Isolation Bacterial Symbiont from *Ulva reticulata* and *Caulerpa racemosa* 
as Candidate of Antimicrobial Producer

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Isolation Bacterial Symbiont from *Ulva reticulata* and *Caulerpa racemosa* as Candidate of Antimicrobial Producer

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ABSTRACT

Isolation of bacterial symbionts from *Ulva reticulata* and *Caulerpa racemosa* was performed by crowded plate technique method using marine agar media. The purpose of this study was to search for antimicrobial-producing bacterial symbiont in green alga *Ulva reticulata and Caulerpa racemosa* from Takalar Coast, South Sulawesi. The isolates of bacterial symbiont were fermented in production media for seven days at 37°C. Antimicrobial activity was done by agar diffusion method using *Bacillus subtilis*, *Staphylococcus*, *Escherichia coli* and *Candida albicans* as microbial test. We found four isolates of bacterial symbiont from *Ulva reticulata* and five isolates from *Caulerpa racemosa*. The isolates of green algae *Ulva reticulata* and *Caulerpa racemosa* have higher antifungal activity against *C. albicans* than antibacterial activity. High antifungal activity was showed by isolates from *C. racemosa* with diameter of zonal inhibition, i.e: BSCR-1 (18.07 mm), BSCR-5 (18.61 mm), BSCR-2 (10.79 mm), isolate BSCR-4 (12.34 mm). and from *Ulva reticulata* was BSUr-3 only that have antifungal activity with diameter of zonal inhibition 14.33 mm.

Keyword: bacterial symbiont, *Ulva reticulata*, *Caulerpa racemosa*, antimicrobial activity
INTRODUCTION

Many pathogenic microorganisms, such as *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* have developed resistance towards antibiotics and this trend has become more and more. A high percentage of the infections contracted in the hospitals are caused by very resistant bacteria, like Methicillin Resistant *Staphylococcus aureus* (MRSA), *Enterococcus faecium* and several microorganisms gram negative resistant to Vancomycin (WHO, 2011).

All these new problems, The scienties or researchers call for more novel antibiotics, the ones is from the marine environment. The oceans are described as a “soup” of fundamentally all types of microbes and an ecosystem of great diversity (Kjer et al. 2010). biologically active compounds to adapt to particular environmental conditions (Penesyan et al, 2010). The marine environment is a habitat for many unique microorganisms, which produce. One of them is from microbial symbiont in marine algae.

Marine algae live in symbiosis with certain microorganisms such as fungi and bacteria. Epiphytic and endophytic microbes live on the surface and in the inner tissues or even in the cell of their hosts, respectively. An endophyte is a bacterial or fungal microorganism, which spends the whole or part of its life cycle colonizing inter- and/or intra-cellularly inside the healthy tissues of the host plant (Tan and Zou, 2001), but bacteria exist only in some marine algae species. Marine macroalgal-bacterial associations range from beneficial, harmful or neutral, over obligate or facultative, to ecto- or endophytic interaction (Goecke et al, 2010).
The bioactive properties of marine algae and marine microorganisms have been analyzed, and in both cases positive results have been obtained. Some seaweed species need vitamins for their growth and possibly the bacteria are partially responsible for the production of these substances; some of them produce antibiotics (Jasti et al., 2005; Penesyan et al., 2010). It is believed that algae and their associated endophytic symbionts should represent a good source of biologically active secondary metabolites (Schulz et al. 2008 and Suryanarayanan et al. 2010). Some of these endophytes may be producing bioactive substances that may be involved in a host-endophyte relationship (Strobel, 2003).

Marine algae have been reported to contain many important compounds which act as antibacterial and antifungal. Seaweeds are classified as Rhodophyta (red algae), Phaeophyta (brown algae) or Chlorophyta (green algae) depending on their nutrient, pigments and chemical composition (Chanda et al, 2010). Some of green algae (strains of Ulva and Caulerpa) were more active than other groups of algae screened for their antibacterial activity and they are potential sources of bioactive compounds and should be investigated for natural antibiotics (Kandhasamy and Arunachalam, 2008; Osman et al, 2010). For example Ulva fasciata which contained sterol alkaloid, phenolic, flavonoid, , terpenoid, glikoside had width spectrum antibacterial activity (Premalatha et al, 2011). Extract of Caulerpa racemosa from India had shown antibacterial activity with <15 mm of zone of inhibition, its bioactive compounds were phenolic, flavonoid, steroid, alkaloid and protein (Srivastava et al, 2010).

Isolation bioactive compound from marine algae need very much samples. By using bacterial endophyte from marine algae, we could produce the bioactive
compound especially novel antibiotic which may be similar with present in the host (macro algae). The purpose of this study was to isolate antibiotic-producing bacterial symbiont in green alga *Ulva reticulata* and *Caulerpa racemosa* from Takalar South Celebes. In this paper, we report the isolation and preliminary screening of bacterial symbiont from *Ulva reticulata* and *Caulerpa racemosa* as antibiotic producer.

**MATERIALS AND METHODS**

**Marine Alga Collection**

The green alga *Ulva reticulata* were collected from Punaga coast and *Caulerpa racemosa* were collected from Putondo coast at Takalar, South Sulawesi at October 2011. Immediately after collection, they were washed in fresh seawater to remove the epiphytes, sand and other extraneous matter. The algae were transported to the laboratory in cool box.

**Isolation of Bacterial Symbiont by crowded plate technique**

The algae were rinsed with sterile seawater, then immersed in Sodium hypochlorite 1% during one minute, then rinsed again with sterile seawater. After surface sterilization, 10 g samples were blended with 90 mL sea water sterile (dilution $10^{-1}$) and then were diluted by serial dilution up to $10^4$. Each dilution sample was poured 1 mL into 20 mL media Marine Agar in petridish, respectively. The media were incubated during 96 hours at 37 °C. After incubation, the colony bacteria which inhibited colony around it, were selected as the antibiotic-producing bacteria. The isolated was purified by multiple streaking method.
Production secondary metabolites by submerged fermentation

The selected bacteria were cultured in sterile Malt Yeast Broth at 37°C for 24 hours. After that, were fermented in sterile production media (2% glucose, 10% amylum dissolved, 2.5% soy powder, 0.1% yeast extract, 0.2% NaCl, aquadest ad 100%), pH 7.0 ± 0.2, using shaker at 120 rpm during six days. The result of fermentation were sonicated 5 minutes and sentrifugated at 3000 rpm during 15 minutes. The supernatant was used as test samples for antimicrobial test.

Antimicrobial Activity assay

All the isolated bacterial symbiont were screen for antimicrobial activity, using microbial collection of Pharmaceutical Microbiology laboratory Faculty of Pharmacy UNHAS, i.e: Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Candida albicans as microbial test. Antimicrobial activity was assay in duplicate using diffusion method with paper disc standard (as reservoir). The discs containing 20 μL samples were place onto the Muller Hinton Agar for antibacterial assay and Potato Dextrose Agar for antifungal test which containing bacterial test and incubated at 37°C for 24 hours. Diameter of inhibition zones formed around the disc were then measured.

RESULT AND DISCUSSIONS

Four bacterial symbiont were isolated from green algae Ulva reticulata from Punaga coast: with code samples i.e: BSUr-1, BSUr-2, BSUr-3 and BSUr-4 and five bacterial symbiont were isolated from green algae Caulerpa racemosa with code samples BSCr-1, BSCr-2, BSCr-3, BSCr-4, and BSCr-5.

After fermentation all bacterial symbiont isolates was assay antimicrobial activity using diffusion methods, the result was showed at table 1 and 2.
Table 1. Antimicrobial activity of supernatant of bacterial symbionts (20 μL/disc) from Ulva reticulata using agar diffusion assay

<table>
<thead>
<tr>
<th>No.</th>
<th>Code samples</th>
<th>Bacterial test</th>
<th>Fungal test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>B. subtilis</em></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>1</td>
<td>BSUr-1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>BSUr-2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>BSUr-3</td>
<td>10.42</td>
<td>9.61</td>
</tr>
<tr>
<td>4</td>
<td>BSUr-4</td>
<td>8.52</td>
<td>7.67</td>
</tr>
<tr>
<td>5</td>
<td>Positive controle</td>
<td>19.56</td>
<td>9.44</td>
</tr>
</tbody>
</table>

Table 2. Antimicrobial activity of supernatant of bacterial symbionts from Caulerpa racemosa using agar diffusion assay

<table>
<thead>
<tr>
<th>No.</th>
<th>Code Samples</th>
<th>Bacterial test</th>
<th>Fungal test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>B. subtilis</em></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>1</td>
<td>BScr-1</td>
<td>12.52</td>
<td>14.53</td>
</tr>
<tr>
<td>2</td>
<td>BScr-2</td>
<td>9.19</td>
<td>10.46</td>
</tr>
<tr>
<td>3</td>
<td>BScr-3</td>
<td>8.45</td>
<td>11.67</td>
</tr>
<tr>
<td>4</td>
<td>BScr-4</td>
<td>-</td>
<td>11.47</td>
</tr>
<tr>
<td>5</td>
<td>BScr-5</td>
<td>13.07</td>
<td>12.96</td>
</tr>
<tr>
<td>6</td>
<td>Positive controle</td>
<td>19.95</td>
<td>8.53</td>
</tr>
</tbody>
</table>

*Antifungal positive controle : Nistatin 100 IU
Antibacterial positive controle : Chlamphenicol 30 ppm

From four bacterial symbiont isolated from U. reticulata, we found only three isolates had antimicrobial activity and category less active according to Ibtissam (2009). BSUr-2 had antibacterial activity against gram negative bacterial test, BSUr-3 had antibacterial activity against gram positive bacterial activity and antifungal activity against C. albicans. BSUr-4 only had antibacterial activity.

From five bacterial symbiont isolated from C. racemosa, Isolat BScr-1 and BScr-5 had higher antimicrobial activity than the other. According to classification antimicrobial activity from Ibtissam et al (2009), they were less active category because diameter of inhibition zone around 10 mm to 16 mm, but antifungal activity were moderately active (diameter of inhibition zone around 16 mm to 20 mm).
We think the bacterial symbiont from Caulerpa sp is potential as candidate antimicrobial resource because we use only supernatant as samples, not yet was extracted the bioactive compound from supernatant. The extract or bioactive compound may be had higher antimicrobial activity.

CONCLUSION

1. We found four isolates bacterial symbiont from Ulva reticulata and five isolates from Caulerpa racemosa from Takalar South Sulawesi.

2. The potent isolate bacterial symbionts as candidate antimicrobial producer were BSCr-1 and BSCr-5 isolates from Caulerpa recemosa.

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REFERENCE


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