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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Research Article

Exploration of Cellulolytic Microbial Consortium as Biodecomposer of Coffee Fruit Skin Waste

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Abstract

Background and Objective: Coffee fruit skin waste contains high cellulose leading to difficulty in degrading them naturally which finally creates environmental pollution. This research is aimed at finding out the compatible cellulolytic microbe in degrading cellulose content of coffee fruit skin waste. **Materials and Methods:** Media Carboxymethyl Cellulose (CMC) was used to select cellulolytic fungi. These fungi were tested through a consortium test on media to check out whether the microbial consortium was compatible or not. Furthermore, the ability of microbial consortium in degrading lignocellulose of coffee fruit skin waste was examined. **Results:** It was found that there were five singular isolates and seven consortium isolates able to degrade cellulose in variative cellulolytic index ranging from 0.43-2.75. In the cellulolytic compatibility test of the seven consortium isolates, there were two compatible consortium isolates with a CCI amount above 1, meanwhile, the consortium test result which was inoculated in PDA media, demonstrated the presence of six consortium isolates of non-synergistic due to the forming of inhibition zone in each isolate. A positive interaction happened between *Trichoderma asperellum* and *Aspergillus japonicus* (TA/AJ). Likewise, at the cellulolytic degradation capability test, consortium TA/AJ can degrade cellulose faster compared to using one isolate. **Conclusion:** The consortium of TA/AJ was the best and proved to be compatible in degrading lignocellulolytic of coffee fruit skin waste.

Key words: Cellulose, clear zone, consortium, cellulolytic, degradation, microbe

Citation: Wisdawati, E., Nildayanti, A. Mursalat, I. Fauliah and H. Aprelia, 2025. Exploration of cellulolytic microbial consortium as biodecomposer of coffee fruit skin waste. Pak. J. Biol. Sci., 28: 302-307.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Coffee (*Coffea* sp.) is one of the crop plantations that holds a significant role in the Indonesian economy. It is an export commodity and has become a source of foreign exchange besides oil and gas. Nowadays, a coffee plantation in Indonesia is ranked 4th as the largest-producing country after Brazil, Vietnam and Colombia¹. Proven through production data, exports and the size of the coffee area. In 2022, production reached 774.960.000 ton, including Robusta coffee at 83% and Arabica at 17%, with a total area of 1.265.930 ha.

Fertilizers should be given to increase coffee production by utilizing environmental residues, such as coffee fruit skin waste. Coffee processing generates 40 to 55% of coffee fruit skin waste; in other words, 1 ton of coffee can be produced from 2 ton². Those are useful for soil fertility since they contain potential nutrients, C level (45.3%), N (2.98%), P (0.18%) and K (2.26%)³. Changing them into fertilizer will add value, but leaving them abandoned might create environmental pollution. Therefore, the solution is to make organic fertilizer from coffee fruit skin waste. Decomposition of coffee fruit skin waste takes longer due to the contents of cellulose 39%, hemicellulose 12%, lignin 23% and minerals⁴. Fermentation is done using decomposer microorganisms to fasten decomposition. Biodecomposer can degrade cellulose, hemicellulose and lignin of plants⁵. Additionally, the microorganism degradation process of the coffee fruit skin waste will produce easily absorbable nutrients⁶.

Microorganisms in decomposing coffee fruit skin waste can be executed in a single or consortium. Compared to a single method, consortium tends to boost up the role of microorganisms more as the mix among microorganisms is more likely to speed up the degradation process of complex compound⁷. The microbial consortium is more stable in various ecological and environmental conditions than a single one. Synergistic growth among consortium microbes can combine microorganisms with the requirements of miscellaneous nutrients needed, working procedures and plant characteristics that finally slow down production costs and increase the biodiversity of microorganisms in the plant rhizosphere and phyllosphere. Implementation of synergistic consortium microorganisms will be successful in solving incompatibility among microorganisms, their competition and adaption to habitat^{7,8}.

Hence, this study aims to improve the role of microorganisms in enhancing the decomposition of coffee fruit skin waste by conducting *in vitro* consortium tests. These tests are intended to explore the consortium's ability to degrade cellulose, hemicellulose and lignin in coffee fruit

skin waste, ultimately contributing to more efficient and sustainable waste management practices.

MATERIALS AND METHODS

Study site and duration: This study was conducted from August to November, 2024. The study was carried out in the Pest and Disease Laboratory, Department of Agricultural Production Technology, Pangkep State Polytechnic of Agriculture, Indonesia.

Materials: Potato Dextrose Agar (PDA), Aquadest, Alcohol 70%, Spritus, NaNO₃, K₂HPO₄, KCl, MgSO₄, Pepton, CMC, Agar, KI, Iodin and waterone.

Methods: This research is divided into several steps.

Isolate rejuvenation: Fungal isolates obtained from coffee fruit skin waste in Potato Dextrose Agar (PDA) media. It is treated as growth media during the incubation of fungal isolates within 7 days.

Test of cellulolytic fungi: A fungal isolate test, which has the potential to degrade cellulose, was carried out, discovering a clear zone around the fungi colonies on selective media, CMC. Purified fungal isolates were grown in selective Media Carboxymethyl Cellulose (CMC) and then incubated at room temperature for 3 days. In the next step, grown isolates on CMC media were dripped by iodine dye⁹. The clear zone that forms around colony fungi indicated that fungal isolates could degrade cellulose. Measurement of both the formed clear zone and fungi colony diameter is intended to find the cellulolytic index activity of fungal isolate. Their amount is calculated through the following formula^{10,11}:

$$\text{Index of cellulolytic activity (IC)} = \frac{\text{Diameter of clear zone (DC)} - \text{Colony diameter}}{\text{Colony diameter (DC)}}$$

Criteria of the cellulolytic amount is considered low if index < 1, average if 1 ≤ index ≤ 2 and high if index > 2¹⁰:

Compatible test with the formula: The compatibility index criteria are calculated based on the following¹⁰:

$$CI = \frac{\text{Combined microorganism growth}}{\text{Single microorganism growth}}$$

where, If CI ≤ 1, then the mixture of bacteria is not compatible and CI ≥ 1, then the mixture of bacteria is compatible.

Fungal consortium test *in vitro*: The test was conducted inside a petri dish containing PDA media. Both fungi were inoculated at a distance of 3 cm. If a growth speed difference occurred, the fungi with slower growth should be inoculated earlier; after 2-3 days, the second isolate was inoculated into PDA media. The interaction between those two fungi was observed after 7 days of incubation.

Test of decomposer microbes on skin fruit coffee waste: Decomposer microbes were added to the making process of coffee fruit skin waste fertilizer, then fermented for 30 days and the decreased cellulose contained in coffee fruit skin waste was analyzed.

RESULTS AND DISCUSSION

Isolated microorganisms from coffee fruit skin waste were *Aspergillus fumigatus*, *Aspergillus* sp., *Penicillium* sp. The consortium test was delivered by using *Trichoderma asperellum* and *Aspergillus japonicus* to obtain consortium isolates that can degrade coffee fruit skin waste and boost plant growth. According to Takala³ and van Nguyen *et al.*⁴, coffee fruit skin waste contains C levels (45.3%), N (2.98%), P (0.18%) and K (2.26%). The plant requires those macronutrients to have optimal growth. On the contrary, some of the nutrients remain in the form of organic compounds, for instance, protein, amino acids and other compounds that cannot be directly absorbed by plants. In addition, coffee fruit skin waste also contains cellulose 39%, hemicellulose 12%, lignin 23% and minerals, in which high lignocellulolytic causes absorbance difficulty. Cellulolytic fungi were capable of decomposing coffee fruit skin waste into inorganic compounds and degrading its lignocellulolytic to be easily absorbed by plants¹². Cellulolytic test results for single and consortium microorganisms are indicated in Table 1.

The result demonstrated that all tested microorganisms, including *Aspergillus fumigatus*, *Penicillium* sp., *Aspergillus* sp., *Trichoderma asperellum* and *Aspergillus*

japonicus, were categorized as fungal isolates that able to decompose and degrade CMC media marked by the forming of the clear zone around fungi colony (Fig. 1). The size of the clear zone was the initial condition of the produced cellulose. The bigger the clear zone, the more cellulose could be obtained and enzyme activity would be higher as well¹³. The same action was taken by Rahardiyan and Moko¹³, showing that several potential fungi in decomposing cellulose cover fungi from *Rhizopus* genus, *Aspergillus*, *Mucor*, *Trichoderma* and *Penicillium*.

The amount of cellulolytic index in a single fungus demonstrated low and high categories. The highest was at *Aspergillus fumigatus* isolate with 2.75 (index >2); three fungi isolates had an average index ($1 \leq \text{index} \leq 2$), which were *Penicillium* sp., *Aspergillus* sp. and *Trichoderma asperellum* with 1.14, 1.91 and 1.14, respectively. Meanwhile, *Aspergillus japonicus* with a cellulolytic index of 0.80 included in a low category cellulolytic index (index <1)^{14,15}. The clear zone indicates the ability of microorganisms to degrade cellulose in CMC media¹⁶. It was formed since CMCase cut the bound of β -1,4-glycosidic in CMC media^{17,18}.

In the test of the fungal consortium, the cellulolytic and compatibility test in which seven couples of the fungal consortium were included, they were *Aspergillus fumigatus*/*Trichoderma asperellum* (AF/TA), *Aspergillus fumigatus*/*Aspergillus japonicus* (AF/AJ), *Penicillium* sp./*Trichoderma asperellum* (P/TA), *Penicillium* sp./*Aspergillus japonicus* (P/AJ), *Aspergillus* sp./*Trichoderma asperellum* (A/TA), *Aspergillus* sp./*Aspergillus japonicus* (A/AJ) and *Trichoderma asperellum*/*Aspergillus japonicus* (TA/AJ). The result demonstrated five couples of incompatible fungal consortiums: AF/TA, P/TA, P/AJ, A/TA, A/AJ and two compatible ones, AF/AJ and TA/AJ (Table 1). The fungal consortium is considered to be incompatible if one or both of the two consortium fungi have a cellulolytic compatibility index under 1 and compatible if both consortium fungi have a cellulolytic compatibility index above 1, which means they

Table 1: Cellulolytic index and cellulolytic compatibility test on single and consortium microorganisms

Isolates	Cellulolytic index (CI)		Cellulolytic compatibility index (CCI)		Compatible/incompatible (C/IC)
<i>Aspergillus fumigatus</i> (AF)	2.75		-		-
<i>Penicillium</i> sp. (P)	1.14		-		-
<i>Aspergillus</i> sp. (A)	1.91		-		-
<i>Trichoderma asperellum</i> (TA)	1.14		-		-
<i>Aspergillus japonicus</i> (AJ)	0.80		-		-
<i>Aspergillus fumigatus</i> / <i>Trichoderma asperellum</i> (AF/TA)	0.64	2.25	0.23	1.97	IC
<i>Aspergillus fumigatus</i> / <i>Aspergillus japonicus</i> (AF/AJ)	2.75	2.64	1.00	3.30	C
<i>Penicillium</i> sp./ <i>Trichoderma asperellum</i> (P/TA)	1.38	0.81	1.20	0.71	IC
<i>Penicillium</i> sp./ <i>Aspergillus japonicus</i> (P/AJ)	0.55	2.11	0.48	2.63	IC
<i>Aspergillus</i> sp./ <i>Trichoderma asperellum</i> (A/TA)	3.5	0.43	1.83	0.38	IC
<i>Aspergillus</i> sp./ <i>Aspergillus japonicus</i> (A/AJ)	1.61	1.73	0.84	2.17	IC
<i>Trichoderma asperellum</i> / <i>Aspergillus japonicus</i> (TA/AJ)	1.52	2.00	1.33	2.55	C

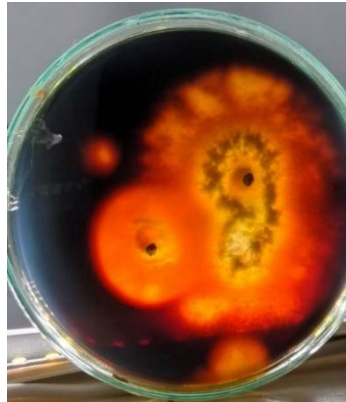


Fig. 1: Clear zone formed at cellulolytic test on CMC media

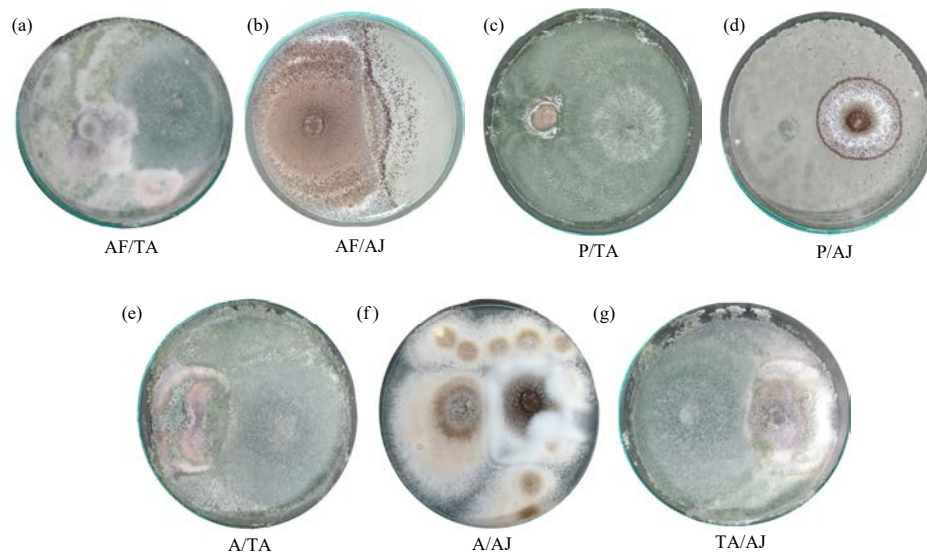


Fig. 2(a-g): Microbial consortium test in PDA Media, (a) *Aspergillus fumigatus* (AF) and *Trichoderma asperellum* (TA), (b) *Aspergillus fumigatus* (AF) and *Aspergillus japonicus* (AJ), (c) *Penicillium* sp. (P) and *Trichoderma asperellum* (TA), (d) *Penicillium* sp. (P) and *Aspergillus japonicus* (AJ), (e) *Aspergillus* sp. (A) and *Trichoderma asperellum* (TA), (f) *Aspergillus* sp. (A) and *Aspergillus japonicus* (AJ), (g) *Trichoderma asperellum* (TA) and *Aspergillus japonicus* (AJ)

are compatible with each other. The index of consortium cellulolytic indicated collective activity from various microorganisms in a consortium to degrade cellulose. An index above 1 indicated that microorganisms showed synergy to produce enzymes in different specifications or worked synergistically to decompose and degrade cellulose, while below 1 or 1 showed better improvement than a single microorganism in degrading cellulose¹⁹.

Cellulose was categorized as a linear polysaccharide which consists of monomer glucose connected by β -1,4-glycosidic bound. Cellulose degradation was carried out enzymatically with cellulose's help in hydrolyzing β -1,4-glycosidic bound in cellulose fiber. Cellulose was degraded into glucose involving an enzymatic work

mechanism by cellulose complex, containing exoglucanase, endoglucanase and β glucosidase. It was also degraded through synergistic mechanism work between exoglucanase and endoglucanase, which hydrolyzed glycosidic bound inside cellulose into glucose and cellobiose. Furthermore, cellobiose got hydrolyzed to form glucose by β -glucosidase. These enzymes were collected from distributed microorganisms in prokaryotic and eukaryotic, including bacteria, fungi and actinomycetes^{17,18}.

Besides the cellulolytic compatibility test of the fungal consortium, a mixed culture compatibility test was applied as well to find the synergistic correlation between two isolates *in vitro* culture. The test results *in vitro* consortium microbes on PDA Media were shown in Fig. 2a-g.

Table 2: Cellulose, hemicellulose and lignin content in the skin fruit coffee, added with microorganisms

Isolates	Cellulose	Hemicellulose	Lignin
Control/C (without microbe)	12.63	11.19	55.51
<i>Aspergillus japonicus</i> (AJ)	7.22	5.42	52.17
<i>Trichoderma asperellum</i> (TA)	10.85	6.16	48.46
Consortium <i>Aspergillus japonicus</i> and <i>Trichoderma asperellum</i> (TA/AJ)	7.49	5.34	45.91

The synergistic correlation is important to handle limitations in competition of space and nutrients among consorted isolates²⁰. The result of *in vitro* culture observation represented that fungal consortium AF/TA, AF/AJ, P/TA, P/AJ, A/TA and A/AJ were incompatible and only TA/AJ cultures were compatible with one another (Fig. 2). At consortium AF/TA, P/TA and A/TA, invasion happened where one of those fungi dominated the growth of other fungi, while at consortium AF/AJ and A/AJ, a demarcation line was formed, limiting the area of each isolate. Incompatible reaction pointed out the presence of an inhibition zone, a ridge of conidia and innovation^{21,22}. There are five types of microbe interaction: Blending each other, partial blend, invasion/alteration, inhibition at the touch point and inhibition at a long distance (inhibitor zone)²³. The TA/AJ was the best consortium to degrade coffee fruit skin waste and could cooperate during degradation; it was also faster than a singular fungal consortium.

The test of cellulose, hemicellulose and lignin contents in coffee fruit skin waste contents for isolate TA/AJ as a single isolate and consortium isolate, is shown in Table 2.

The test of cellulose, hemicellulose and lignin contents in coffee fruit skin waste contents, which applied singular and fungal consortium, signified that the utilizing of microorganisms could decrease cellulose, hemicellulose and lignin content of coffee fruit skin waste compared to zero microorganism addition. Moreover, using a fungal consortium was more effective than a single fungus in degrading the contents of cellulose, hemicellulose and lignin of coffee fruit skin waste (Table 2). Microbial treatments reduced cellulose, hemicellulose and lignin content compared to the control. The consortium (*A. japonicus*+ *T. asperellum*) showed the highest lignin degradation (45.91%), followed by *T. asperellum* (48.46%) and *A. japonicus* (52.17%). This suggests the consortium was the most effective in lignocellulose breakdown.

Consortium fungi TA/AJ could be used to develop the decomposing process of coffee fruit skin waste. Compatible Consortium fungi were more effective and faster in performing the decomposing process and increased compost production²⁴.

CONCLUSION

The TA/AJ consortium was identified as the most effective combination for degrading the lignocellulosic components of coffee fruit skin waste. This consortium demonstrated superior performance compared to individual isolates, highlighting the benefits of microbial synergy. The findings suggest that microbial consortia are a promising approach for enhancing the biodegradation of agricultural waste. This study contributes to developing sustainable waste management strategies by utilizing compatible microbial consortia.

SIGNIFICANCE STATEMENT

This research discovered cellulolytic fungi that possess cellulolytic compatibility characteristics and could have synergy with one another without showing any inhibition. Those Consortium fungi that have the cellulolytic compatibility character could increase the cellulose degradation process of coffee fruit skin waste compared to utilizing only a singular isolate. They are expected to be developed as decomposers in coffee skin waste processing, therefore the waste can be degraded quickly with zero environmental pollution.

ACKNOWLEDGMENTS

This research was funded by Applied Research, Vocational Product Research (P2V), Funding from the Academic Directorate of Vocational Higher Education (APTV), Ministry of Education, Culture, Research and Technology for Fiscal Year 2024 with the Number Decree of Director of the Pangkep State Polytechnic of Agriculture: 601/PL.22/PG/2024 with number of contract: 156/PL.22.7.1/SP.PG/2024.

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