral tissue (Fig. 2a-d; i-p).

Based on DNA sequencing analysis, isolate S.KS.07 was identified as a bacterium closely related to *C. firmus*, with a 99.93% similarity. *C. firmus* has demonstrated the ability to support the recovery of corals infected with WS, although complete restoration to their original condition was not achieved. The healing process in treated corals was indicated by their ability to recover after the final inoculation day. The lesions, initially characterized by white discoloration, gradually faded, replaced by brown pigmentation or brown spots (Figures 3g-h). Inoculation

with *C. firmus*-containing isolates successfully halted the spread of WS, allowing the coral to undergo a recovery process. A similar study was conducted by Ushijima et al. (2023) on the potential of *Pseudoalteromonas* sp. strain McH1-7 as a probiotic for corals infected with stony coral tissue loss disease (SCTLD). In laboratory trials, McH1-7 effectively halted or slowed disease progression by up to 68.2% in treated *Montastraea cavernosa* fragments and prevented disease transmission by 100%. While previous research has shown promising results in controlling diseases like SCTLD (Ushijima et al., 2023), the effectiveness of *C. firmus* in combating white syndrome represents a new frontier in coral disease management *C. firmus* is a Gram-positive bacterium that thrives in both terrestrial and marine environments. Several studies have revealed its antimicrobial potential and ability to inhibit disease progression. For example, the combination of *C. firmus* with silica particles has been proven to suppress seed rot disease in rice *Oryza sativa* (Kang et al., 2023). Additionally, research on bacteria from the *Cytobacillus* genus, which serves as an epibiont on the brown alga *Hydroclathrus* sp., has demonstrated its potential as an antimicrobial agent. This bacterium can inhibit the growth of several pathogenic bacteria, including *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, as well as the pathogenic fungus *Candida albicans* ATCC 90028 (Ethica et al., 2023; Saied et al., 2022). Furthermore, research conducted by Ethica et al. (2023) indicated that *Cytobacillus* HI03-3b plays a role in polysaccharide and protein degradation and ecologically inhibits the growth of pathogenic bacteria, thereby protecting its algal host from predation. *C. firmus* is primarily utilized in agriculture and aquaculture, both for disease prevention in crops and as a bioindicator of water quality in contaminated environments.

To date, no references or studies have reported that *C. firmus* forms a symbiotic relationship with coral biota. Therefore, the findings of this study can be considered a novel discovery in coral reef ecosystem management, particularly in the field of disease control. Research conducted by Gao et al. (2024) reported that bacterial isolation from the shrimp *Litopenaeus vannamei* farming system (water, shrimp, and sediment) revealed an association with *C. firmus*. This bacterium functions as a probiotic in shrimp aquaculture and has been shown to inhibit the growth of several pathogenic bacteria, including *Vibrio cholerae, V. owensii, V. fluvialis, V. campbellii, V. rotiferianus, Photobacterium damselae, V. vulnificus* and *V. parahaemolyticus* (Gao et al., 2024). While *C. firmus* has been previously studied in agricultural and aquaculture contexts. This study provides the first evidence of its potential as a probiotic for corals, offering a sustainable and eco-friendly alternative to chemical disease control methods

Based on this study, *C. firmus* has the potential to be used as a biocontrol agent for white syndrome (WS) in corals. This bacterium functions as an environmentally friendly natural probiotic. The use of natural probiotics provides ecological benefits as it does not harm the environment and can serve as an alternative to pesticides or other chemical agents that may have negative impacts on the ecosystem.

CONCLUSION

Coral-associated bacteria with bioactive compounds that effectively target the pathogens responsible for White Syndrome (WS) belong to 2 classes, 3 families, 5 genera, and 5 species. These bacteria exhibit high phylogenetic similarity to *Bacillus tequilensis* (99.79% and 99.93%), *Micrococcus luteus* (99.43%), *Cytobacillus firmus* (99.93%), *Staphylococcus arlettae* (99.79%), and *Priestia aryabhattai* (99.86%). Among these, *Cytobacillus firmus* stands out as a promising biocontrol agent for managing WS in corals.

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PROBIOTIČKI POTENCIJAL MIKROBIOTE KORALJA: PRIRODNA OBRANA PROTIV IZBJELJIVANJA KORALJA

SAŽETAK

Zdrave bakterije povezane s koraljima proizvode antimikrobne spojeve koji mogu inhibirati patogene uzročnike bolesti. Ovaj potencijal biološke kontrole osobito je važan u borbi protiv smrtonosne bolesti izbjeljivanja koralja (BS). Cilj ovog istraživanja bio je identificirati korisne bakterije iz zdravih koralja koje bi mogle poslužiti kao agensi za suzbijanje izbjeljivanja koralja. Uzorci zdravih i BS-inficiranih koralja prikupljeni su u vodama Sawopudoa, jugoistočni Sulawesi, Indonezija. Bakterije su izolirane metodama razmazivanja i prenošenja na hranjivu podlogu, a njihova antimikrobna aktivnost ispitana je *in vitro* pomoću agar-čep testa. Izolati s izraženim zonama inhibicije identificirani su sekvenciranjem 16S rRNA

gena. Najperspektivnije sojeve potom se testiralo *in vivo* inokulacijom BS-inficiranih koralja. Od šest izolata s izraženom antimikrobnom aktivnošću, najučinkovitiji su pokazali visoku sličnost s vrstama *Bacillus tequilensis*, *Micrococcus luteus*, *Cytobacillus firmus*, *Staphylococcus arlettae* i *Priestia aryabhattai* (sličnost od 99,43% do 99,93%). *Cytobacillus firmus* pokazao je najveću učinkovitost *in vivo*, formirajući zonu inhibicije promjera 16,6 mm i potičući obnovu do 20% zaraženog tkiva koralja. Ovi rezultati naglašavaju potencijal probiotičkih bakterija kao održive strategije za ublažavanje izbjeljivanja koralja i zaštitu koraljnih grebena.

Ključne riječi: simbiotske bakterije koralja, bolest koralja, *Cytobacillus firmus*, biološka kontrola, izbjeljivanje koralja

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