Effect of soil fungi in agricultural land on anti-spoilage fruit bacteria

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ABSTRACT

In the course of the investigation for antimicrobial metabolite producing fungi, 17 fungi were isolated from four different soil samples collected at agricultural land along the Sein Pan Myaing Road, Dagon University Campus Area, Yangon Division. In the preliminary studies are biochemical tests and antimicrobial activities with the isolated fungi. Eight kinds of pathogenic bacteria were isolated from spoilage fruits: orange, apple and grape fruits. These isolated spoilage fruit bacteria were tested with the isolation of 17 different soil fungi. In the present research studies, soil fungi with the anti-spoilage fruit activities were screened and isolated.

INTRODUCTION

Microbes play important roles in the ecosystem. They can live in the air, on land and in fresh or salt water environments (Vaupotic et. al., 2008). Fungi grow best in environments that are slightly acidic. Soil samples are good substrata for isolating microbes. Secondary metabolites can be seen in several human medical applications such as antibiotic penicillin (Gibbms & Rokas, 2013).

Microbial spoilage may due to the plant pathogens acting on stems, leaves, flowers, or root of the plant, on the fruit or other special parts used as foods. Spoilage of fresh fruits and vegetables usually occurs during storage and transport. Fruits may become contaminated with pathogenic and spoilage microorganisms either during their growing in fields, orchards, vineyards, or greenhouses, or during harvesting, postharvest handling, and distribution (Beuchrt, 2002).

Diseases are caused by biotic agents such as microbial pathogens and nematodes and abiotic factors such as unfavorable environmental conditions. Diseases due to biotic causes are more numerous than those due to abiotic causes (Ferman and Rodriguez, 1993). The aim and objectives are to isolate and characterize the soil microorganisms, to investigation the varieties of fungi in different soil samples and to study antimicrobial activities of isolated strains.

MATERIALS AND METHODS

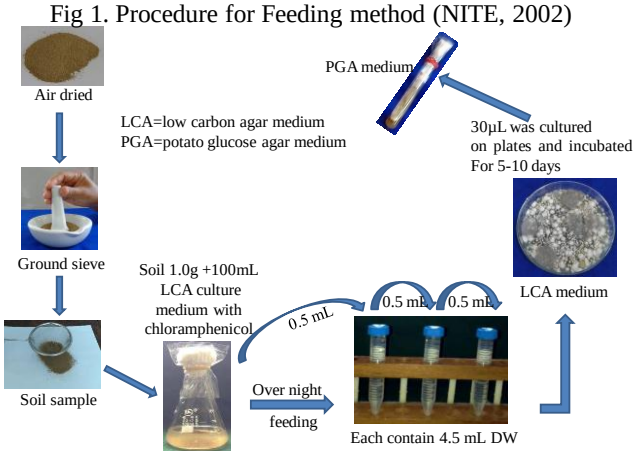
Isolation of soil samples

Four different soil samples were collected at agricultural land along the Sein Pan Myaing Road in Dagon University. The isolation of fungi were undertaken by feeding method (NITE, 2002).

Feeding method (NITE, 2002)

Soil sample (1.0g) was poured onto 100 ml LCA liquid culture medium and it was incubated overnight and 20 ml of 70% methanol was added. After shaking for 2 minutes, 30µL sample was cultured on plates low carbon agar medium (LCA medium) and incubated for 5-10 days as shown in figure (1).

Fig 1. Procedure for Feeding method (NITE, 2002)



Collection and Isolation of spoilage fruit bacteria

The spoilage fruit of apple, orange and grape were collected at Thingangyun Market and isolated bacteria using by the serial dilution method.

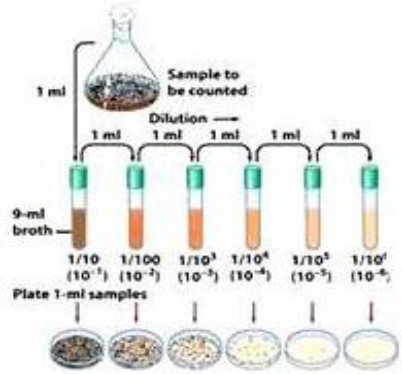


Fig 2. Serial dilution of spoilage fruit bacteria

Preliminary study for antimicrobial activities by paper disc diffusion assay (Ando, 2004)

The isolated fungi were inoculated on seed medium and incubated at room temperature for 3 days. Twenty mL of seed culture was transferred into the fermentation medium and incubated at room temperature for 7 days. Twenty µL of fermented broth was put on paper disc and placed on assay plate containing test organisms and the plates were incubated for 24 hrs at 28 □ C (Figure 3).

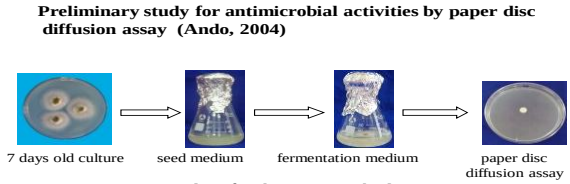


Fig.3.Procedure for the antimicrobial activity test

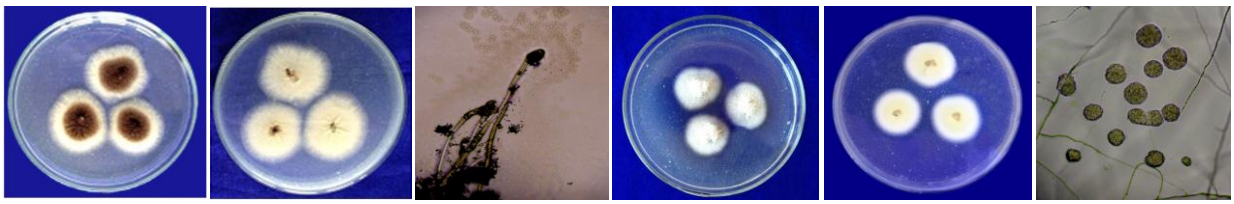
RESULRS

Isolation of microorganisms from soil samples

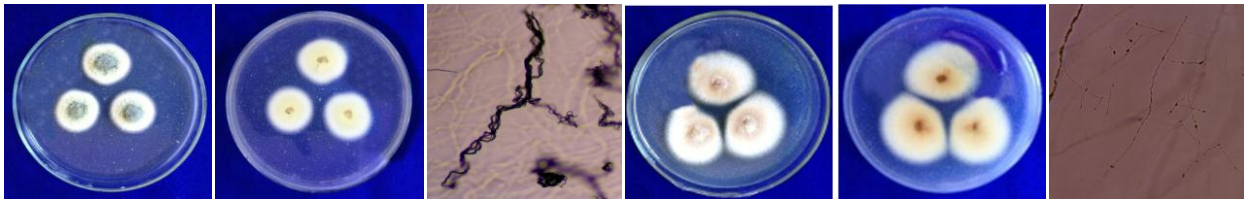
In this studies, 17 kinds of fungi were isolated from four different soil samples from the agriculture land along the Sein Pan Myaing Road, Dagon University as shown in Table 2 and Figure 4-6.

Table-1 Numbers of fungi isolated from four different soil samples by feeding method (NITE, 2002)

Soil No.	Isolated fungi	Soil type	pH	location	Collected date and place
1	FS 1, FS 2,FS 3	Silty clay loam	6.14	N 16º55′04.7″ E 096 12′53.7″	29.9.2016 Along the Sein Pan Myaing Road, Dagon University Campus.
2	FS 4, FS 5,FS 6, FS 7	Silty clay loam	5.07	N 16º55′05.9″ E 096 12′51.0″	
3	FS 8, FS 9,FS 10, FS11	Silty clay loam	5.75	N 16º55′07.3″ E 096 12′56.2″	
4	FS 12, FS 13, FS 14, FS 15, FS 16, FS 17	Silty loam	6.45	N 16º55′10.4″ E 096 12′57.3″	
Total fungi - 17					

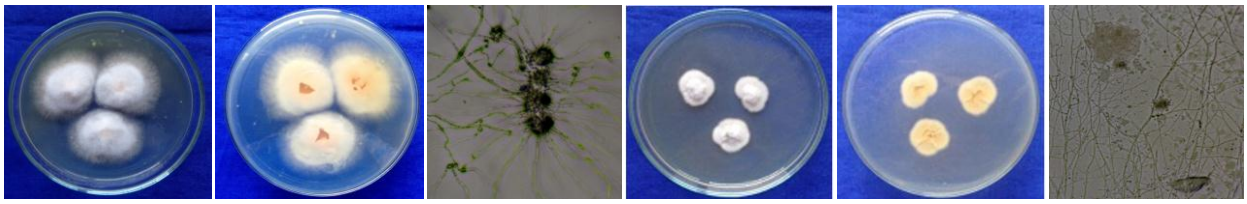


FS-1 FS-2



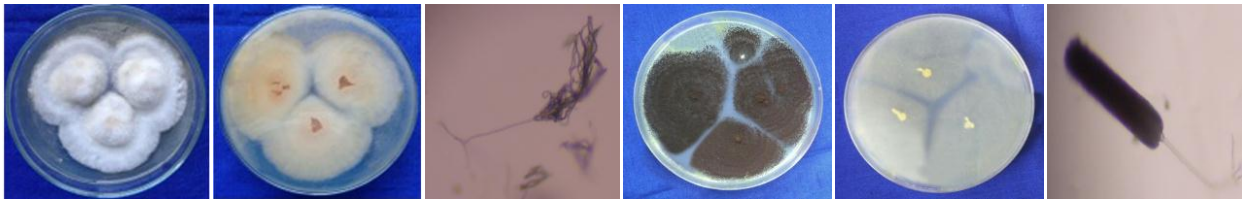
FS-3 FS-4

Fig 4.Colony characteristic and photomicrograph (x400) of FS-1, FS-2, FS-3 and FS-4

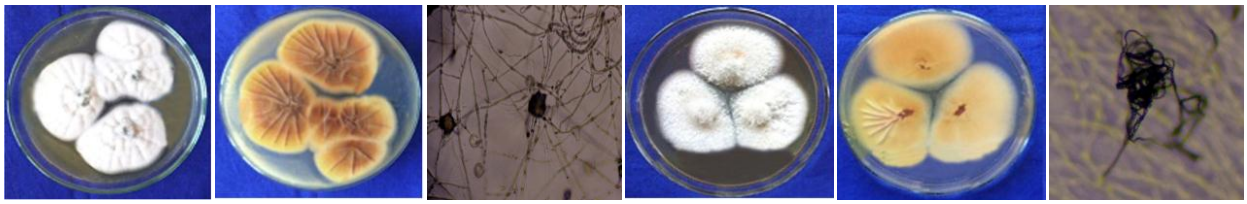


FS-5 FS-6

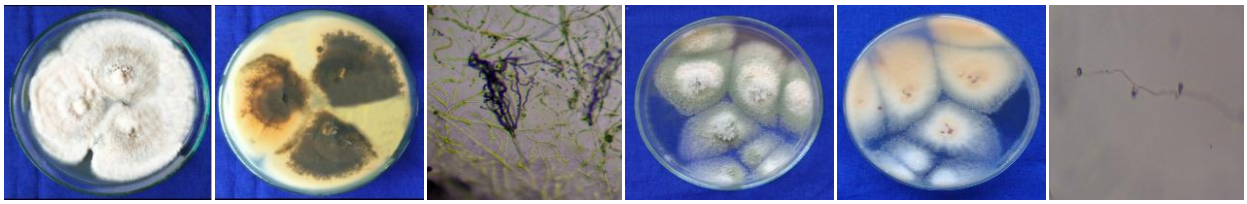
FS-7 FS-8



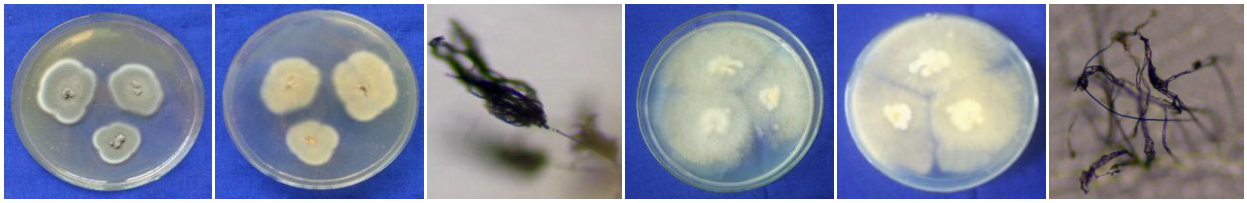
FS-9 FS-10



FS-11 FS-12

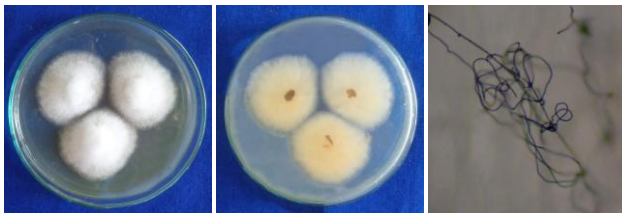


FS-13 FS-14



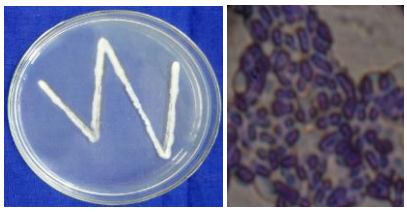
FS-15 FS-16

Fig 5.Colony characteristic and photomicrograph (x400) of FS-5 to FS-16

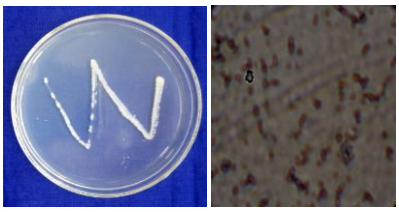


FS-17

Fig 6.Colony characteristic and photomicrograph (x400) of FS-17

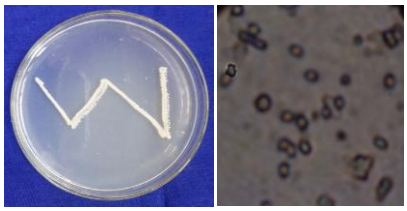


PS-1

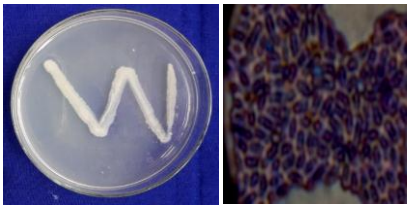


PS-2

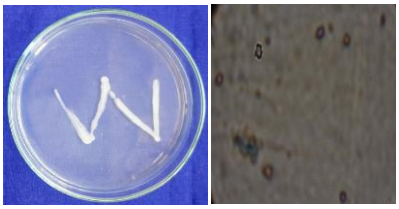
PS-3



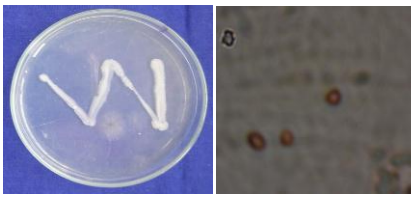
PS-4



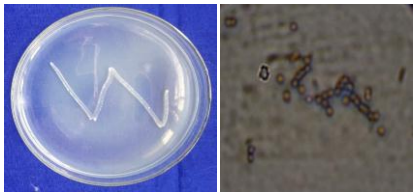
PS-5



PS-6



PS-7

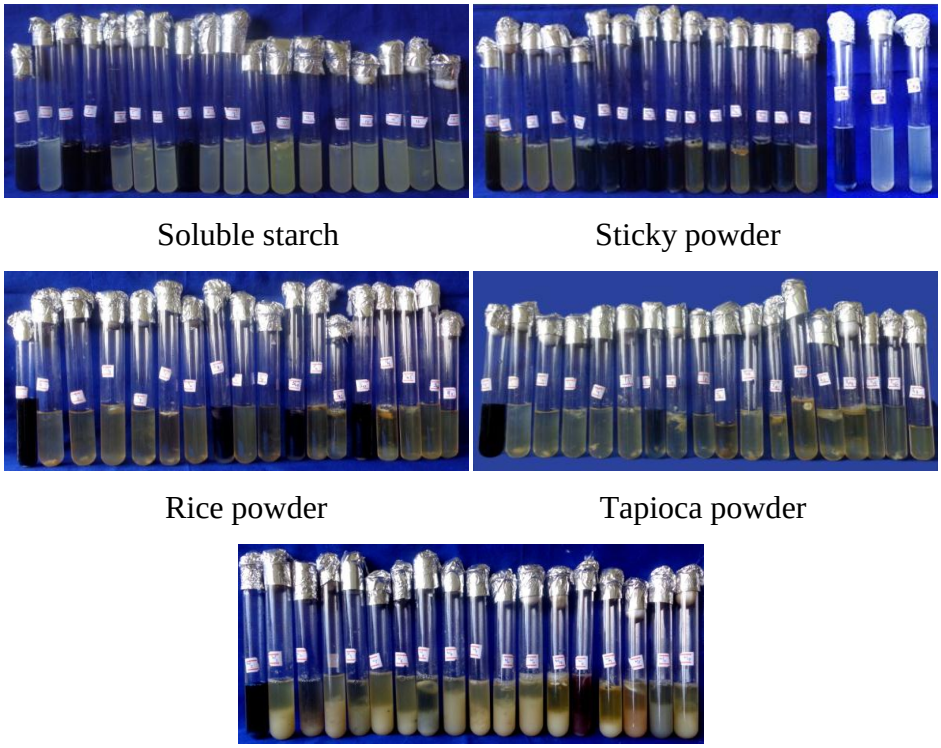


PS-8

Figure 7.Cultural characteristic and cell morphology of isolated from spoilage apple, orange and grap fruit

Biochemical characterization of isolated fungi

These isolated 17 different soil fungi were characterized by biochemical tests and antimicrobial activities as shown in table 4 and figure 9.

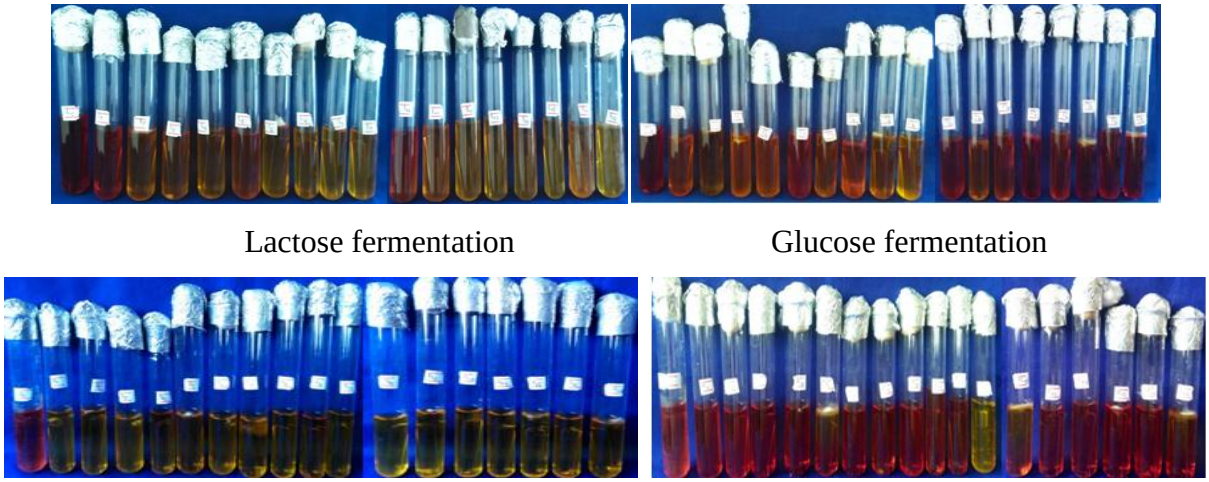


Soluble starch Sticky powder

Rice powder Tapioca powder

Wheat powder

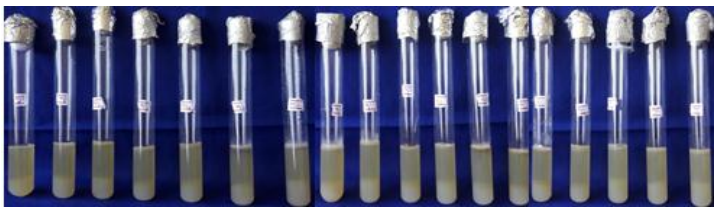
Fig 8. Biochemical test for starch hydrolysis activities of isolated fungi



Lactose fermentation Glucose fermentation

Dextrose fermentation Ribose fermentation

Fig 9.Biochemical test for acid fermentation of isolated fungi

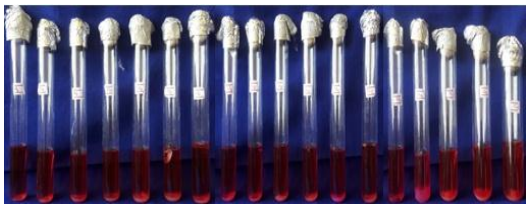


H_2S

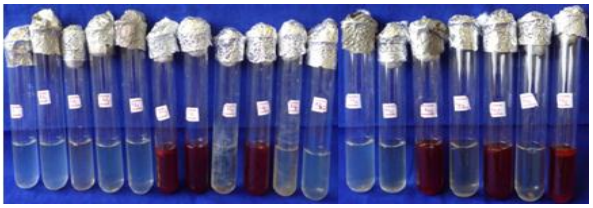
Mannitol salt broth

Methyl red

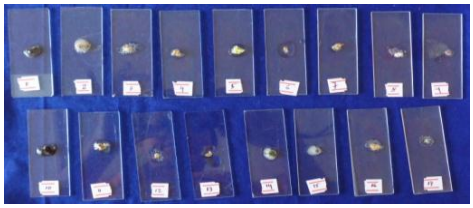
VP



Urea hydrolysis



Nitrate reduction



Catalase test

Casein hydrolysis

Fig 10. Biochemical test for H_2S, Mannitol salt broth, Methyl red, VP, Urea hydrolysis, Nitrate reduction, Catalase test and Casein hydrolysis of isolated fungi

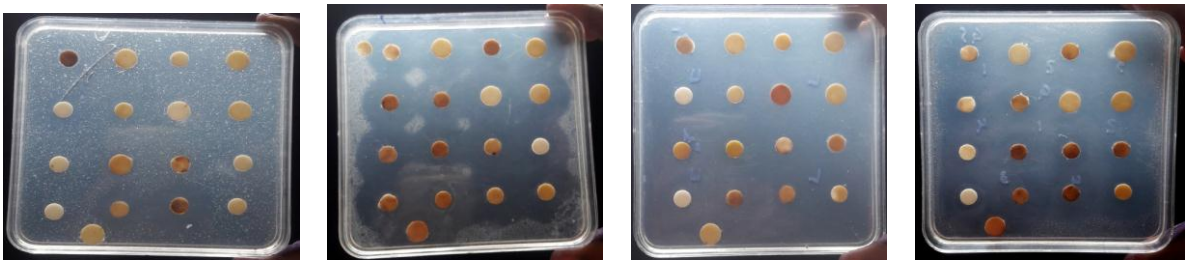
Table 2.Biochemical characteristic of isolated soil fungi

Tests	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Soluble starch	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+
Sticky powder	+	+	+	-	-	-	-	-	+	-	+	-	-	+	-	+	+
Rice powder	+	+	+	+	+	+	-	+	+	-	+	+	-	+	+	+	+
Tapioca powder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Wheat powder	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Lactose gas	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
Glucose gas	+	+	+ +	+	-	+	+	+	+	-	+	-	-	+	-	-	-
Dextrose gas	+ +	+	+ +	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ribose gas	-	-	-	-	+ +	-	-	-	-	-	+ +	+	-	-	-	-	+
H_2S	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
Mannitol salt Broth	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-
Methyl Red	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Voges-Proskauer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urea	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	-
Nitrate Reduction	-	-	-	-	+	+	-	+	-	-	-	-	+	-	+	-	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
Casein Hydrolysis	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-

+ = positive , - = negative

Test for antimicrobial activity of isolated fungi

These isolated 17 different fungi were tested with *Agrobacterium tumefaciens, Bacillus subtilis, Candida albicans, Micrococccus luteus, Escherichia coli, Pseudomonas fluroescens* as shown in table 5 and figure .



Candida albicans *Agrobacterium tumefaciens* *Bacillus subtilis* *Micrococccus luteus*

Figure11. Antimicrobial activities of isolated soil fungi

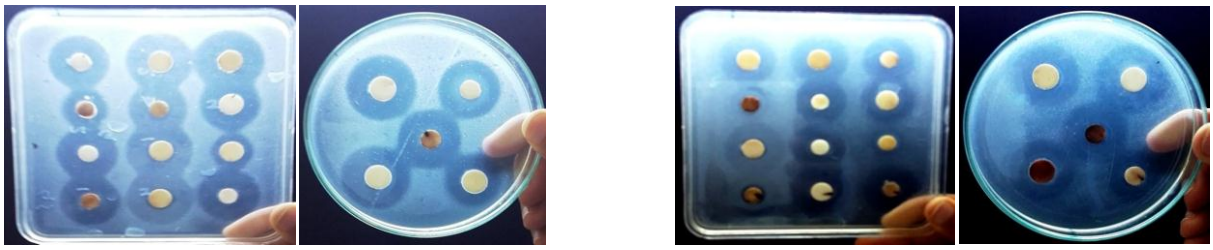


Figure 12.Isolated soil fungi against on PS-5 and PS-8 (isolated from spoilage orange and grape)

DISCUSSION AND CONCLUSION

In the present study, four different soil samples were collected at along North East of Dagon University Campus. These soil samples were used by feeding method (NITE, 2002), for the isolation of fungi. The isolation techniques for isolating effectively new or interesting microorganisms from natural substrata are expected to emerge from the field research. There is no rule for the isolation of microorganisms. Isolation method should be the best way for isolating microbes (Moncalvo, 1997). In the investigation of microbes were isolated 17 different fungi by using LCA and PGA media. A wide range of fungi occur in the soil environment and they have various ecological functions. Most of these fungi can grow on artificial media *in vitro.* These abilities to isolate microorganisms from soil samples and specific media have been developed to select for certain groups of microorganisms.

And then, these isolated 17 different fungi were biochemically testes such as starch hydrolysis, acid fermentation test, H_2S, mannitol salt broth, methyl red, Voges-Proskauer, urea test, Nitrate Reduction, Catalase test, Casein Hydrolysis. The biochemical capabilities of microorganisms are so much, and a wide variety of new or unusual compound may be produced by various microbial isolates (Crueger and Crueger, 1989).

The result of various biochemical tests for 17 different fungi of all soluble starch tests, strain no. FS-2, FS- 3 and FS 7 are negative and all the other showed positives reactions. In

sticky powder test, strain No. FS-4, FS-5, FS-6, FS-7, FS-8, FS-10, FS-12, FS-13 and FS-15 which showed negative and the remaining fungi were found positive. In rice powder, strain No. FS-7, FS-10 and FS-13 were negative and the other strains showed positive. Tapioca powder hydrolysis observed all strains were positive and only strain No. FS-13 was negative and the remaining strains were showed positive in wheat powder hydrolysis. Soil samples can be considered as a new source for isolation of microorganisms because there is much possibility of finding new microorganisms. Therefore, soil samples were utilized for the isolation of starch hydrolyzing enzyme producing microorganisms (Antranikian, 1990).

In the acid fermentation test, expect strains No. FS-1 and FS-10 which are negative on lactose gas test. Strain No. FS-1, FS-2, FS- 3, FS- 4, FS-6, FS-7, FS-8, FS-9, FS-11 and FS-14 possess glucose gas reaction and in all strains possess the dextrose gas test. In the result of ribose gas test, strain No. FS-5, FS-11 and FS-12 are positive and all the others were negative. In H_2S test, the microbes reduce sulfer-containing compounds to sulfide during the prosses of metabolism. Thus, strain No. only FS-5 can break down the hydrogen sulfide more than the other isolated fungi. The test of mannitol salt broth, strain No. FS-7, FS-8 and FS-13 showed positive reaction and all the others were negative reaction. If mannitol has been used by the microbes, acidic byproducts will be produced and the pH of the medium in which the microbe sits will drop (website 6). Methyl red and Voges-Proskauer tests were found negative result. Expect from the strain No. FS-5, FS-8, FS-13, FS-15 and FS-17 and others possess motility. Subsequently, the urease hydrolysis showed strain no. FS-11, FS-13 and FS-14 were positive. In the nitrate reduction test, strain No. FS-5, FS-6, FS-8, FS-13, FS-15 and FS-17 have positive result. Expect from the strain No. FS-13, others fungi possess catalase reaction. In the last test of casein hydrolysis, strain No. FS-3 and FS-9 showed positive and the others do not possess the casein hydrolysis reaction.

However, these isolated 17 different fungi tested with six kinds of antimicrobials such as *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Micrococccus luteus* and *Pseudomonas fluroescen*. Among them, *Escherichia coli* and *Pseudomonas fluroescens* were showed not against on these isolated fungi but they found against on *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Candida albican* and *Micrococccus luteus.* In the antimicrobial activities, FS-2, FS-4, FS-7, FS-12, FS-13 and FS-17 were not against on *Candida albicans* but FS-15 showed the best inhibitory zone (16.20 mm). However, *Agrobacterium tumefaciens* and *Micrococccus luteus*, No.FS-17 showed the best inhibitory zone (45.60 mm) and (40.12 mm) respectively. *Bacillus subtilis* were displayed significant inhibitory zone with all of these isolated fungi in antimicrobial test.

Subsequently, eight kinds of bacteria were isolated spoilage fruit from apple, orange and grape by using the serial dilution method. These isolated bacteria PS-1 and PS-5 were gram positive and bacillus, while PS- 4 gram negative and short rod but the others are gram negative and cocci respectively. These isolated 17 different fungi from soil samples were tested with eight kinds of spoilage fruit bacteria. These isolated 17 different fungi possess activity against spoilage fruit bacteria PS-5 and PS-8 isolated from orange and grape. Among them, fungus FS-14 showed the best inhibitory zone on PS-5 (29.50 mm) and FS-11 found on PS-8 the best inhibitory zone of (30.33 mm) respectively. Therefore, among these isolated 17 different fungi showed the best antimicrobial activity FS-15 and FS-17 showed the best against *Agrobacterium tumefaciens*, *Micrococccus luteus* and *Candida albicans*. However, soil fungi FS-11 and FS-14 were found

possess the antimicrobial activity on antispoilage fruit bacteria on PS-5 and PS-8 with the inhibitory zone of (29.50mm and 30.33mm) respectively. Therefore, it was found that soil fungi can possess the best inhibition of the growth of antispoilage fruit bacteria. Among these isolated soil fungi which possess the best antimicrobial activity will be selected for further investigation.

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