Optimization of antagonistic activity of the extracellular compound *Serratia plymuthica* UBCF_13 against phytopathogenic fungi through the addition of carbon and nitrogen

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Abstract. *Serratia plymuthica* UBCF_13 is one of the biocontrol bacteria that can suppress the growth of phytopathogenic fungi. Bacterial antifungal activity is influenced by several environmental factors, including the addition of carbon and nitrogen sources. This study aimed to determine the carbon and nitrogen sources that can encourage the maximum antagonistic activity of the extracellular compound from *S. plymuthica* UBCF_13. Carbon and nitrogen sources used in this study were glucose, sucrose, glycerol, ethanol, peptone, ammonium sulfate, tryptone, and yeast extract, with a concentration of 2 %. Bacterial extracellular compounds were applied to phytopathogenic fungi [*Colletotrichum gloeosporioides*, *Sclerotium rolfsii*, and *Fusarium oxysporum*] to see their antifungal activity. Data were statistically analyzed using one-way variance with DNMRT at the 5 % level. The best antifungal activity against *C. gloeosporioides* resulted from the addition of carbon source [ethanol] 22.28 % and nitrogen source [peptone] 16.94 %. The best antifungal activity against *S. rolfsii* fungi resulted from the addition of carbon source [ethanol] 14.67 % and nitrogen source [ammonium sulfate] 15.53 %. The inhibition ability of the extracellular bacterial compounds of *S. plymuthica* UBCF_13 in recording the growth of *S. rolfsii* with the addition of carbon and nitrogen is fairly low. *S. plymuthica* bacteria are less able to produce antifungal compounds that can suppress the growth of *S. rolfsii* fungi. The best antifungal activity against *F. oxysporum* fungi resulted from the addition of carbon source [ethanol] 8.47 % and nitrogen source [ammonium sulfate and tryptone] 28.25 % & 26.88 %.

Keywords: Carbon sources, Extracellular compound, nitrogen sources, phytopathogen fungi, *S. plymuthica* UBCF_13.
1. Introduction

*Serratia plymuthica* UBCF_13 bacteria is a species of *Serratia* bacteria which is a collection of the Biotechnology Laboratory, Faculty of Agriculture, Andalas University. This bacterium was isolated from the leaf phyllosphere of *Brassica juncea* L. Solok, West Sumatra [1]. *Serratia plymuthica* bacteria is one of the gram-negative bacteria which can act as a biological control agent in the presence of antagonistic activity against several pathogenic fungi [2]. *S. plymuthica* UBCF_13 was first tested in vitro for its ability to suppress the growth of *Colletotrichum gloeosporioides* through colony application and this bacteria was able to suppress fungal growth with an inhibitory power of 34.48 % [1], but it was unable to suppress the growth of *Sclerotium rolfsii* and *Fusarium oxysporum* [3].

A bacterium can show antifungal activity if it is given optimal environmental conditions. One of the optimal environmental conditions is adequate sources of nutrients such as carbon and nitrogen. The needs for the types of carbon and nitrogen sources in the production of metabolite compounds by each bacterial species are specific. The type of nitrogen source needed will depend on the biosynthetic pathway required to produce a particular type of metabolite [4]. Modification of culture conditions through the addition of carbon and nitrogen has been tested to assess its effect on the antifungal ability of *S. plymuthica* bacteria UBCR_12. The addition of glucose and peptone as a source of carbon and nitrogen has been shown to increase the antagonistic activity of bacteria against *C. gloeosporioides* fungi [5,6].

2. Materials and methods

2.1 Preparation of materials

*S. plymuthica* bacteria were cultured on *King's B* [pH 7], *C. gloeosporioides* fungi, *Sclerotium rolfsii*, and *Fusarium oxysporum* were cultured on PDA [pH 7]. All tools and materials are autoclaved.

2.2 Modification of culture media and production of extracellular compounds of *S. plymuthica* bacteria UBCF_13

Culture media modification was carried out by adding 2% carbon sources or nitrogen to the culture media for *S. plymuthica*. For the production of extracellular compounds, a single colony of bacteria was added to 50 ml of each modified medium. Then the cultures were incubated for 24 hours [100 rpm, RT].

2.3 Extraction of the extracellular compounds of *S. plymuthica* bacteria UBCF_13

30 ml of incubated bacterial culture were centrifuged. Then the supernatant was sterilized using a 0.22 µm syringe filter and transferred to a 2 ml Eppendorf tube and ready to be applied.

2.4 Activity assay of the extracellular compounds of *S. plymuthica* bacteria UBCF_13

When the fungal culture was two days old, as much as 50 µl of the cell-free extracellular compound was applied to the application hole 3 cm from the center of the pathogenic fungi.
2.5 Observation and data analysis
The culture density was observed after passing 24 hours of the incubation period [OD_{600nm}]. Observation of inhibitory power was carried out starting from the first day after application until the next seven days and then calculated using the formula [7].

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\text{Inhibition (\%)} = \frac{\text{control diameter} - \text{treatment diameter}}{\text{control diameter}} \times 100 \%
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The data obtained were analyzed using one-way ANOVA with the help of SPSS software version 16.0 [8] and using the DNMRT advanced test with a significance level of 5%.

3. Result and Discussion
3.1 Growth of S. plymuthica Bacteria UBCF_13 on Each Addition of Carbon and Nitrogen Nutrients
Measurement of bacterial growth was carried out to see the effect of adding carbon and nitrogen nutrients to an increase in the number of secondary metabolites produced. The addition of a carbon source to the growing medium could result in a decrease in the OD value during the 24-hour incubation period for Enterobacter cloacae SG1 bacteria [9]. From the results obtained, the OD value is not always directly proportional to the addition of carbon and nitrogen nutrient sources to the bacterial growth medium and the OD value is not always directly proportional to the number of metabolites produced. In the treatment that produced the smallest OD, when tested with the fungus C. gloeosporioides, the highest inhibition value was obtained against this fungus. However, it has very low inhibitory power against two other fungi, namely Fusarium oxysporum and Sclerotium rolfsii.

**Figure 1.** Illustration of placement of pathogenic fungi and bacterial extracellular compounds in the activity assay medium
3.2 Effect of Addition of Carbon and Nitrogen Nutrition to the Antagonistic Activity of the S. plymuthica Bacterial Extracellular Compound strain UBCF_13 against the C. gloeosporioides Phytopathogenic Fungus

The effect of increasing carbon and nitrogen sources is different for each fungal pathogen, some are suppressing the growth of fungi and on the other hand, there is no antagonistic activity at all. The addition of galactose and glucose to the growing medium can increase the antimicrobial activity of C. kutscheri and C. xerosis, while the addition of ribose and lactose to the growing medium suppresses its antimicrobial activity [10]. From this study, it was found that the addition of ethanol nutrients to the growth media for S. plymuthica bacteria had a significant effect on inhibiting the growth of C. gloeosporioides fungi and was significantly different from other treatments. In the addition of nitrogen nutrients, the addition of peptone showed a significantly different value from other treatments with an inhibitory value of 16.94 % on the 8th day after application.
Figure 3. Inhibition of extracellular compound of *S. plymuthica* strain UBCF_13 against *C. gloeoeporioides* with the addition of 2 % carbon on the 8th day after application. The data released is an average value of 5 replications. Bar error presenting SD value.

Figure 4. Inhibition of extracellular compound of *S. plymuthica* strain UBCF_13 against *C. gloeoeporioides* with the addition of 2 % nitrogen on the 8th day after application. The data released is an average value of 5 replications. Bar error presenting SD value.

3.3 Effect of Addition of Carbon and Nitrogen Nutrition to the Antagonistic Activity of the *S. plymuthica* Bacterial Extracellular Compound strain UBCF_13 against the *F. oxysporum* Phytopathogenic Fungus

Based on variance using a completely randomized design, it was found that the addition of ethanol had a significant effect on the inhibition value of *F. oxysporum* growth by *S. plymuthica* bacteria and was significantly different from other treatments. In the addition of nitrogen, the addition of ammonium sulfate and tryptone did not appear to be significantly different, but significantly different from other treatments.

Figure 5. Inhibition of extracellular compound of *S. plymuthica* strain UBCF_13 against *F. oxysporum* with the addition of 2 % carbon on the 8th day after application. The data released is an average value of 5 replications. Bar error presenting SD value.
Figure 6. Inhibition of extracellular compound of \textit{S. plymuthica} strain UBCF\_13 against \textit{F. oxysporum} with the addition of 2\% nitrogen on the 8th day after application. The data released is an average value of 5 replications. Bar error presenting SD value.

3.4 Effect of Addition of Carbon and Nitrogen Nutrition to the Antagonistic Activity of the \textit{S. plymuthica} Bacterial Extracellular Compound strain UBCF\_13 against the \textit{S. rolfsii} Phytopathogenic Fungus

The inhibition ability of the extracellular bacterial compounds of \textit{S. plymuthica} UBCF\_13 in recording the growth of \textit{S. rolfsii} with the addition of 2\% carbon is fairly low. This indicates that these bacteria are less able to produce antifungal compounds that can suppress the growth of \textit{S. rolfsii} fungi. \textit{S. rolfsii} fungus is known to belong to a fungus that has a high level of pathogenicity and has a wide range so that it causes major losses to farmers [11-14]. Referring to this theory, in this study \textit{S. rolfsii} fungi had a rapid development compared to \textit{C. gloeoesporioides} and \textit{F. oxysporum}. This fungus has rapid growth in environments with high humidity and a temperature range of 25-35 C [15-17].

Figure 7. Inhibition of extracellular compound of \textit{S. plymuthica} strain UBCF\_13 against \textit{S. rolfsii} with the addition of 2\% carbon on the 8th day after application. The data released is an average value of 5 replications. Bar error presenting SD value.
Figure 8. Inhibition of extracellular compound of S. plymuthica strain UBCF_13 against S. rolfsii with the addition of 2 % nitrogen on the 8th day after application. The data released is an average value of 5 replications. Bar error presenting SD value.

4. Conclusions
The best antifungal activity against C. gloeosporioides resulted from the addition of carbon source [ethanol] and nitrogen source [peptone]. The best antifungal activity against S. rolfsii fungi resulted from the addition of carbon source [ethanol at 1 day after application] and nitrogen source [ammonium sulfate at 8 days after application]. The inhibition ability of the extracellular bacterial compounds of S. plymuthica UBCF_13 in recording the growth of S. rolfsii with the addition of carbon and nitrogen is fairly low. S. plymuthica bacteria are less able to produce antifungal compounds that can suppress the growth of S. rolfsii fungi. The best antifungal activity against F. oxysporum fungi resulted from the addition of carbon source [ethanol] and nitrogen source [ammonium sulfate and tryptone].

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