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## Effectiveness of marine fungal symbiont isolated from soft coral *Sinularia* sp. from Panjang Island as antifungal

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### Abstract

Soft corals are invertebrates living on coral reefs that produce bioactive compound that can be used such as an antibacterial, antifungal, anti-tumor, and anti-inflammatory that are beneficial for the pharmaceutical industry. Several studies have shown that fungi are very rich of natural chemical compounds that are potential as a source of new drugs, but there is few research in marine fungal symbionts. In this research we collected a soft coral *Sinularia* sp. as source of marine fungal symbionts from Panjang island of the North Java Sea. A total of 15 fungi were successfully isolated and screened using overlay method against pathogenic fungi *Candida Albicans* and *Aspergillus flavus*. The results revealed that a fungal symbiont was able to inhibit the growth of pathogenic fungi. Phytochemical tests showed that phenolic, triterpenoid and flavanoid compounds were detected within fungal extract. Molecular identification based on 18s rRNA gene showed that the active fungal was closely related to *Aspergillus unguis*.

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### 1. Introduction

The oceans are the source of a large group of structurally unique natural products that are mainly accumulated invertebrates that are common to coral reef ecosystems, such as sponges, tunicates, bryozoans, soft corals and mollusks. This diversity has been the source of unique chemical compounds with the potential for industrial

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development as pharmaceuticals, cosmetics, nutritional supplements, molecular probes, enzymes and agrichemicals. Thus, coral reef represents a virtually un-exploited resources for discovery of even more novel compounds with useful applications [1]. Coral reef is a productive ecosystem with high biodiversity in the sea and being targeted to find a useful bioactive compound. [2]. Soft corals are marine invertebrates that rich products bioactive compound that has the potential of biotechnology. Soft corals also had potential antiviral, antimicrobial and another [3,4, 5,6]. Examples of bioactive compounds soft coral *Sinularia* sp. are *Flexibilide* and *sinulariolide* from soft coral *Sinularia flexibilis* as antimicrobial [7].

Natural products from marine invertebrates greatly expand the chemical diversity available for biotechnological exploitation. Despite a gradual reduction in activities by the pharmaceutical industry in the field of natural compound research in the past 25 years, an average of two new natural compounds were approved as drugs per year during this period [9]. [10] mentioned marine fungi are prolific resources of natural products. In recent years, marine fungi have been explored more intensely to obtain novel and biologically active compounds. [11] mentioned that microbe symbiotically associated with soft corals can synthesize secondary metabolites similar to host. This allows the fungal symbionts to produce antifungal compounds that may be used to combat fungal pathogen. It has been considered that the problem of supply has hampered the development of most secondary metabolites from marine invertebrates, thus, it is important to highlight the possible role of marine fungie symbionts with soft corals in providing an alternative to the commercial medicine worlds.

Here, we report the antifungal potential of a fungal symbiont of softcoral *Sinularia* sp. againts fungal pathogens *Candida albicans* and *Aspergillus flavus*.

## 2. Materials And Methods

### 2.1 Collection of samples and fungal isolation

Colonies of soft coral *Sinularia* sp. were collected from Panjang Islands, North Java Sea, Indonesia by scuba diving . Upon collection colonies were put into sterile plastic bags (Whirl-Pak, Nasco, USA) and put into cool-box . The tissues were then crushed using a sterile mortar. The homogenized tissues were serially diluted 10<sup>-4</sup> until 10<sup>-6</sup>, spread on MEA medium containing vancomisin and chloramphenicol antibiotics, incubated at room temperature for 4 days. On the basis morphological features, colonies were randomly picked and purified by streak plates. [6,11].

### 2.2. Antifungal test

Antifungal test of soft coral-fungal symbiont against patogen fungal was performed by overlay method. [6]. Fungal pathogenic (*Candida albicans* and *Aspergillus flavus*) used in this study were obtained from Tropical Marine Biotechnology, Diponegoro University. Culture of fungal pathogen in the logarithmic phase was mixed with Zobell soft agar medium (1% v/v), which were then poured on to the respective agar surface previously inoculated with soft coral-symbiont fungal and incubated for 4 days. The plates were incubated at room temperature for 2 days. Antifungal activity was defined by the formation of inhibition zones around the fungal colonies.

### 2.3. Molecular Identification.

DNA extraction, amplification and sequencing were also carried out at the Tropical Marine Biotechnology UPT Diponegoro University. The DNA genomes from misselia fungal isolates were extracted through chelex , The DNA concentrations were quantified and qualified by using Nanodrop. 18S rRNA fragments were generated by using universal primer ITS1 - ITS4 with base sequence ITS1: 5'- CTT GGT CAT TTA GAG GAA GTAA-3' dan ITS4 : 5'- TCC TCC GCT TAT TGA TAT GC-3'. [13, 14,15]. These primers were used to obtain 400- 800 bp rDNA fragments for sequencing purposes. The DNA amplification was performed by Taq polimerase Master Mix with approximately 2 µg of total genome as a template. PCR was conducted as modification from [16] : initial denaturation (5 min at 95 ° C ), 32 cycles of primer denaturation (1 min at 95 ° C), annealing (1 min at 57,1 ° C), and elongation (1 min at 72 ° C) followed by a final elongation step (7 min at 72 ° C). [16] . Purification of

PCR products and subsequent sequencing analysis were conducted according to the method of [16] The determined DNA sequence of strains were then compared for homology to the BLAST database.

## 2.4. Phytochemical test

Chemical tests were carried out on the fungal extract using standard procedures to identify the constituents as described by [17], [18].

## 3. Results and Discussion.

### 3.1. Antifungal Test

There were 15 isolates from symbiont fungal soft coral tested, however only 1 isolate was found to inhibit the growth of 2 Pathogenic fungal. (Table 1, Fig 1).

Table 1. Antifungal activity of fungal symbiont

No	Isolat Code	<i>A.Flavus</i>	<i>C.Albicans</i>
1	SCPPB 1.10	+	+

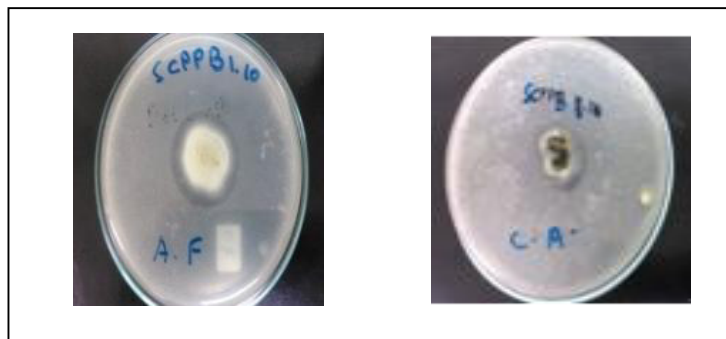


Fig 1. Antifungal activity of fungal symbiont

### 3.2. Molecular Identification

Molecular identification of the active soft coral isolate based on 18S rRNA, revealed that it is belonging to the members of genus *Aspergillus* (Table 2 and Fig 2)

Table 2. Molecular Characterization of fungal symbiont

Isolates	Close Relative	Simillarty	Acc. Number
SCPPB 1.10 (NF-2)	<i>Aspergillus unguis</i>	99%	JF731256.1

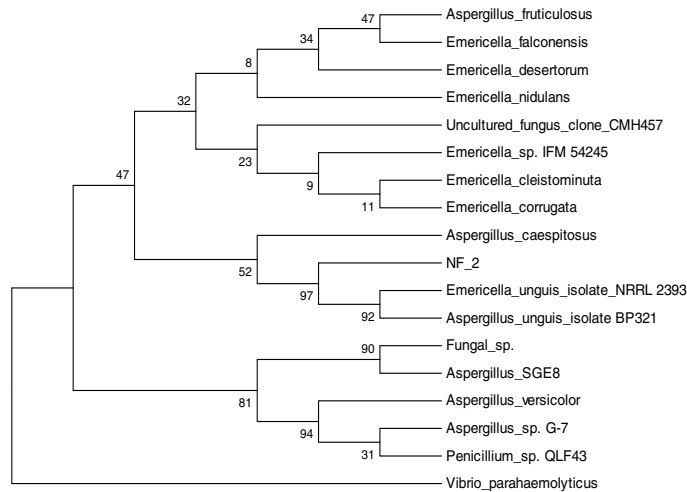


Fig 2. Neighbor-joining phylogenetic tree 18s rRNA of the active fungal symbionts.

### 3.3. Phytochemical Test

There were 5 isolates from antifungal test, however only one to phytochemicals test. (Table 3)

Table 3. Phytochemicals Test of the active fungal symbiont

Parameter	SCPPB 1.10 Extraction
Phenolic	+
Triterpenoid	+
Flavonoid	+

## 4. Discussion

Inhibitory interactions among coral associated fungus that occur on the coral surface are of great interest to search for secondary metabolite-producing fungal Isolation and screening for secondary metabolite-producing fungal in coral reef ecosystems have been neglected until now.

Reef invertebrates, being sessile and soft-bodied organisms, are susceptible to invasion by microbial pathogens such as bacteria and fungi; hence they need to possess defence mechanisms against such pathogens. Given that invertebrates also lack cell-based immune responses and are continuously exposed to a broad array of potentially deleterious microorganisms, it is reasonable to hypothesise that the production of bioactive secondary metabolites could act as the fundamental mechanism of antimicrobial defence [19]. As part of the effort to explore the potential of microbial symbionts of reef invertebrates, we successfully screened these symbionts for biological activity against pathogenic fungal. It is interesting to note that symbionts come from different invertebrates and at least one isolate showed antifungal activity against two pathogenic fungal. These results have prompted the search for bioactive compounds from microbial associated with different invertebrates in a sustainable manner, without harming the coral reefs. These findings were in accordance with the highlights of microbial associates of invertebrates with medical potential [20].

Flavonoid are derivatives of phenol compounds. Activity of phenolic compounds is by damaging lipids in the plasma membrane of microorganisms. The plasma membrane is semipermeable and serves to control the transport of various metabolites into and out of cells. Disorder or damage to the structure of the plasma membrane can inhibit

or impair the ability of the plasma membrane as an osmotic barrier and disrupt a number of biosynthetic processes required in the membrane.[21] Phenol compound works by denaturation of cell proteins and cell membranes, and is fungistatic or fungicidal dependent concentration. At a concentration of 0.1-2% phenol damage the cytoplasmic membrane causing leakage metabolites and besides that inactivate a number of enzymes. At high levels of phenols causing coagulation of proteins and cell membrane lysis would experience.

Function of these terpenoid compounds are antibacterial, antifungal, antiviral, and can be used in the treatment and therapy. Triterpenoid compound that is dominated by the number chain terpenoid compounds 3 times the chain terpenoids, which have the best antimicrobial activity.

*Sinularia flexibilis* extracts has five components that is diterpenes *flexibilida* terpenoids, *dihydroflexibilida*, *sinulariolida*, *epi-sinulariolida*, and *epi-sinularilida* acetate, that proved have antimicrobial activity [22] Consistently, fungi isolated from sponges account for the highest number (28%) of novel compounds reported from marine isolates of fungi [23]. Marine isolates of fungi evidently are a rich source of chemically diverse natural products which has not been consequently exploited so far. Among a number of metabolites from sponge-associated fungi with promising biological activities are the cytotoxic gymnastatins and the p56lck tyrosine kinase inhibitor ulocladol

Molecular phylogenetic analysis shows that the active isolates belonged to members of the genera *Aspergillus*, *Emericella*, *Fungal*, *Penicillium*, and *Uncultured fungie*. It is interesting to note that the active softcoral isolate SCPPF 1.10, which is closely related to *Aspergillus* species. [23]. *Aspergillus versicolor*, isolated from *Xestospongia exigua* was found to be rich source of novel polyketides. Additionally, many *Aspergillus* and *Penicillium* spp. are known to produce extracts with a wide variety of activities. Decreasing the number ubiquitous species isolated could represent a valid method to increase the probability of producing novel chemistry to use the discovery of new pharmacologically active metabolites.

We do know that Marine-derived fungi (MDF) associated with marine invertebrates produce numerous novel bioactive metabolites, including antitumor, antibiotic, antifungal, antialgal, antiinsect, antioxidant and acetylcholine esterase inhibitors [24,25,26,,27,28] Sponge-associated fungal species produce novel metabolites with unique bioactivities in comparison with their terrestrial conspecifics [29]. To cite a few examples *Aspergillus niger* elaborates seven new diterpenoids [29], *Aspergillus versicolor* synthesises a novel lipopeptide [25]

*Aspergillus* elaborates many polyketides, nonribosomal peptides, isoprenoids and lipopeptides of pharmaceutical importance [30] and is an ideal platform for expressing many other fungal and non-fungal metabolites [31,32] moreover, the molecular regulation of secondary metabolism in the model organism *Aspergillus nidulans* Winter is very well understood [33,34]. Such background knowledge of the secondary metabolism of this genus will help significantly in exploring the chemical diversity of microbe symbiotic .

## 5. Conclusion

Marine fungi symbionts from soft corals provides evidence of antifungal potential against pathogenic fungi. Marine fungal symbionts soft corals could overcome the acknowledged supply problem as marine natural product with search secondary metabolites .

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