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Inhibition of Exophitic and Endophytic Microbials Against *Fusarium decemcellulare* Brick. CAUSES OF APPLE ROT DISEASE (*Malus domestica*Borkh)

I Made Sudarma*, Ni Wayan Suniti* and Ni NengahDarmiati*
**Staff Lecturer in Agroecotechnology Study Program Faculty of Agriculture, Udayana University Jl. PB. Sudirman Denpasar-Bali, email: sudarma_made@ymail.com*

Abstract
Postharvest apple rot disease is a serious problem in Indonesia, especially in Bali, considering that religious ceremonies always use fruit as offerings for ceremonial purposes. Fruit rot was first discovered in Bali caused by the fungus Fusarium decemcellulare as a result of DNA sequencing analysis with 100% homology from the genebank. Visualization of the amplified F.decemcellulare DNA showed 650 bp DNA fragments. Exophytic and endophytic microbial diversity indexes were 1.2491 and 2.7930, respectively, with exophytic and endophytic microbial dominance indexes being 0.8958 and 0.7390, respectively. The results of the in vitro inhibition test of exophytic and endophytic fungi against the highest pathogenic F. decemcellulare were A. niger and Rhizopus sp. each of 83.33±00% and the endophytic microbe is the fungus Rhizopus sp. of 81.48±9.21%. The results of the in vivo inhibition of exophytic and endophytic microbes against pathogens were obtained in treatment C (Rhizopus sp.) with a percentage of damage of 6±2.24% and significantly different from the control (given the pathogen).

Key words:*Fusarium decemcellulare, diversityand dominance index, inhibition ability, exophytic and endophytic.*

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I. Introduction

Apple rot disease is often encountered when the fruit is stored (post-harvest) which damages the skin of the fruit and makes it look less attractive. This is very disturbing when offerings will be used for religious ceremonies in Bali. The fungus *F. decemcellulare* that causes apple rot was first discovered in Bali, while this pathogen was first reported to attack apples in Korea (Seung-Yeo *et al*. 2017). Besides attacking apples, pathogens can also attack cacao flowers (Del Castillo *et al*., 2016), attack lotus fruit plants (*ZizyphusMauritiana*) in India (Singh and Singh, 2018), the cause of late death in manga in China, the results of research during 2009- 2011 (Yan-Xiang *et al*., 2013), and attacked the longan (*Dimocarpus longan*Lour.) in Puerto Rico (Zakaria, 2022).

Exophytic microbes (fungi, bacteria, and viruses) that live on flowers are thought to affect pollination, and the attraction of bees due to the presence of these microbes (Cullen *et al*., 2021). Endophytic fungi are fungi that grow in plant tissues while exophytic fungi are surface fungi that can live saprophytically but do not cause disease in plants. Phylloplanefungiare fungi that grow on plant surfaces (Langvard, 1980). There are groups of phylloplanefungi: resident (stay silent) and casual (coincidentally). Residents can reproduce on healthy leaf surfaces without affecting the host, while casuals land on leaf surfaces but cannot grow (Leben 1965).

Endophytic microorganisms, commonly known as 'endophytes', represent a very diverse group of organisms that reside within plant tissues in a broadly mutualistic manner. The immense biodiversity of endophytes coupled with their multifaceted interactions with related organisms (e.g. other endophytes, invading pathogens, insects, pests and feeders) often leads to the coevolution of certain functional traits. These traits range from synergism, physical and chemical communication, to allelopathy, which is part of an overall mutualistic network of endophytes with related microorganisms (Li *et al.*, 2016).

`Generally, endophytic microorganisms are present in the tissues of most host plants. The associations of endophytic organisms with their host plants are varied and complex. Endophytic microbial organisms often contribute to the normal health and development of their host plants in exchange for relatively preferential niches. Several groups of endophytic microorganisms have been described as mutualists that protect plants from biotic stress. Endophytes contribute to and may also be responsible for the adaptation of host plants to environmental stresses. Over the past two decades endophytes have been targeted as valuable sources of novel bioactive compounds and secondary metabolites (Tadych, and White, 2019).

II. Materials And Methods

Location and Time Study

The research was carried out in two places: 1) looking for sick and healthy fruit specimens from the Batubulan market and supermarkets. 2) Laboratory of Plant Diseases and Agricultural Biotechnology Laboratory. The research was carried out from April to August 2021.

Molecular Identification

a. DNA Extraction

DNA extraction followed the procedure of Doyle and Doyle (1987), 0.2 g of pathogenic fungal mycelium samples were ground with liquid nitrogen and powdered fungal pathogens were put into Eppendorf tubes. Then 500 L of CTAB buffer and 50 µl -mercaptoethanol were added, then mixed until homogeneous using a vortex. To lyse the cell wall, heating is carried out at a temperature of 70ºC for 60 minutes where every 10 minutes it is back and forth to speed up the lysis process. Then cooled down to room temperature. Then 500 µl of chloroform isoamylalcohol (24:1) was added to the tube and mixed until homogeneous by vortex and centrifuged at 12,000 rpm for 15 minutes. The supernatant obtained was transferred to a new Eppendorf tube by adding 500 µl of sodium acetate, mixed until homogeneous by vortex and centrifuged again at 12,000 rpm for 10 minutes.The supernatant was transferred to an eppendorf tube and then 500 µl of sodium acetate and isopropanol were added, mixed until homogeneous by vortex and centrifuged again at 12,000 rpm for 10 minutes. The tube was shaken gently to bind DNA and incubated at -20 C for 30 minutes. The DNA threads obtained were precipitated by centrifugation for 10 minutes. The supernatant was discarded, the pellet was washed with ethanol (70%) then centrifuged at 8,000 rpm for 5 minutes. The ethanol was removed and the pellets were dried. The pellet was resuspended with 50 µl of TE buffer and stored at -20ºC for further use in the DNA amplification process.

b. DNA amplification

DNA amplification was carried out on a Thermo Cycle PCR machine. Amplification was carried out using universal primers to detect the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA), namely forward primer ITS1 (5'- CTTGGTCATTTAGAGGAAGTAA-3') and reverse primer ITS4 (5'-TCCTCCGCTTATTGATATGC-3') with target size results amplification was 490 bp (Doyle and Doyle, 1987). The DNA amplification reaction was carried out with a total volume of 25 µl consisting of 1 µl of DNA, 2.5 µl of buffer 10x and Mg2+, 0.5 µL of 10 mM dNTP, 1 µl of each primer, 12.5 µl of Taq DNA (10 units/ µl), and 9.5 µl H_2O. The amplification conditions were divided into several stages, namely predenaturation at 94^{o}C for 3 minutes, followed by 30 cycles of amplification, each cycle consisting of strand separation/denaturation of DNA at 94^o C for 1 minute, primer attachment/annealing at 45^o C for 1 minute, DNA synthesis at 72^o C. for 2 minutes. Especially for the last cycle plus the synthesis step for 10 minutes, then the cycle will end with a temperature of 4^o C.

c. DNA electrophoresis

The amplified product was analyzed using Blued electrophoresis with 1% agarose gel (0.5xTris-Borate EDTA/TBE). Electrophoresis was carried out at 100 volts for 28 minutes and then the agarose gel was incubated in dye containing ethidium bromide (1%) for 15 minutes, then washed with H_2O for 10 minutes. The results of the electrophoresis were visualized with an ultraviolet transilluminator. The DNA bands formed on the results of the electrophoresis were documented with a digital camera.

d. DNA Sequence Analysis

The amplification product was sent to Macrogen (Singapore) for nucleotide sequencing. The tracing results were then analyzed using the basic local alignment search tool (BLAST) program with an optimization program to obtain DNA base sequences that have homology with DNA sequences found on the National Center for Biotechnology Information (NCBI) website. The nucleotide sequences obtained were then analyzed using ClustalW multiple alignment on Bioedit sequence alignment editor software version 7.0.5. Homology results close to 100% similarity categorized as the same species as the sample species.

Isolation of Endophytic and Escophyte Fungi

Isolation of endophytic fungi, plant parts such as fruitwere washed with sterile running water, then the plant parts were sterilized with 0.525% sodium hypochlorite for 3 minutes, and 70% alcohol for 2 minutes, then rinsed with sterile water for 1 minute. and then placed on PDA media (which was first given an anti-bacterial antibiotic, namely livoploxacin with a concentration of 0.1% (w/v). The fungus that emerged from the leaf pieces was transferred to a test tube containing PDA to be stored and classified by morphospecies. Exophytes can be done by spraying plant parts (fruit, leaves and stems).The washing water is collected, then in a tube, then

taken, from a 1 ml tube it is grown into a PDA which has previously been filled with livoploxacin with a concentration of 0.1% (w/v).

Identification of Endophytic and Exophytic Fungi

The stored endophytic and exophytic fungi were then grown in Petri dishes containing PDA and repeated 5 times. Cultures were incubated in the dark at room temperature (±27^{o}C). Isolates were identified macroscopically after 3 days of age to determine colony colour and growth rate, and microscopic identification to identify septa on hyphae, spore/conidia shape and sporangiophores. Fungal identification using reference book Samson *et al*. (1981); Pitt and Hocking (1997); Barnett and Hunter (1998); and Indrawati*et al*. (1999) and identification of actinomycetes Miyadoh (1997).

Inhibitory Test of Endophytic and Exophytic Microbes Against Pathogens

The endophytic and exophytic microbes that were found were tested for their inhibition against the growth of pathogenic fungi using the dual culture technique (in one Petri dish, one pathogenic fungus was grown each flanked with two endophytic fungi). The inhibitory power can be calculated as follows (Dollar, 2001; Mojica-Marin et al., 2008):

$$\text{Inhibition ability (\%)} = \frac{A - B}{A} \text{ x } 100$$

Where: A = pathogen colony diameter in single culture (mm)
B = pathogen colony diameter in dual culture (mm)

Endophytic and Exophytic Microbial Prevalence

Determining the prevalence of endophytic and exophytic microbial was based on the frequency of endophytic and exophytic microbial isolates found in healthy fruit per Petri dish, divided by all isolates found times 100%. The prevalence of isolates will determine the dominance of endophytic microbes present in healthy mango fruit.

Determining Diversity and Dominance Index

The diversity and dominance of contaminant fungi can be determined by calculating the Shannon-Wiener diversity index (Odum, 1971) and the dominance of microbes is calculated by calculating the Simpson index (Pirzan and Pong-Masak, 2008).

(1) Microbial diversity index

Microbial diversity index was determined by the Shannon-Wiener diversity index, namely by the formula (Odum, 1971):

$$H' = - \sum_{i=1}^{s} Pi \ ln \ Pi.$$

Where:
H' = Diversity Shannon-Wiener index
S = Number of genera
Pi = ni/N as the proportion of the ith species (ni = Total number of individuals of total microbial species i, N = Total number of individuals in total n)

The criteria used to interpret the diversity of Shannon-Wiener (Ferianita-Fachrul et al., 2005) are: H' value < 1, meaning low diversity, H' value 1 – 3 means diversity is moderate and H' value > 3 means diversity is classified as high or (Table 1).

Table1.Criteria forweightingenvironmentalquality(Tauruslina*etal*.,2015)

Diversityindex	Communitystructure conditions	Category	Scale
>2,41	Verystable	Verygood	5
-2,4	Morestable	Good	4
1,21 –1,8	Stableenough	Currently	3
0,61 –1,2	Lessstable	Bad	2
<0,6	Unstable	Verybad	1

(2) Dominance index

Microbial dominance index was calculated by calculating the Simpson index (Pirzan and Pong-Masak, 2008), with the following formula:

$$C = \sum_{i=1}^{s} Pi2$$

Where:
C = Simpson index
S = Number of genera
Pi = ni/N is the proportion of individuals of type i and all individuals (ni = the total number of individuals of type i, N = the number of all individuals in the total n).

Furthermore, the species dominance index (D) can be calculated using the 1-C formulation (Rad *et al.* 2009).The criteria used to interpret the dominance of soil microbial species are: close to 0 = low index or lower dominance by one microbial species or there is no species that extremely dominates other species, close to 1 = large index or tends to be dominated by several microbial species (Pirzan and Pong-Cook, 2008).

***In Vivo* Antagonist Test**

In vivo antagonistic test of endophytic and exophytic microbial that have the best inhibitory power was used by dipping into a suspension of spores of antagonistic fungi (each according to treatment), then dipping into suspension of spores of pathogenic fungi. The best treatment of endophytic/exophytic fungi was found, among others:

A = control (without smearing with antagonist) + pathogen
B = antagonist treatment 1 (250 ml aqua (10% sugar solution) + spore suspension 1 Petri dish) + pathogen
C = antagonist treatment 2 (250 ml aqua (10% sugar solution) + spore suspension 1 Petri dish) + pathogen
D = antagonist treatment 3 (250 ml aqua (10% sugar solution) + spore suspension 1 Petri dish) + pathogen
E = antagonist treatment 4 (250 ml aqua (10% sugar solution) + spore suspension 1 Petri dish) + pathogen
F = antagonist treatment 5 (250 ml aqua (10% sugar solution) + spore suspension 1 Petri dish) + pathogen
G = without any treatment.

All treatments were repeated 5 times. The experiment was designed with a completely randomized design (CRD), and after the analysis of variance (ANOVA) was carried out, it was continued with the Duncan Multiple Range Test (DMRT) at 5% and 1% levels. Damage parameters were measured by the formulation: sick fruit that was given a stab divided by all punctures (20 stabs) times 100%.

III. Results And Discussion

Molecular Identification

DNA fragments measuring 650 bp were successfully amplified from 4 fungal samples using the universal primer ITS1/ITS4 (Figure 1). The amplified DNA sample was then used for the sequencing stage to determine the fungal species. Sequencing analysis confirmed that the fungus was *Fusarium decemcellulare* with 100% homology to several *F. decemcellulare* isolates in the genebank (Table 2).

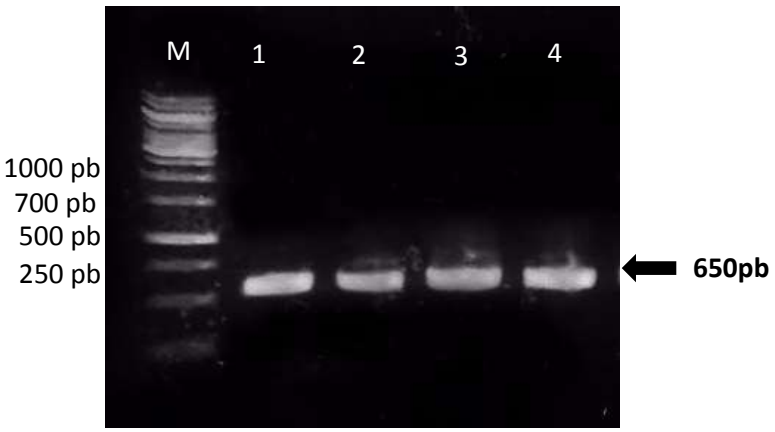

Figure 2. Visualization of amplified *Fusarium decemcellulare* DNA using ITS1/ITS4 universal primer on 1% agarose gel. M: DNA marker (1kb ladder); Sample no. 1-4 (*F. decemcellulare*)

Table 2. Homology (%) of nucleotide sequences of *F.decemcellulare* isolates with several isolates that have been reported in GenBank

Sequence	A (%)	B (%)	C (%)	D (%)	E (%)	F (%)	G (%)
A	ID	100	100	98	98	100	78
B	100	ID	100	98	98	100	78
C	100	100	ID	98	98	100	78
D	98	98	98	ID	100	98	78
E	98	98	98	100	ID	98	78
F	100	100	100	98	98	ID	78
G	78	78	78	78	78	78	ID

Where: A = *F. decemcellulare* Bali, B = JX171453 *F. decemcellulare* Chi, C = JX171567 *F. decemcellulare*Chi, D = LC212975 *F. dececellulare*Kor, E = LC214751 *F. decemcellulare*Kor, F = MN24943 *F. decemcellulare* Chi, dan G = JX171473 *Microceralavarum* USA.

Phylogenetic analysis showed that *F. decemcellulare* isolates from Bali formed one group with 3 isolates from China and separated from isolates from South Korea. This shows that the isolates have very close homology to the isolates from the genebank, so that the genetic diversity can be said to be low (Figure 2).

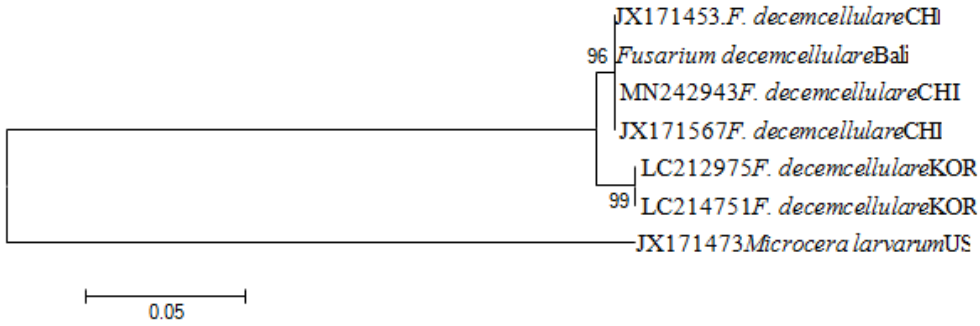

Figure 2. Phylogenetic analysis of *Fusarium decemcellulare* isolate based on partial nucleotide sequence alignment from internal transcribed spacer 1, 5.8S ribosomal RNA gene using Mega 6.06 (Neighbor Joining Algorithm with 1,000 bootstraps replicates).

The oldest ecosystem in the world found in the tropics is one of the causes of disease is *F. decemellulae* (Ploetz, 2005). This fungus as a pathogen of apple rot was first reported in Korea. In 2014, abnormal brown spots were observed on Hongro apples in a field in Gyeongsangbuk-do Province and during low-temperature storage. The spots are round, light brown in color, and different from previously reported symptoms of apple disease (Lee *et al.,* 2017).

DiseaseIncidence

The apple rot disease found (Figure 3A) was brown to black, curved inward and the rot was slightly dry (Figure 3B). The results of the isolation showed that this symptom was caused by a fungus. The colour of the mycelium that grew on Petri dishes was pink with a rather slow growth (Figure 3C), after being observed under a microscope it turned out that macroconidia were curved (Figure 3D). The identification of the pathogen was *F. decemcellulare* (Table 2).

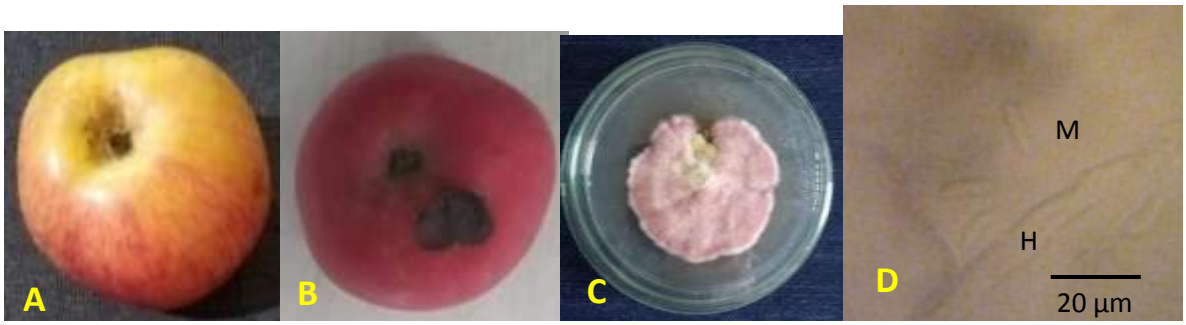

Figure 3. Study of disease, (A) healthy apples, (B) sick apples with rot symptom, (C) fungal mycelium and (D) club-shaped pathogenic conidia (M = macroconidium; H = hypha)

Populations of Exophytic and Endophytic Microbes

Exophytic microbial populations found in healthy apples were 18 species, of which the most were *Lasiodiplodiatheobromae* with 10 isolates, followed by *Actinomycetosporasuccina* (Actinomycetes) with 6 isolates and *Rhizopus* sp. as many as 5 isolates (Table 3; Figure 4), while the endophytic fungi found 3 species, including *Rhizopus* sp. the most with 23 isolates, followed by *A. niger* with 3 isolates and finally *L. theobromae* with 1 isolate, each with the highest prevalence in exophytic fungi of 22% was *L. theobromae* and in endophytic microbes *Rhizopus* sp. by 85%. (Table 3; Figure 5).

Table 3. Populations of exophytic and endophytic microbes in healthy apples

No.	Name of exophytic mirobes	Number of isolates	Name of endophytic microbes	Number of isolates
1	*Actinomyces bovis* (Actinomycetes)	2 (4%)*	*Aspergillus niger*	3 (11%)
2	*Actinomycetosporasuccina* (Actinomycetes)	6 (13%)	*Lasiodiplodiatheobromae*	1 (4%)
3	*Actinomyces israelii* (Actinomycetes)	1 (2%)	*Rhizopus* sp.	23 (85%)
4	*Arthrinium* sp.	1 (2%)		
5	*Asanoairimotensis (Actinomycetes)*	1 (2%)		
6	*Aspergillus niger*	2 (4%)		
7	*Catenulispora subtropic* (Actinomycetes)	1 (2%)		
8	*Colletotrichum* sp.	1 (2%)		
9	*Lasiodiplodiatheobromae*	10 (22%)		
10	*Micromonospora* sp. (Actinomycetes)	2 (4%)		
11	*Micromonospora jinlongensis*(Actinomycetes)	1 (2%)		
12	*Nocardia asteroides* (Actinomycetes)	1 (2%)		
13	*Nonomuraeamonospora* (Actinomycetes)	4 (9%)		
14	*Rhizopus sp.*	5 (11%)		
15	*Sacharomonospora* sp. (Actinomycetes)	2 (4%)		
16	*Streptomyces eurocidicus*(Actinomycetes)	1 (2%)		
17	*Streptomyces roseoverticillatus (*Actinomycetes)	3 (7%)		
18	*Streptosporagium* sp. (Actinomycetes)	1 (2%)		
	Jumlah	45		27

*Numbers in brackets indicate the prevalence of microbes found

Sudarma*et al*. (2021a) stated in the results of his research that 7 species of exophytic microbes were found, dominated by the fungus *Rhizopus* sp. as many as 18 isolates, while endophytic fungi were found as many as 4 species which were dominated by *Rhizopus* sp. and *A. niger* with 6 isolates each. *Rhizopus* sp. dominates in endophytes, this is because cosmopolitan conidia are easily spread and infect fruit tissues, while in exophytes *L.theobromae* dominates because these fungi are pathogenic in several fruit commodities (Sudarma*et al*, 2022).

Exophytic and Endophytic Microbial Diversity and Dominance Index

The microbial diversity index of healthy apple fruit exophytes is 1.249, this means that the condition of the commodity structure is quite stable with a medium category and a scale of 3 (Table 1), while the dominance index is 0.896, meaning that there are species that dominate, namely *L. theobromae* as many as 10 isolates (Table 4). The endophytic microbial diversity index in healthy apples was 2.793, which means the condition of the commodity structure was more stable with a good category and a scale of 4. The Simpson dominance index was 0.739, meaning that there was a dominant species, namely *Rhizopus* sp. as many as 23 isolates (Table 4).

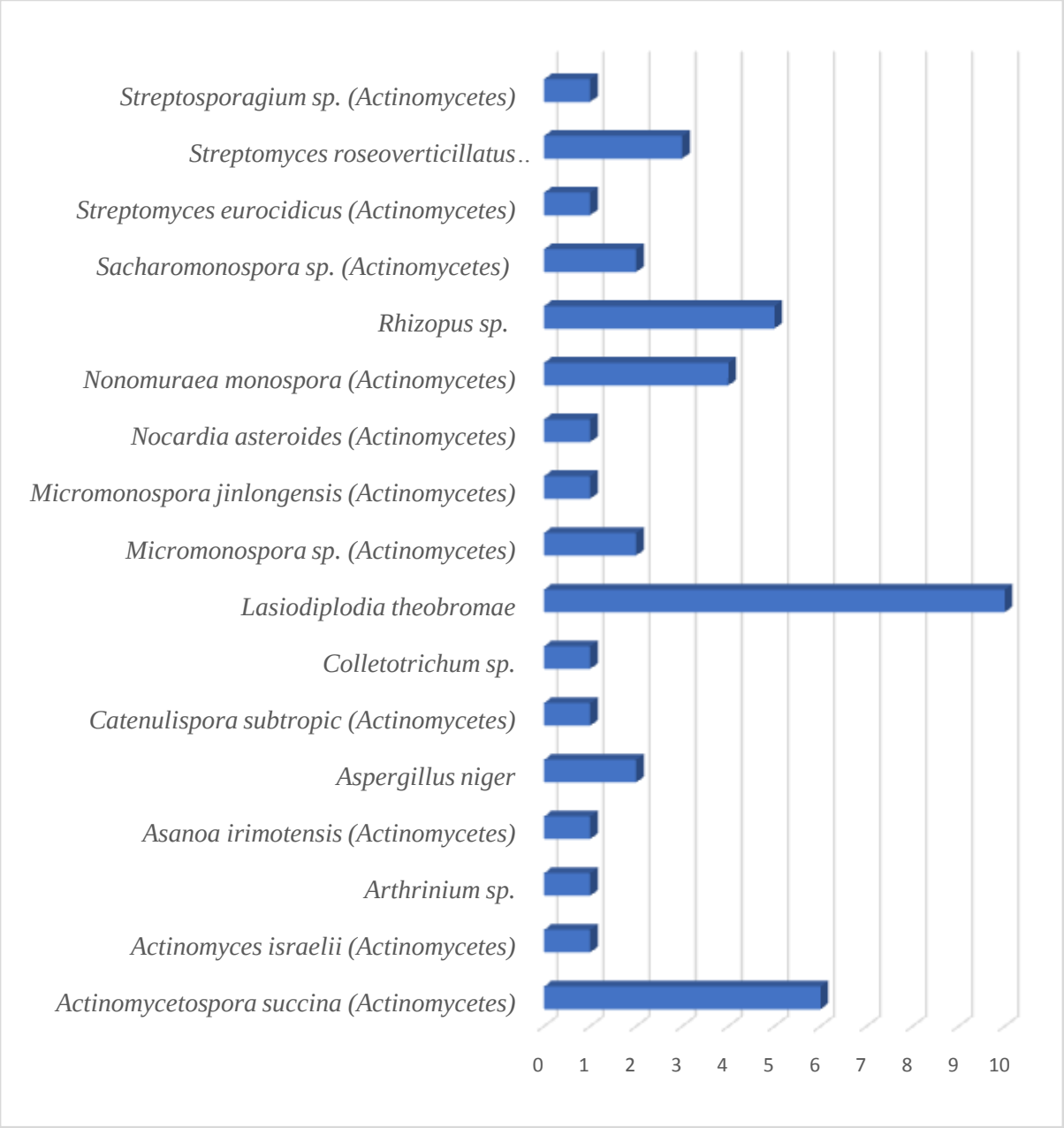

Figure 4. Exophytic microbial population in healthy apples

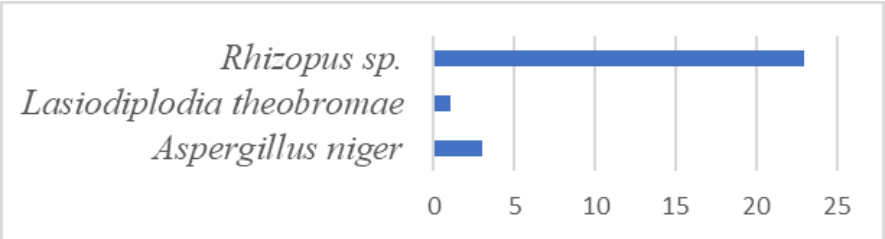

Figure 5. Population of endophytic fungi in healthy fruit

Table 4. Indices of diversity and dominance of exophytic and endophytic microbes in healthy apples

Index	Exophytic	Endophytic
H' (diversity index)	1.2491	2.7930
D (dominance index)	0.8958	0.7390

The research results of Sudarma*et al.* (2019) found that the diversity and dominance index of exophytic fungi in healthy sugar-apple fruit was 2.3742 and 0.8667, respectively, while for endophytic fungi the index of diversity and dominance was 2.6356 and 0.6489, respectively. Likewise, the research results of Sudarma*et al.* (2021b) found that the diversity and dominance index for exophytic microbes was 2.450 and 0.4078, respectively, while for endophytic microbes the diversity and dominance index was 1.876 and 0.580, respectively. Almost all the dominant fungi are *Rhizopus* sp. this indicates that the fungus *Rhizopus* sp. Many postharvest fruits are found both exophytic and endophytic. The more species found, the greater the chance to inhibit pathogens from attacking plants, so that apples can be saved from pathogen attacks.

In Vitro Inhibition of Exophytic and Endophytic Microbes

In vitro inhibition of exophytic and endophytic microbes against pathogen A showed that the best exophytes were *A. niger* and *Rhizopus* sp. each of 83.33±00%, while the best endophytic microbes were *Rhizopus* sp. of 81.48±9.21% (Table 5).

Aspergillus niger and *Rhizopus* sp. still showed the highest inhibition against pathogens. Inhibition was competitive because no seed zone was found as a sign of antibiotic inhibition. According to Wahdania*et al.* (2016) found the inhibition of *A. nger* against cocoa pod rot pathogen (*Phytophthora palmivora*). *Aspergillus niger* produces a-amylase and glucoamylase enzymes that allow starch to be broken down into simple glucose and then fermented into ethanol. *A. niger* also produces enzymes such as amylase, amyloglucosidase, pectinase, cellulose, glycosides that can break down amino acids and CO2.

Vigianti (2015) succeeded in isolating Indonesian tempeh fungi, which is a soybean-based food product fermented by *Rhizopus oligosporus*. The role of *R.oligosporus* as the main fungus in soybeans is very important, thus changing its composition from soybean substrate to a more nutritious food and containing many enzymes and bioactive compounds, including antibacterial compounds.

Table 5. Inhibition of exophytic and endophytic microbes against pathogens *in vitro*

No.	Name of exophytic microbes	Inhibition ability (%)	Name of endophytic microbes	Inhibition ability (%)
1	*Actinomyces bovis* (Actinomycetes)	-	*Aspergillus niger*	72.22±5,56
2	*Actinomycetosporasuccina* (Actinomycetes)	-	*Lasiodiplodiatheobromae*	77.78±00
3	*Actinomyces israelii* (Actinomycetes)	-	*Rhizopus* sp.	81.48±9,21
4	*Arthrinium* sp.	-		
5	*Asanoairimotensis (Actinomycetes)*	-		
6	*Aspergillus niger*	88.33±00		
7	*Catenulispora subtropic* (Actinomycetes)	66.67±00		
8	*Colletotrichum* sp.	66.67±00		
9	*Lasiodiplodiatheobromae*	-		
10	*Micromonospora* sp. (Actinomycetes)	66.67±11,79		
11	*Micromonospora jinlongensis*(Actinomycetes)	-		
12	*Nocardia asteroides* (Actinomycetes)	-		
13	*Nonomuraeamonospora* (Actinomycetes)	71.11±4,19		
14	*Rhizopus sp.*	83.33±00		
15	*Sacharomonospora* sp. (Actinomycetes)	-		
16	*Streptomyces eurocidicus*(Actinomycetes)	-		
17	*Streptomyces roseoverticillatus (*Actinomycetes)	-		
18	*Streptosporagium* sp. (Actinomycetes)	-		

Furthermore, the results of research by Endrawati and Kusumaningtyas (2017) stated that Rhizopus sp. is a fungus that easily grows in soil, vegetables and fruits as well as fermented processed products. Rhizopus sp. can increase the nutritional value of feed ingredients. *Rhizopus* sp. has long been known in Indonesia, especially for the manufacture of tempeh. Several studies on *Rhizopus* sp. have opened up opportunities for the use of *Rhizopus* sp. for other functions. The fungus *Rhizopus* sp. can suppress the growth of the toxigenic fungus *Aspergillus flavus* and degrade aflatoxins. *Rhizopus* sp. can also produce compounds that can inhibit pathogenic bacteria and function as antioxidants. *Rhizopus* sp. absorbs some mineral elements and converts them into organic minerals so that they can improve mineral absorption in the body better. The use of fermented feed ingredients by *Rhizopus* sp. in livestock showed better results than without fermentation. *Rhizopus* sp. Sudarma*et al*. (2018) stated that the diversity of exophytic fungi plays an important role in controlling disease-causing pathogens, especially sugar apple fruit rot. The greater the diversity, the more stable the ecosystem, and the more opportunities to inhibit pathogens.

Fendiyanto and Satrio (2020) stated that *Aspergillus niger* has the ability, as a biological agent, to suppress food spoilage pathogens in many foods, including bread, however, there are still few reports of antagonistic tests on bread, especially between *A. niger* as a biological control agent against rotting fungus. Therefore, to analysis the growth antagonist test of *A. niger* against food spoilage fungi, it is hoped that the shelf

life of bread can be extended and mycotoxin contamination can be avoided. Antagonist test is a test that utilizes the characteristics of microorganisms that grow faster than pathogens or produce antibiotic compounds. The fungus with the highest inhibitory value was *Hyphopichiaburtonii*, while the lowest was *Saccharomyces cerevisiae*. These findings suggest that *A. niger* can be used as a biological control in extending bread storage in the future.

***In Vivo* Inhibition of Exophytic and Endophytic Microbes**

The inhibition of exophytic and endophytic microbes *in vivo* against pathogens was as follows: treatment C (*Rhizopus* sp.) was able to suppress the highest among other treatments, and was not significantly different from treatment A (without pathogens), this indicatesthat*Rhizopus* sp. (Table 6) with very fast growth in Petri dishes can compete well against pathogens. Likewise, it can be seen in Figure 5.4, that treatment C (*Rhizopus* sp.) was very significant in suppressing the growth of pathogens (Figure 6).

Table 6. Inhibition of exophytic and endophytic microbes against pathogens *in vivo*

Treatment	Avarage of damage persentage	Notation	
		DMRT 5%*	DMRT 1%*
K-P tanpapatogen)	5±0	a	a
K+P (denganpatogen)	98±4.47	f	f
A (*Colletotrichum* sp.)	20±7.07	b	b
B (*A. niger*)	72±8.37	d	d
C (*Rhizopus* sp.)	6±2.24	a	a
D (*Micromonospora* sp., Actinomycetes)	44.48±5.48	c	c
E (*Lasiodiplodiatheobromae*)	86±5.48	e	e

*Note: the same letter in the same column means that the test level is not significantly different from 5% and 1% is very significant.

Figure 5.8. Effect of exophytic and endophytic microbial treatment on pathogens A, (K-P = without pathogens, K+P = with pathogens, A = *Colletotrichum* sp., B = *A. niger*, C = *Rhizopus* sp., D = *Micromonospora* sp., Actinomycetes., and E = *L. theobromae*) 3 days after inoculation

Sriherwanto*et al.* (2017) stated that solid fermentation using *Rhizopus* sp. has the potential to be further developed as a biofloating agent in the manufacture of floating fish feed. Moensaku*et al.* (2021) stated that based on the results of the study, it was stated that the mold in red bean tempeh was fermented by *Rhizopus oligosporus* and *Rhizopus* was antagonistic against pathogenic bacteria. The results of this study strengthen the benefits of tempeh as a functional food.

IV. Conclusion

The pathogen found to cause apple rot disease was the fungus *Fusarium decemcellulare* with DNA fragments measuring 650 bp each. The diversity index of exophytic and endophytic fungi was 1.2491 and 2.7930, respectively, with the dominance index of exophytic and endophytic fungi being 0.8958 and 0.7390, respectively. The results of in vitro inhibition of exophytic and endophytic fungi against pathogens (*F. decemcellulare*) were the highest *A. niger*and *Rhizopus* sp. each of 83.33±00% and the endophytic microbe is the fungus Rhizopus sp. of 81.48±9.21%. The results of the in vivo inhibition of exophytic and endophytic microbes against pathogens were obtained in treatment C (*Rhizopus* sp.) with a percentage of damage of 6±2.24% and was very different from the control (given the pathogen).

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