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Dr. IR. HILDA KARIM, MP UNM <hilda.karim@unm.ac.id>

AOMI-D-21-00198 - Submission Confirmation

1 message

Archives of Microbiology (AOMI) <em@editorialmanager.com> Wed, Feb 17, 2021 at 5:58 PM
Reply-To: "Archives of Microbiology (AOMI)" <niranjana.muralimohan@springernature.com>
To: Hilda Karim <hilda.karim@unm.ac.id>

Dear Dr. Karim,

Thank you for submitting your manuscript, "Antagonistic Activity and Characterization of Indigenous Soil Isolates of Bacteria and Fungi Against Onion Wilt Incited by Fusarium sp.", to Archives of Microbiology.

The submission id is: AOMI-D-21-00198
Please refer to this number in any future correspondence.

During the review process, you can keep track of the status of your manuscript through the Editorial Manager website.

Your username is: HKarim-392
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Thanks again.

With kind regards,
Springer Journals Editorial Office
Archives of Microbiology

Now that your article will undergo the editorial and peer review process, it is the right time to think about publishing your article as open access. With open access your article will become freely available to anyone worldwide and you will easily comply with open access mandates. Springer's open access offering for this journal is called Open Choice (find more information on www.springer.com/openchoice). Once your article is accepted, you will be offered the option to publish through open access. So you might want to talk to your institution and funder now to see how payment could be organized; for an overview of available open access funding please go to www.springer.com/oafunding.

Although for now you don't have to do anything, we would like to let you know about your upcoming options.

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Dr. IR. HILDA KARIM, MP UNM <hilda.karim@unm.ac.id>

Thank you for your approval - [EMID:58b33073d4a97384]

1 message

Archives of Microbiology (AOMI) <em@editorialmanager.com> Wed, Feb 17, 2021 at 5:58 PM
Reply-To: "Archives of Microbiology (AOMI)" <niranjana.muralimohan@springernature.com>
To: Hilda Karim <hilda.karim@unm.ac.id>

Dear Dr. Karim,

Thank you for approving the changes and returning your submission entitled "Antagonistic Activity and Characterization of Indigenous Soil Isolates of Bacteria and Fungi Against Onion Wilt Incited by Fusarium sp.".

You will be able to check on the progress of your paper by logging on to Editorial Manager as an author. The URL is https://www.editorialmanager.com/aomi/.

Thank you for submitting your work to this journal.

Kind regards,

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Dr. IR. HILDA KARIM, MP UNM <hilda.karim@unm.ac.id>

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1 message

Archives of Microbiology (AOMI) <em@editorialmanager.com> Wed, Feb 17, 2021 at 5:56 PM
Reply-To: "Archives of Microbiology (AOMI)" <niranjana.muralimohan@springernature.com>
To: Hilda Karim <hilda.karim@unm.ac.id>

Dear Dr. Karim,

The PDF for your manuscript, "Antagonistic Activity and Characterization of Indigenous Soil Isolates of Bacteria and Fungi Against Onion Wilt Incited by Fusarium sp." is ready for viewing.

In order to formally submit your manuscript to the journal, you must approve the PDF.

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Your manuscript will then be formally submitted to the journal.

Thank you very much.

With kind regards,
Springer Journals Editorial Office
Archives of Microbiology

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Dr. IR. HILDA KARIM, MP UNM <hilda.karim@unm.ac.id>

Registration Welcome Notification for Archives of Microbiology

1 message

Archives of Microbiology (AOMI) <em@editorialmanager.com> Tue, Feb 16, 2021 at 9:43 PM
Reply-To: "Archives of Microbiology (AOMI)" <niranjana.muralimohan@springernature.com>
To: Hilda Karim <hilda.karim@unm.ac.id>

Dear Dr. Karim,

Please be informed you have been registered by our editorial team as a user on the Editorial Manager site for Archives of Microbiology. Information about Archives of Microbiology can be found on the journal website, or by selecting Journal Overview from the top navigation bar at https://www.editorialmanager.com/AOMI/.

Editorial Manager is the manuscript submission and peer-review tracking system through which individuals are invited to review, to write articles for the journal, or to process submissions.

Your username is: HKarim-392

For security reasons, passwords are never sent by email. To set a password, please click this link:
https://www.editorialmanager.com/aomi/l.asp?i=131804&l=47EZ54L1

If you forget your password, you can click the 'Send Login Details' link on the Editorial Manager Login page at https://www.editorialmanager.com/AOMI/.

You can change your password and other personal information at: https://www.editorialmanager.com/AOMI//info_update.asp

With best regards,
Springer Nature
Journals Editorial Office

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Dr. IR. HILDA KARIM, MP UNM <hilda.karim@unm.ac.id>

How to finalize your submission to Archives of Microbiology

1 message

Archives of Microbiology (AOMI) <em@editorialmanager.com> Tue, Feb 16, 2021 at 9:43 PM
Reply-To: "Archives of Microbiology (AOMI)" <niranjana.muralimohan@springernature.com>
To: Hilda Karim <hilda.karim@unm.ac.id>

Dear Dr. Karim,

Thank you for choosing to submit your manuscript (Antagonistic Activity and Characterization of Indigenous Soil Isolates of Bacteria and Fungi Against Onion Wilt Incited by Fusarium sp.) to Archives of Microbiology. There are a few more steps to complete your submission.

At this stage, please ensure that your files meet the submission requirements in the Instructions for Authors on our journal's homepage and that you provide a new cover letter.

NEXT STEPS
1. Login
Log in to https://www.editorialmanager.com/AOMI/ with your username Your username is: HKarim-392. For security reasons, passwords are never sent by email. To set a new password, please click this link:
https://www.editorialmanager.com/aomi/l.asp?i=131803&l=HHM0ZBWM.

2. Edit submission
Go to 'Submissions sent back to author' and click 'Edit Submission'.

3. Final check
All the relevant sections should have been pre-populated, but it is worth checking that all the required information is correct.

Please do this WITHIN 7 DAYS from receiving this email. If you require more time, please reply to this email to let us know. If we haven't received a reply within 7 days, we will assume you do not want to proceed with the submission to Archives of Microbiology

With kind regards,

Journal Editorial Office
Archives of Microbiology

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Dr. IR. HILDA KARIM, MP UNM <hilda.karim@unm.ac.id>

Your submission to Archives of Microbiology - [EMID:4cedc713532b869d]

1 message

Submission Editor <em@editorialmanager.com> Tue, Feb 16, 2021 at 9:42 PM
Reply-To: Submission Editor <submissioneditor@springernature.com>
To: Hilda Karim <hilda.karim@unm.ac.id>

Subject: Your submission to Archives of Microbiology

Dear Dr. Karim,

Thank you for letting me know you would like to submit your manuscript (Antagonistic Activity and Characterization of Indigenous Soil Isolates of Bacteria and Fungi Against Onion Wilt Incited by Fusarium sp.) to Archives of Microbiology.

I have initiated the submission to the journal on your behalf.

Next steps:

The journal will contact you shortly to explain how to finalize your submission. When you finalize your submission, you will have the opportunity to revise your manuscript and upload a new cover letter.

I would appreciate your time to answer a few quick questions about the service - click here to complete our survey: https://springernature.eu.qualtrics.com/jfe/form/SV_3UejQncTro7c3T7?T=1&M=CMIC-D-20-02548&C=INDONESIA

I wish you every success with your manuscript.

With kind regards,

Mrudula Mohare
Editorial Submission Advisor
Springer Nature

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Dr. IR. HILDA KARIM, MP UNM <hilda.karim@unm.ac.id>

Major Revisions requested AOMI-D-21-00198

3 messages

Archives of Microbiology (AOMI) <em@editorialmanager.com> Thu, Apr 1, 2021 at 3:16 PM
Reply-To: "Archives of Microbiology (AOMI)" <niranjana.muralimohan@springernature.com>
To: Hilda Karim <hilda.karim@unm.ac.id>

Dear Dr. Karim,

We have received the reports from our advisors on your manuscript, "Antagonistic Activity and Characterization of Indigenous Soil Isolates of Bacteria and Fungi Against Onion Wilt Incited by Fusarium sp.", which you submitted to Archives of Microbiology.

Based on the advice received, I have decided that your manuscript could be reconsidered for publication should you be prepared to incorporate major revisions. When preparing your revised manuscript, you are asked to carefully consider the reviewer comments which can be found below, and submit a list of responses to the comments. You are kindly requested to also check the website for possible reviewer attachment(s).

Please make sure to submit your editable source files (i. e. Word, TeX).

In order to submit your revised manuscript, please access the Editorial Manager website.

Your username is: HKarim-392
If you forgot your password, you can click the 'Send Login Details' link on the EM Login page at https://www.editorialmanager.com/aomi/.

We look forward to receiving your revised manuscript before 31 May 2021.

With kind regards,
Erko Stackebrandt
Editor in Chief
Archives of Microbiology

COMMENTS FOR THE AUTHOR:

Reviewer #1: The manuscript entitled as "Antagonistic activity and characterization of indigenous soil isolates of bacteria and fungi against onion wilt incited by Fusarium sp." under manuscript number AOMI-D-21-00198 - [EMID:8ee391b5fb6ef552], describe describes the potential antifungal role of indigenous soil isolates of bacteria and fungi against Fusarium sp. The work is based on in vitro screening of bacterial and fungal isolates, thereafter, the molecular identification of the selected isolates showing prominent activity. To my mind, this is a valuable work showing basis for formulating the ecofriendly and non-chemical base management strategies against plant pathogens. I recommend this study for publication. However, I have a few general questions and suggest some minor revisions of the prepared manuscript below.

No need to give statistical analysis values in the abstract. (The four tested microbial isolates were able to significantly inhibit Foc activity in vitro based on the ANOVA test, with values $\alpha = 0.05$, and $n = 3$).
Line 43: mention the disease (Management of this disease can be focused)
Any fungal pathogen isolated from a diseased plant, a pathogenicity test must be done. The identification of the fungal pathogen must be confirmed by a molecular experiment.
A separate paragraph showing the information regarding statistical analysis in material and method section is missing.
I recommend adding a clearer picture of fungus spores Fusarium oxysporum used for identification.
Information about the microscope, its magnifying scale etc. is missing in Fig. 3
Try to show the % similarity rate in 4 and 5.
The author should show consistency in reference style according to journal's format instruction. In some references full name of the journal was used while in some places the journal name was abbreviated. Some references have journal name in italics while others have plain text.
Overall, the manuscript should be carefully and deeply revised for grammar and English use, since minor mistakes are found in some parts of the paper.

Structurally, the introduction need some modifications. The authors concentrated more on crop introduction. Specific information regarding the pathogen and the isolated microbes should be added. Trichoderma is very famous for

biological control activity against a wide range of pathogens. The discussion part can be improved by taking information from recently published review such as:

https://doi.org/10.3390/microorganisms8050617
http://dx.doi.org/10.3390/microorganisms8030401
Conclusion is very short, a little intro and one sentence suggestion should be brought in.

Reviewer #2: In general, I find the work interesting since it is proposed to search for and characterize native microorganisms with the potential to control Fusarium in onion. However, progress is incipient and more information needs to be generated to build a good article. For this reason I consider that this manuscript should be considered as a short communication.
It is necessary to clarify different doubts that arise in the manuscript. Throughout the manuscript it is mentioned that the crop is shallot, while in the title it is mentioned onion. The clearer question is why not identify the phytopathogenic fungus as the antagonists were identified. It is inappropriate to say that F. oxysporum f. sp. cepae when the evidence is insufficient. The comments per section are:

Tittle. Use the most suitable name onion or shallot.

Abstract. Do not use the Fusarium oxysporum f.sp. cepae because there is not enough evidence.
Key words. I suggest include Bacillus subtilis and shallot disease. Omit tuber rot disease and phytopathogenic fungi.

Introduction.
In rows 64-66, P. aeruginosa and T. harzianum are repetitive. Avoid this. Omit the underlying paragraph.
Materials and methods
Because the onion is attacked by different species of Fusarium, identification by STI analysis is necessary.
In "In vitro Tests of Fusarium Antagonist Isolates" authors say observation of the inhibition zone (growth inhibition, GI) was done every two days. This is not zone inhibition, is growth inhibition and two days interval is much time interval.
In PCR amplification. It is really 10 µl volume of the reaction? Please confirm.

Results
On culture and morphological characterization are not enough for identification of Fusarium isolate. The "In vitro tests of Antagonist Microbes vs. Foc Fungi" needs rewrite. Punctual comments are in the manuscript. The text of the manuscript is not properly related to a table or figure. It seems that antagonism by fungus C3 is not the show in the Table 1. In Fig. 2, the C3 fungus shows greater inhibition.
Fig. 2. The growth of the antagonists seems little for 7 days, particularly for bacteria and T. asperellum of which there are strains that in 72-96 hours already covered the Petri dish. Fig. 3 Which treatment? vs fungus or bacterium?
In the case of fungi, no antagonist overgrowth on Fusarium?

Discussion
Focus the discussion more on the results obtained. Es muy especulativa pues aborda cuestiones que no se estudiaron en este trabajo. In the scientific literature, there is much information on the mode of action of T. asperellum. There is also enough information on how Trichoderma species inhibit the growth and pathogenicity of Fusarium. We must focus on the relationship Trichoderma (T. asperellum, mainly) - Fusarium.
Some information is results. Please move the underlying paragraph (207-211) to results.
In this section use the name of each bacterium or fungus.
This is a starter work. Much information is missing before thinking of a consortium. Do not rush.

Conclusions
The work is simple and the antagonism of bacteria and fungi was little characterized. Therefore, I consider it appropriate to say that 2 bacteria and 2 fungi with potential for Fusarium biocontrol were isolated is enough. Use the names of the microorganisms instead of the codes. Avoid using Foc as there is a lack of adequate identification of the phytopathogen.

Administrator
Consult the Instructions to Authors fop proper reformatting of the manuscript (headings, spacing, journal abbreviation in the list of references)
Compare isolates to type strains only (Blast option) and denote the type strains by a superscript T behind the accession numbers
Provide accession numbers for the 16S rRNA and ITS sequences

There is additional documentation related to this decision letter. To access the file(s), please click the link below. You may also login to the system and click the 'View Attachments' link in the Action column.
https://www.editorialmanager.com/aomi/l.asp?i=137930&l=ETYKSOKQ

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Dr. IR. HILDA KARIM, MP UNM <hilda.karim@unm.ac.id>

AOMI-D-21-00198R1 : Your PDF Has Been Built

2 messages

Archives of Microbiology (AOMI) <em@editorialmanager.com> Sun, May 16, 2021 at 10:54 PM
Reply-To: "Archives of Microbiology (AOMI)" <niranjana.muralimohan@springernature.com>
To: Hilda Karim <hilda.karim@unm.ac.id>

Dear Dr. Karim,

The PDF for your manuscript, "Antagonistic Activity and Characterization of Indigenous Soil Isolates of Bacteria and Fungi Against Onion Wilt Incited by Fusarium sp." is ready for viewing.

In order to formally submit your manuscript to the journal, you must approve the PDF.

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Click on that category, view your submission and approve it. In the unlikely case of conversion issues please contact the Journal's Editorial Office by clicking the "CONTACT US" link on the journal EM home page.

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Thank you very much.

With kind regards,
Springer Journals Editorial Office
Archives of Microbiology

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Archives of Microbiology (AOMI) <em@editorialmanager.com> Sun, May 16, 2021 at 11:01 PM
Reply-To: "Archives of Microbiology (AOMI)" <niranjana.muralimohan@springernature.com>
To: Hilda Karim <hilda.karim@unm.ac.id>

[Quoted text hidden]

UNM

Dr. IR. HILDA KARIM, MP UNM <hilda.karim@unm.ac.id>

Submission Confirmation

1 message

Archives of Microbiology (AOMI) <em@editorialmanager.com> Sun, May 16, 2021 at 11:03 PM
Reply-To: "Archives of Microbiology (AOMI)" <niranjana.muralimohan@springernature.com>
To: Hilda Karim <hilda.karim@unm.ac.id>

Dear Dr. Karim,

We acknowledge, with thanks, receipt of the revised version of your manuscript, "Antagonistic Activity and Characterization of Indigenous Soil Isolates of Bacteria and Fungi Against Onion Wilt Incited by Fusarium sp.", submitted to Archives of Microbiology

The manuscript number is AOMI-D-21-00198R1.

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We will inform you of the Editor's decision as soon as possible.

With best regards,
Springer Journals Editorial Office
Archives of Microbiology

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Dr. IR. HILDA KARIM, MP UNM <hilda.karim@unm.ac.id>

Your Submission AOMI-D-21-00198R1

4 messages

Archives of Microbiology (AOMI) <em@editorialmanager.com> Wed, Jun 16, 2021 at 3:54 PM
Reply-To: "Archives of Microbiology (AOMI)" <niranjana.muralimohan@springernature.com>
To: Hilda Karim <hilda.karim@unm.ac.id>

Dear Dr. Karim,

We have received the reports from our advisors on your manuscript, "Antagonistic Activity and Characterization of Indigenous Soil Isolates of Bacteria and Fungi Against Onion Wilt Incited by Fusarium sp.", submitted to Archives of Microbiology.

Based on the advice received, I have decided that your manuscript can be accepted for publication after you have carried out the corrections as suggested by the reviewer(s).

Below, please find the reviewers' comments for your perusal.

You are kindly requested to also check the website for possible reviewer attachment(s).

Please make sure to submit your editable source files (i. e. Word, TeX).

Please submit your revised manuscript using the Editorial Manager system.

Your username is: HKarim-392
If you forgot your password, you can click the 'Send Login Details' link on the EM Login page at https://www.editorialmanager.com/aomi/.

I am looking forward to receiving your revised manuscript before 15 Aug 2021.

With kind regards,
Erko Stackebrandt
Editor in Chief
Archives of Microbiology

COMMENTS FOR THE AUTHOR:

Reviewer #2: Comments and suggestions were adequately addressed. The manuscript is ready for publication

Editor: In contrast to line 134 in which potentially pathogenic fungal isolates are mentioned the following tree shows only one isolate (fungi01??). If this strain is 100% related to Fusarium oxysporum strain KG_26 why does it branch so distantly?

Accession numbers must be provided and give in the trees. Compare the bacterial isolates to type strains only (BLAST option) and show only closely related type strains in the tree. For type strains of Bacillus see: https://lpsn.dsmz.de/genus/bacillus

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Authors may need to take specific actions to achieve compliance with funder and institutional open access mandates. If your research is supported by a funder that requires immediate open access (e.g. according to Plan S principles) then you should select the gold OA route, and we will direct you to the compliant route where possible. For authors selecting the subscription publication route our standard licensing terms will need to be accepted, including our self-archiving policies. Those standard licensing terms will supersede any other terms that the author or any third party may assert apply to any version of the manuscript.

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Dr. IR. HILDA KARIM, MP UNM <hilda.karim@unm.ac.id>

AOMI-D-21-00198R2 : Your PDF Has Been Built

1 message

Archives of Microbiology (AOMI) <em@editorialmanager.com> Mon, Aug 16, 2021 at 12:40 AM
Reply-To: "Archives of Microbiology (AOMI)" <niranjana.muralimohan@springernature.com>
To: Hilda Karim <hilda.karim@unm.ac.id>

Dear Dr. Karim,

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With kind regards,
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Reply-To: "Archives of Microbiology (AOMI)" <niranjana.muralimohan@springernature.com>
To: Hilda Karim <hilda.karim@unm.ac.id>

Dear Dr. Karim,

We acknowledge, with thanks, receipt of the revised version of your manuscript, "Antagonistic Activity and Characterization of Indigenous Soil Isolates of Bacteria and Fungi Against Onion Wilt Incited by Fusarium sp.", submitted to Archives of Microbiology

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Reply-To: "Archives of Microbiology (AOMI)" <niranjana.muralimohan@springernature.com>
To: Hilda Karim <hilda.karim@unm.ac.id>

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Editor in Chief
Archives of Microbiology

COMMENTS FOR THE AUTHOR:

Editor: as indicated in my last comments accession numbers for 16S rRNA gene sequences and ITS sequences must be provided and the isolates O n l y compared to type strains. Strain X2 for example in not the type strain of B. subtilis (see https://lpsn.dsmz.de/species/bacillus-subtilis) and the number EG 1303 is not the one for B. velezensis (see https://lpsn.dsmz.de/species/bacillus-velezensis) just to give two examples. Delete all non-type strain entries- Note also that for a proper assignment the 16S rRNA gene sequences should be at least 800 nucleotides long and in case the sequences are shorter indicate in the text that the names of species are tentative.

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Archives of Microbiology (AOMI) <em@editorialmanager.com> Sun, Oct 10, 2021 at 3:21 PM
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To: Hilda Karim <hilda.karim@unm.ac.id>

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Archives of Microbiology (AOMI) <em@editorialmanager.com> Thu, Oct 14, 2021 at 2:02 PM
Reply-To: "Archives of Microbiology (AOMI)" <niranjana.muralimohan@springernature.com>
To: Hilda Karim <hilda.karim@unm.ac.id>

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Dr. IR. HILDA KARIM, MP UNM <hilda.karim@unm.ac.id> Fri, Oct 29, 2021 at 7:22 AM
To: "Archives of Microbiology (AOMI)" <niranjana.muralimohan@springernature.com>

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eproofing@springernature.com <eproofing@springernature.com> Thu, Dec 23, 2021 at 2:06 AM
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To: hilda.karim@unm.ac.id

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Article Title : Antagonistic activity and characterization of indigenous soil isolates of bacteria and fungi against onion wilt incited by *Fusarium* sp.
DOI : 10.1007/s00203-021-02663-2
AOMI-D-21-00198R3

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Journal: Archives of Microbiology.
DOI : 10.1007/s00203-021-02663-2
Title : Antagonistic activity and characterization of indigenous soil isolates of bacteria and fungi against onion wilt incited by *Fusarium* sp.

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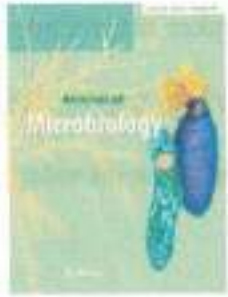

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ORIGINAL PAPER

Antagonistic activity and characterization of indigenous soil isolates of bacteria and fungi against onion wilt incited by *Fusarium* sp.

Hilda Karim[1] · Andi Asmawati Azis[1] · Oslan Jumadi[1]

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Abstract

Tuber rot disease due to phytopathogen *Fusarium oxysporum* f. sp. cepae (Foc) infection is one of the main factors causing the decreasing global onions production. This study aims to find bacteria and fungi candidates with Foc antagonistic activity through in vitro tests using dual culture techniques. A total of three bacterial isolates and three fungal isolates isolated from the rhizosphere of healthy onion plants showed the ability to inhibit *Fusarium oxysporum* growth. LC648364 isolate had an average inhibitory capability of 65.93%. At the same time, LC648367 and LC648368 fungal isolates can inhibit the growth of *F. oxysporum* by as much as 74.82% and 67.76%, respectively. Molecular analysis based on 16S rRNA markers showed three isolates belonging to the *Bacillus*. The LC648364 isolates are closely related to species *Bacillus* sp. strain LLB-17, LC648365 is closely related to *B. subtilis* strain S11 and LC648366 is closely related to *B. cereus* strain EM6. For the fungi, based on internal transcribed spacer (ITS) gene markers, there are three isolates. The LC648367 isolate is closely related to *Aspergillus tubingensis*, LC648368 is closely related to *Trichoderma asperellum* and LC648369 is closely related to *Issatchenkia orientalis*. This study can be used to develop indigenous microbial consortiums as biological control agents for phytopathogenic fungi *Fusarium* tuber rot on onion.

Keywords *Fusarium* · *Bacillus* · *Aspergillus tubingensis* · *Trichoderma asperellum* · Onion disease

Introduction

Onions (*Allium cepa* var *ascalonicum* L) are one of the world's main commodities with production reaching 96.77 million tons per year. However, productivity fluctuates almost every year. In Indonesia, several regions show fluctuations in the amount of production each year (BPS 2018). Various factors, especially unfavorable environments, such as drought, salinity, climate, nutritional imbalance and plant diseases, are the main obstacles in the production of onions (Abdelrahman et al. 2016). Among a number of diseases caused by pathogens, *Fusarium* tuber rot or wilt disease caused by *Fusarium oxysporum* f. sp. *cepae* (*Foc*) is the most damaging and a serious threat to onion production worldwide (Abdelrahman et al. 2016; Chand et al. 2017; Kalman et al. 2020). Symptoms caused by *Foc* include plants wilting rapidly, newly formed leaves curling and turning yellow, plants almost collapsing, white fungi colonies appearing at the base of the rotting layered bulb (Brayford 1996; Taylor et al. 2016). *Foc* is a pathogenic fungus that can infect a very wide range of plants as the hosts (Summerell et al. 2011; Armitage et al. 2018). This fungus can form chlamydospores so that it can last a long time in the soil (Brayford 1996; Cremer 2000; Kalman et al. 2020).

Management of *Fusarium* tuber rot or wilt disease can be focused on integrating different prevention methods, including the use of mixed crops, crop rotation systems, use of pathogen-resistant cultivars, use of chemical fungicides and the use of biological agents (Mc Govern 2015; Gupta et al. 2020). In practice, the use of synthetic fungicides by onion farmers has not been fully effective because of the residue left on crops, environmental pollution, and killing other organisms that are not targeted. Moreover, the continuous use of synthetic fungicides can lead to the emergence of resistant pathogenic populations (Mehnaz et al. 2013; Fournier et al. 2020; Tleuova et al. 2020).

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✉ Hilda Karim
hilda.karim@unm.ac.id

[1] Department of Biology, Faculty of Mathematics and Natural Science, Universitas Negeri Makassar, Jl. Daeng Tata Raya, Makassar 90222, Indonesia

Biological control using microbes that are antagonistic to pathogenic fungi is the right alternative because it does not have a negative impact on the environment (Lecomte et al. 2016; Jamil et al. 2020; Kalman et al. 2020).

Utilization of microbes as biological control agents ideally uses the potential of indigenous natural enemies with the hope that these microbes will work more effectively and are supported by appropriate environmental factors, do not cause changes in ecosystems, and are cheaper to formulate (Kalman et al. 2020). Therefore, the diversity of microbes from the root area and their propagation followed by their release back into the rhizosphere is a conservation measure that will provide promising prospects for biological disease control (Raaijmakers et al. 2009; Kandel et al. 2017.).

In the last decade, research on biocontrol and microbial metabolite products for pest and pathogen control has intensified (Jangir et al. 2018). Generally, this microbial group belongs to the genera *Bacillus*, *Pseudomonas*, *Streptomyces* and *Trichoderma* (Ramyabharathi et al. 2020; Jangir et al. 2018; Kalman et al. 2020). This group of microbes is able to act as a biocontrol agent in reducing pathogenicity through a number of mechanisms, such as antibiotic production, root colonization, induction of systemic resistance systems in the host, production of extracellular cell wall breakdown enzymes and formation of resistant spores (Ongena and Jacques 2008; Beneduzi et al. 2012). A number of studies have reported that the application of microbes, both bacteria and fungus, is effective in suppressing the growth of *Fusarium* pathogens, including using *Bacillus* sp. (Jangir et al., 2018), *Pseudomonas aeruginosa* DRB1 and *Trichoderma harzianum* CBF2 antagonist *Foc* Tropical Race 4 (Foc-TR4) (Wong et al. 2019). Further, Khan et al. (2020a) report that secondary metabolites produced by *Trichoderma* spp., such as harzianolides, peptaibols, gliotoxin, trichokonin, and several volatile compounds, have functioned as antifungal, stimulating plant growth and increasing resistance to pathogens.

This study aims to evaluate the antagonistic activity of indigenous microbial strains isolated from onion growing areas in Enrekang Regency, South Sulawesi, Indonesia. In vitro analysis was conducted using *Fusarium* isolates which were isolated from onion plants showing symptoms of tuber rot. All isolates that showed potential in inhibiting the growth of the *F. oxysporum* pathogen were identified molecularly using specific primers for the 16S rRNA gene and the nuclear ribosomal internal transcribed spacer (ITS) region using specific primers ITS1 and ITS4. The isolates obtained are expected to be able to contribute to the inventory of genetic diversity in the region, with possible future applications for the control of *Fusarium* pathogens in plants, especially in onion.

Materials and methods

Isolation of *Fusarium* tuber rot

Fusarium tuber rot were isolated from onion rhizosphere soil samples which showed tuber rot symptoms in the onion cultivation area in Enrekang regency. The isolation was carried out based on techniques described in Miao et al. (2016) using potato dextrose agar medium (PDA, Merck) and incubated for 5 to 7 days at 25 ± 2 °C. Isolates were determined based on their microscopic morphological characteristics. Microscopic observation using the fungal slide culture method was used to observe the hyphae growth under a microscope (Harris 1986).

Fusarium-antagonist bacterial and fungal isolations

Fusarium tuber rot-antagonist bacteria and fungi were both isolated from healthy rhizosphere areas of onion plants by the serial dilution method. The rhizosphere bacteria isolation technique is based on Jangir et al. (2018) with modifications. The dilution results were grown in Nutrient Agar (Merck) medium at 30 °C for 48 h, whereas the fungal isolation technique is based on Miao et al. (2016) by growing the results of 10^{-3} dilution in PDA medium at 25 ± 2 °C for 5–7 days. Next, the bacterial and fungal isolates were purified in the same medium and maintained at 4 °C. Further preservation used glycerol stock (25%) and was stored at a temperature of –80 °C. All the isolates which were successfully identified were characterized based on morphological, biochemical parameters and molecular identification.

In *vitro* tests of *Fusarium*-antagonist isolates

Fusarium tuber rot-antagonist microbes screening was conducted using the dual culture method (Skidmore and Dickinson 1976). A culture block with a diameter of 8 mm from antagonist isolate and another from *Fusarium* isolate was placed opposite to each other in a PDA medium, 3 cm away from the edge of the Petri dish. As a control, a single *Fusarium* culture disk was placed alone in another Petri dish without the antagonist isolate. The Petri dish was then incubated at a temperature of 25 ± 2 °C for 5–7 days. Observation of growth inhibition (GI) was done every two days. Observation was terminated when the colony in the control reached maximum growth. The percentage of GI was calculated using the formula:

$$\mathrm{GI} = \left[(R1 - R2)/R1\right] \times 100\%$$

In which, R1 is the radius of radial growth to the opposite direction in the control Petri dish and R2 is the radius

of radial growth in the treated petri dish. The tests were done three times to acquire the mean of the inhibition zone for each isolate.

The GI data were analyzed using one-way ANOVA with values $\alpha = 0.05$ and $n = 3$.

DNA extraction and PCR amplification

Isolation of fungal genomic DNA was carried out using the Plant Genomic DNA Mini Kit (Geneaid) in accordance to the manufacturer's standard protocol. The nuclear ribosomal internal transcribed spacer (ITS) region was amplified using a universal primer set (ITS 1: 5'-TCC GTA GGT GAA CCT GCG G-3' and ITS 4: 5'-TCC TCC GCT TAT TGA TAT GC-3') (White et al. 1990). The PCR reaction consisted of 1 µl DNA template (100 ng/µl), 5 µl NZYTaq II 2× Green Master Mix, 0.25 µl ITS 1 primer (10 pmol/µl), 0.25 µlITS 4 primer (10 pmol/ µl), 3.5 µl dH_2O so that the total reagent volume was 10 µl. PCR was run with a thermal cycler for pre-denaturation at 95 °C for 5 min, for denaturation at 95 °C for 30 s, for annealing at 52 °C for 30 s, for extension at 72 °C for 30 s, the reaction being repeated for 35 cycles, and post-PCR at 72 °C for 5 min.

The total bacterial genome was isolated using Presto™ Mini gDNA Kit (Geneaid). According to the manufacturer's protocol, the 16S rRNA gene amplification was performed using specific primer pairs (63 F: 5'-CAG GCC TAA CAC ATG CAA GTC-3' and 1387 R: 5'-GGG CGG WTG GTA CAA GGC-3'). The mix composition and PCR program were made the same as the ITS gene amplification procedure in fungi. PCR products were analyzed using 1% agarose gel in 1 × TAE buffer. The gel was then electrophoresed at a voltage of 100 V for 28 min and stained using ethidium bromide staining. The visualization of the electrophoresis results was carried out using a UV-Transilluminator. PT Bioneer Indonesia conducted the PCR product sequencing.

Construction of phylogenetic trees

The 16S and ITS sequences for all bacteria and fungi were constructed to determine their evolutionary relationships based on phylogenetic analysis. Multiple sequence alignments were performed using Bio Edit's CLUSTAL W program. Phylogenetic tree construction was carried out using the neighbor-joining method from the MEGA version 10.0 program. Each clade obtained was then determined using bootstrap analysis with 1000 replications and then Kimura's two-parameter model was used. The nucleotide sequences in this study have been deposited in the DNA databank of Japan (DDBJ, URL: http://www.ddbj.nig.ac.jp/) under Accession No. LC648364 through LC648369.

Results

Isolation and identification of fungal pathogens

The isolates suspected as *Fusarium* were isolated from the rhizosphere of the onion plants which showed tuber rot symptoms. Observation of the morphology of fungal isolates was based on the characteristics described which include parameters of color, colony, texture, and air hyphae. All parameters showed characteristics matching *Fusarium oxysporum*. Furthermore, the observations showed that on the upper surface, the mycelium was purple, while the lower surface was white. In addition, microscopic characteristics, such as macroconidia, microconidia and chlamydospores, were successfully observed under a microscope at magnification of 400× (Fig. 1) with the appearance of a colorless round microconidium, and a crescent-shaped macroconidium that was colorless and had 3–5 septa while chlamydospores are single-celled.

Further identification was carried out by molecular method and based on the results of sequencing analysis has been confirmed that the pathogen isolated is *Fusarium*

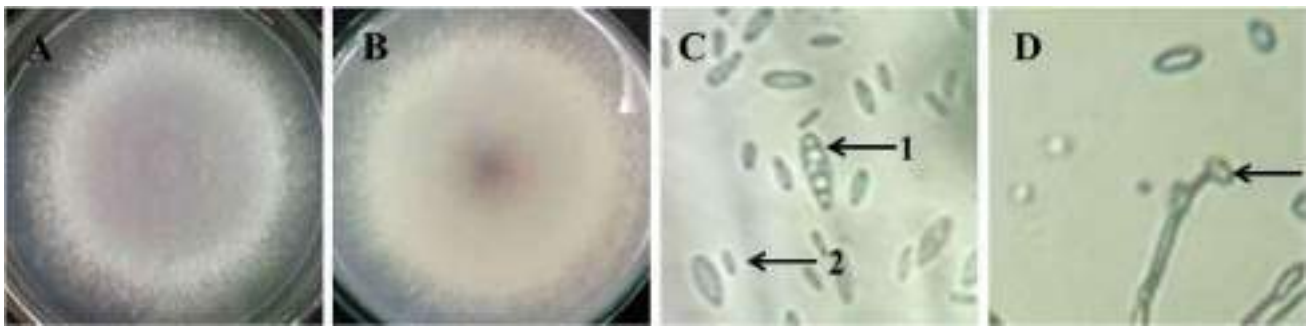

Fig. 1 Morphological and microscopic characteristics of 7-day-old *F. oxysporum* isolated from the rhizosphere of onion plants. **a** The upper surface of the colony; **b** the basal surface of the colony; **c** microconidia (1) and macroconidia (2) microscope observation at × 400 magnification; **d** Chlamydospores (× 400 magnification)

oxysporum which has 96.6 % similarity identity to *Fusarium oxysporum* strain KG_26 (Fig. 2).

In vitro tests of antagonist microbes isolates

A total of three fungi isolates and three bacterial isolates were isolated from rhizosphere soil samples. From the results of initial in vitro testing against *Fusarium oxysporum*, three isolates of fungi and three isolates of bacteria showed inhibitory activity reaching 50% against *F. oxysporum* mycelium growth. In the second test, the percentage of inhibitory potential was measured against the growth of *F. oxysporum* grown in dual culture with the test isolate. Tests were carried out three times to determine the average inhibition. From the results of analysis of variance (ANOVA) on all isolates, it was found that almost all tested microbes had inhibitory activity above 50%. Isolate LC648367 showed the highest inhibitory activity of *F. oxysporum* with an average of 74.82%, whereas the inhibitory activity of fungi against *F. oxysporum* was discovered to have a higher growth rate than the bacterial activity. The lowest inhibitory activity was shown by the LC648369 fungal isolate with an inhibition value of 41.12%. All data are presented in Table 1.

The capability of bacteria and fungi to inhibit *F. oxysporum* growth seems to be correlated with different growth rates. Visually, the growth of fungi in colonizing the growth medium was seen to be faster than bacteria (Fig. 3). From all isolates of bacteria and fungi, LC648364 and LC648367 can be considered to have the best potential as antagonists in suppressing *F. oxysporum* growth *in vitro*.

Further analysis was carried out to determine the capability of the isolates to suppress the growth of *F. oxysporum* mycelium. Microscopic observations were carried out on the outer part of the *F. oxysporum* mycelium growth zone. From the observations, it was found that hyphae damage occurred which is assumed to have been due to the activity of the antifungal compounds produced. In contrast to the control, hyphae in *F.oxysporum* were seen to undergo fragmentation (Fig. 4).

Molecular identification of bacterial and fungal isolates

The three bacterial isolates were analyzed molecularly to identify species based on their evolutionary relationships. The phylogenetic tree construction from the alignment results of 16S gene amplification products with the GenBank database showed that all *F. oxysporum* antagonist bacteria were related to the genus *Bacillus* and all of them belong to different species evolutionarily (Fig. 5). There are three isolates belonging to the genus *Bacillus*. The LC648364 isolate is closely related to species *Bacillus* sp. strain LLB-17 with a gene similarity rate of 96%, LC648365 is closely related to *B. subtilis* strain S11 with the 97 % similarity identity and LC648366 showed a closer relationship with species *B. cereus* strain EM6 with level of 97 %.

The results of the BLAST analysis were different for each fungal sample. From the results of phylogenetic constructs, it was found that the LC648367, LC648368, and LC648369 samples were of different species. The LC648367 isolate is closely related to *Aspergillus tubingensis* with a similarity rate of 99.6%, LC648368 is closely related to *Trichoderma asperellum* with a similarity 99.2% and LC648369 is closely

Table 1 Percentage of inhibition of *F. oxysporum* growth by bacteria and fungi from the rhizosphere of onion plants on PDA medium

Isolate code access number	Inhibition (%)				
	Day 1	Day 3	Day 5	Day 7	Mean
LC648364	54.84	65.39	70.61	72.87	65.93[b]
LC648365	49.34	59.67	61.04	65.38	58.86[c]
LC648366	49.30	51.70	58.58	65.53	56.28[c]
LC648367	68.35	73.77	77.65	79.51	74.82[a]
LC648368	62.12	65.32	70.79	72.79	67.76[b]
LC648369	25.60	41.00	46.81	51.08	41.12[d]

Numbers followed by the same notation do not show a significant difference based on analysis of variance with values $\alpha = 0.05$ and $n = 3$

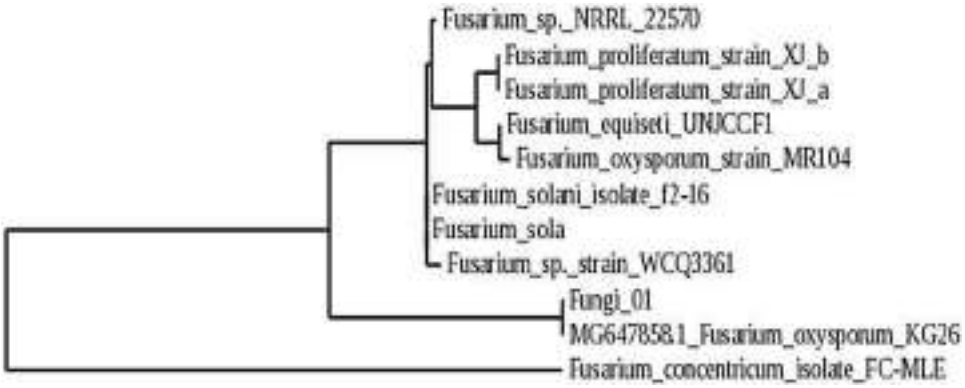

Fig. 2 Phylogenetic tree of the pathogenic fungus (*Fusarium oxysporum*)

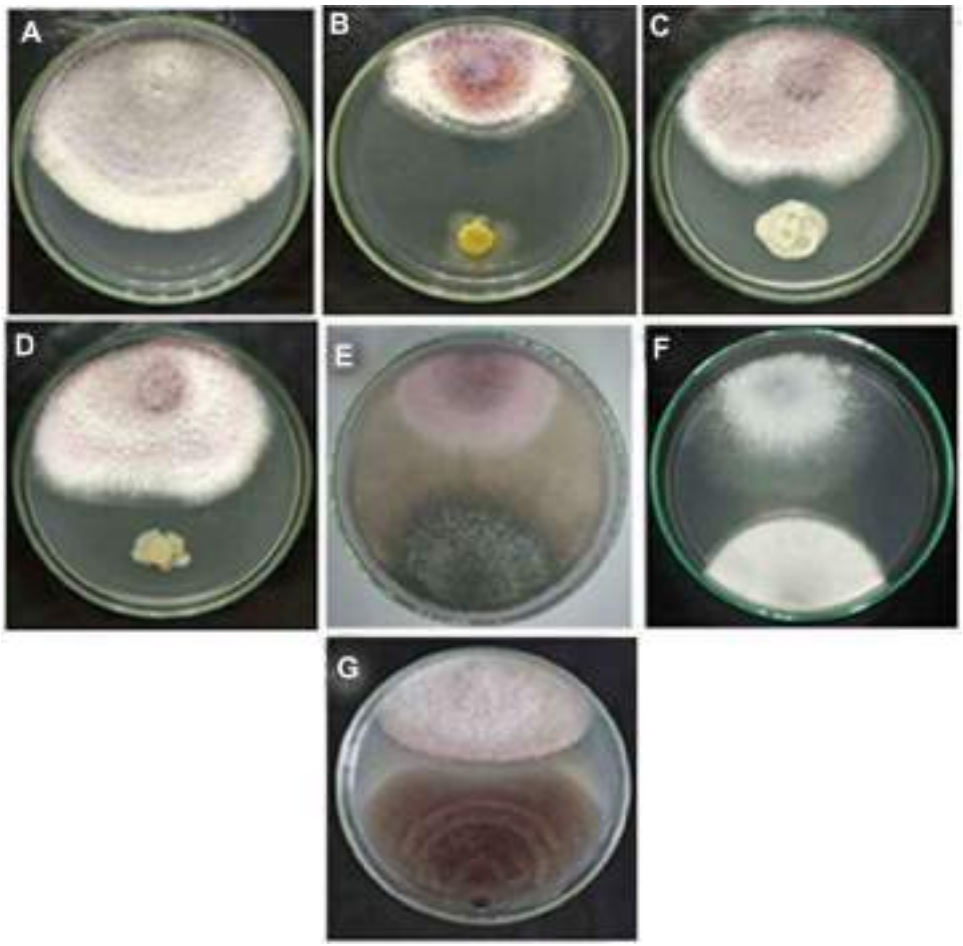

Fig. 3 Inhibitory activity of bacteria and fungi against the pathogen *Fusarium oxysporum*. Origin of onion rhizosphere in PDA medium on day 7. **a** Control; **b** bacterial LC648364, **c** bacteria LC648365, **d** bacteria LC648366, **e** fungus LC648367, **f** fungus LC648368, **g** fungus LC648369

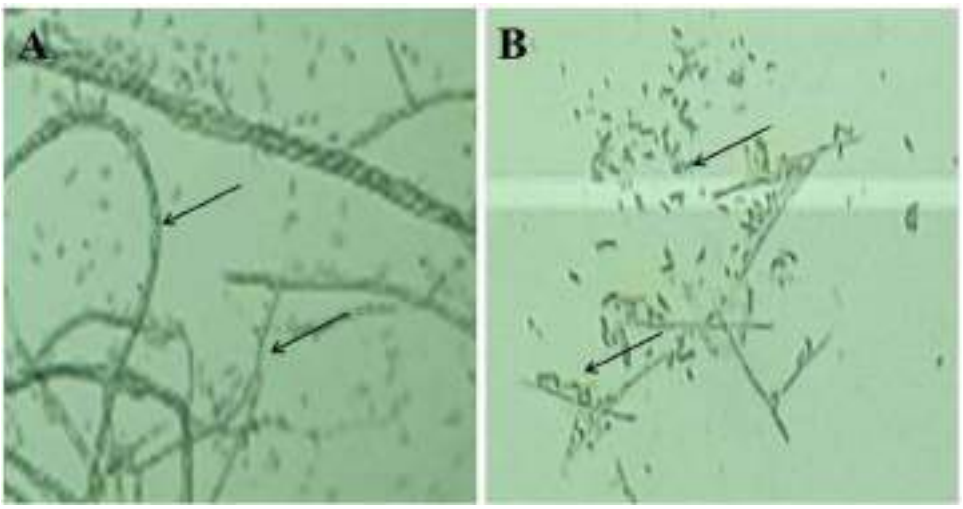

Fig. 4 Comparison of growth conditions for *Fusarium* hyphae at day 7 in PDA medium. **a** Hyphae condition in control; **b** the condition of the *F. oxysporum* hyphae tested. Arrows show hyphae in both treatments. Microscope observation at × 100 magnification

related to *Issatchenkia orientalis* with a similarity level of 99.2% (Fig. 6).

Discussion

Fusarium oxysporum f. sp. cepae (Foc) is one of the most severe diseases (Cramer 2000; Wang et al. 2019) which affects all phases of plant development at pre- and

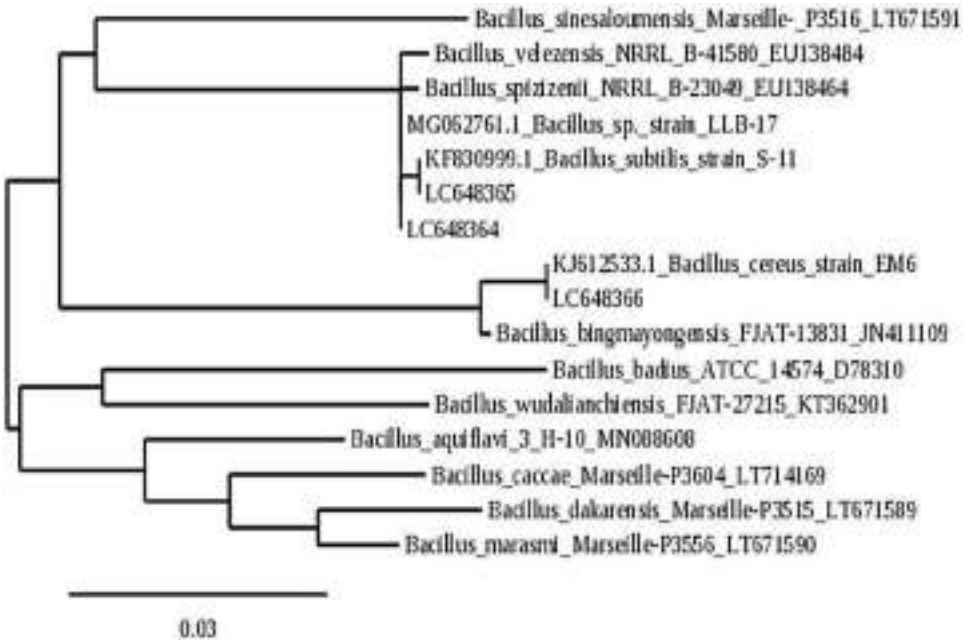

Fig. 5 Phylogenetic trees constructed using neighbor-joining method at 1000 times bootstrap using Kimura's two-parameter model. The results showed the position of the isolates and related *Bacillus* species based on the 16S rRNA gene sequence

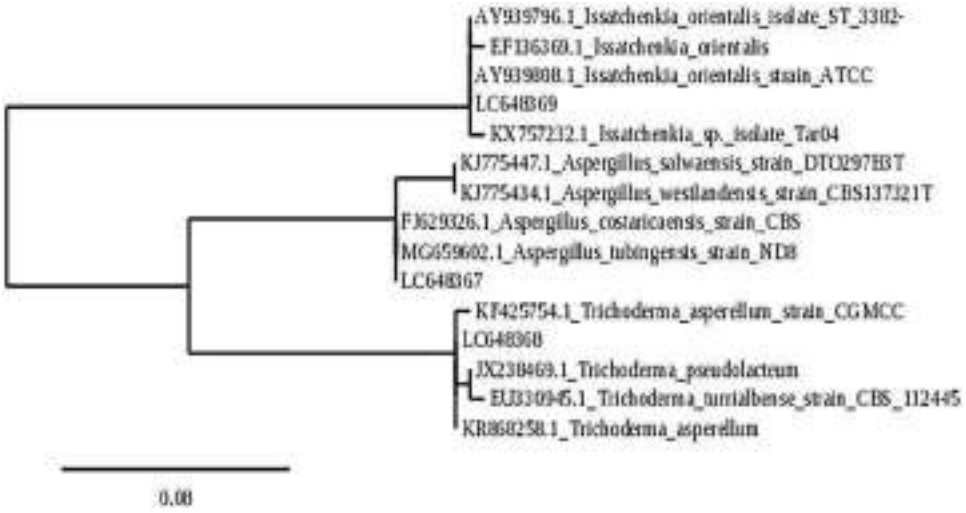

Fig. 6 Phylogenetic trees constructed by neighbor-joining method at 1000 times bootstrap using Kimura's two-parameter model. The results show the position of the related isolates based on the ITS gene sequence

post-harvest stages from damping off and delayed seedling emergence to bulb rot (Galeano et al. 2014; Wang et al. 2019). Warm temperatures (28–32 °C optimum) can induce infection and climate change will predict increase it (Cramer 2000). *Fusarium oxysporum* was white floccose mycelia. Some isolates produced dark violet pigment in the agar (this character was observed for 1H (less virulent) and 13 M (avirulent) isolates). Microconidia were formed in false heads on short monophialides. Thin-walled macroconidia were approximately straight and slightly tapered at the ends (Ghanbarzadeh et al. 2014).

Fusarium wilt or tuber rot in onion plants due to *Foc* infection causes enormous losses annually to global agriculture. It can survive in the soil for many years because like many other *Fusarium* species, Foc produces resilient, long-lived chlamydospores (Brayford 1996; Cramer 2000; Armitage et al. 2018) so that the treatment using synthetic fungicide is not entirely effective (Fig. 1d) which is resistant to extreme environmental stress (Gupta et al. 2020). This is also considered uneconomical and a source of environmental pollution, so that alternative pathogen control with antagonistic microbes (biocontrol) is more promising and sustainable (Abbey et al. 2018; Fournier et al. 2020; Tleuova et al. 2020). In this study, a number of indigenous microbes showed antagonistic activity against *F. oxysporum* growth in vitro (Fig. 3).

Three bacterial isolates and three fungal isolates showed inhibitory activity of *F. oxysporum* mycelium growth. LC648364 (*Bacillus* sp. strain LLB-17) was significantly ($p < 0.05$) able to inhibit the growth of radial mycelium *F.*

oxysporum when compared to controls with inhibition percentages of 65.93%. When compared with bacterial isolates, the *F. oxysporum* inhibition capability of fungal isolates was much higher. Although the rate of bacterial cell proliferation is faster, the expansion capability of fungal hyphae in the test medium is much faster, so this is thought to be correlated with its antagonistic activity in suppressing the growth of *F. oxysporum* mycelium. Kalman et al. (2020) reported that the *Foc* growth rate reached 0.83–0.87 cm/day. The activity of rhizosphere bacteria in suppressing pathogen growth can be through a number of mechanisms of action, including synthesis of hydrolytic enzymes, such as chitinase, β-1,3-glucanase, and proteases, that can lyse pathogenic fungal cells (Lopez et al. 2020), (2) competition for nutrition and colonization of the rhizosphere niche (Rana et al. 2019), and (3) production of siderophores and antibiotics (Kumar et al. 2018; Panchami et al. 2020). But generally, the mechanism of inhibitory action by bacteria occurs due to the synthesis of a number of bioactive compounds, particularly antibiotics (Jangir et al. 2018; Panchami et al. 2020; Ramyabharathi et al. 2020).

From the results of molecular analysis using 16S rRNA markers, it was found that the three bacterial isolates were included in the genus *Bacillus* (Fig. 5). The isolate with the highest inhibitory capability, LC648364 has evolutionary similarity to *Bacillus* sp. strain LLB-17. The interesting thing is that isolate LC648364 has a percent identity of 94% when compared to *Bacillus* sp. strain LLB-17, where both share the same branch. A number of studies have reported the capability of *Bacillus* to suppress the growth of various phytopathogenic fungi so that it is commonly used as a biocontrol agent in both monoculture and consortium forms (Khan et al. 2017). Cucu et al. (2019) reported that *B. subtilis* QST713 was able to suppress the growth of *F. oxysporum* f. sp. *lycopersici* (*Fol*). *Bacillus* sp. B44 Anatagonist *Fol* (Jangir et al. 2018). In contrast to bacteria, of the three antagonistic fungi isolates tested with the dual culture method, isolates LC648367 and LC648368 showed significant inhibitory activity while isolate LC648369 was the lowest among the three (Table 1) with an inhibitory percentage of 41%. The results of molecular analysis showed that the LC648367 isolate had high homology (99.4% –100% similarity) (Gupta et al. 2020) with *Aspergillus tubingensis* strain ND8, whereas LC648368 and LC648369 are identical to *Trichoderma asperellum* strains CHI3 and *Issatchenkia orientalis*.

The application of fungi in controlling the growth of the *F.oxysporum* pathogen is not only related to its high proliferation capability so that it is able to colonize the environment quickly, especially habitats exposed to pathogens (rhizosphere, phyllosphere, and plant organs) but is also related to its capability to produce bioactive compounds (Ghorbanpour et al. 2018). A number of previous studies have reported that *A. tubingensis* has antifungal activity. Zhao et al. (2018) reported that *A. tubingensis* QF05 was able to inhibit the activity of the pathogenic fungus *Botrytis cinerea* in tomato plants, whereas Kriaa et al. (2015) reported that the activity of glucose oxidase (β-D-glucose: oxygen-oxidoreductase EC 1.1.3.4) which was partially purified from *A. tubingensis* CTM 507 effectively inhibited *F. solani*. This enzyme activity causes the mycelium to undergo lysis, cytoplasmic vacuolization, premature formation of chlamydospores, and mycelium induction through anastomosis between hyphal filaments.

The inhibitory activity of *F. oxysporum* by the fungus LC648368 with a percentage of 41.12% was strong. The results of molecular analysis showed that LC648368 had an evolutionary relationship with *T. asperellum* with a similarity percentage reaching 99.2% with *T. asperellum* strain CHI13. The mechanism of inhibitory action by *Trichoderma* can be either direct contact or the result of diffusion of the compound being excreted into the environment. *Trichoderma* species have antagonistic activity which are production of anti-microbial metabolites, faster physiological conformation, spatial and nutrient competition, mycoparasitism, and antibiosis by enzymes and secondary metabolites (Verma et al. 2007). *Trichoderma* is one of the fungi that has the capability to produce a number of metabolites that can inhibit or kill pathogenic fungi, so it is the most common biocontrol agent (Ghorbanpour et al. 2018). A number of bioactive compounds with the antifungal activity of *Trichoderma* have been reported, such as 3-octanone and 1-octen-3-ol, which are both fungistatic and strong fungicides (Okkull et al. 2003), 6-pentyl-2H-pyran-2-one produced by *T. koningii*, *T. harzianum*, *T. virens*, and *T. viride* (Worsatit et al. 1994) and sesquiterpenes from *T. harzianum* (Lee et al. 2016).

De la cruz-Quiroz et al. (2018) reported that there are two mechanisms to inhibit the activity of *Phytophthora capsica* and *Colletotrichum gloeosporioides* by *Trichoderma*, namely the production of antibiotic compounds, which work during the growth of *Trichoderma* hyphae to touch the phytopathogenic biomass, and the second is the mycoparasitic mechanism, which works when these organisms come into contact. Furthermore, Das et al. (2019) reported that *T. asperellum* was able to effectively inhibit the growth of *Ralstonia solani* and *Phytophthora capsica* through mycelium colonization of pathogens. *T. asperellum* was also reported to be able to suppress the growth *of F. oxysporum* f. sp. cucumerinum (May et al. 2019). Cotxarrera et al. (2002) also reported that *T. asperellum* was able to effectively inhibit the growth of *Fusarium oxysporum* f. sp. *lycopersici* by antibiosis, mycoparasitism and competition for nutrients in wilt. In addition, Khan et al. (2020b) reported that the inhibition of pathogenic fungi growth by *Trichoderma* spp. includes interactions between

secondary metabolites and hydrolytic enzymes can induce expansion of cell death, competition for nutrients, and inhibition of enzymes that play a role in the synthesis of the cell wall of pathogenic fungi.

From this research, all tested isolates have great potential to be applied as a field biocontrol to suppress *F. oxysporum*. However, the capability for antifungal activity by both bacteria and fungi can be further optimized through bioformulation in the form of a consortium. A large number of studies have stated that the application of fungi and a number of bacteria, especially *Bacillus*, are able to inhibit or even kill the growth of phytopathogens through a number of mechanisms (Cucu et al. 2019; Karuppiah et al. 2019; Jangir et al. 2018). Furthermore, Wong et al. (2019) stated that a BCA consortium (biological control agents) is more effective in controlling plant pathogens than single strains due to the involvement of various modes of action of antagonists in suppressing phytopathogens. Apart from acting as a biocontrol agent against phytopathogens, the application of fungi and bacteria as biocontrol agents is also correlated with supporting plant growth through the mechanism of action of providing metabolites synthesized by bacteria, for example phytohormones, or facilitating the absorption of certain nutrients from the environment (Beneduzi et al. 2012; Jangir et al. 2018). However, further testing is still needed to obtain a more comprehensive understanding of all isolates obtained.

Conclusion

A total of three bacterial isolates and three fungal isolates isolated from the rhizosphere of healthy onion plants showed the ability to inhibit *Fusarium oxysporum* growth. Based on the molecular study, LC648364 isolates are closely related to species *Bacillus* sp. strain LLB-17, LC648365 is closely related to *B. subtilis* strain S11, LC648366 is closely related to *B. cereus* strain EM6, LC648367 is closely related to *Aspergillus tubingensis*, LC648368 is closely related to *Trichoderma asperellum* and LC648369 is closely related to *Issatchenkia orientalis*. The study shows that LC648364 and LC648367 can be considered to have the best potential as antagonists in suppressing *F. oxysporum* growth. The microbial consortium used in this study could be developed as a biological control agent for *F. oxysporum* on onion.

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Declarations

Conflict of interest The authors declare that there is no conflict of interest.

Ethical statement Hereby, I Dr. Hilda Karim consciously assure that for the manuscript Antagonistic Activity and Characterization of Indigenous Soil Isolates of Bacteria and Fungi Against Onion Wilt Incited by *Fusarium* sp. the following is fulfilled: (1) This material is the authors' own original work, which has not been previously published elsewhere. (2) The paper is not currently being considered for publication elsewhere. (3) The paper reflects the authors' own research and analysis in a truthful and complete manner. (4) The paper properly credits the meaningful contributions of co-authors and co-researchers. (5) The results are appropriately placed in the context of prior and existing research. (6) All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such using quotation marks and giving proper reference. (7) All authors have been personally and actively involved in substantial work leading to the paper and will take public responsibility for its content. I agree with the above statements and declare that this submission follows the policies of Solid-State Ionics as outlined in the Guide for Authors and in the Ethical Statement.

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