OPTIMIZING FERMENTATION CONDITIONS OF SELECTED SOIL FUNGUS *PENICILLIUM RUGULOSUM* AGAINST *ESCHERICHIA COLI*

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Abstract

In this research work, eighteen fungi were isolated from nine soil samples collected in Magway Township Magway Region, by using chemical treatment dilution and soil dilution methods. All isolated fungi were tested with ten kinds of test organisms were using paper disc diffusion assay method. In the study of fungus KFO-06, *Penicillium rugulosum* showed the highest antibacterial activity on *Escherichia coli*. Therefore, this fungus was selected for further investigation. In the study of carbon and nitrogen utilization of *Penicillium rugulosum* for the growth, tapioca powder was excellent for carbon source and peanut cake was excellent for nitrogen source. However, it was observed that the highest antibacterial activity with obtained by using glycerol as carbon source and yeast extract as nitrogen source. Six fermentation media were used for fermentation study and it was found that FM-I showed the highest antibacterial activity on test organism *Escherichia coli* than other medium. In the fermentation studies 72 hrs seed cultures and 15% size of inoculums were optimized for the fermentation.

Keywords Fermentation, Optimization, Antibacterial activity

Introduction

Microorganisms play a major role in soil ecosystem along as fungi, bacteria, protists, small invertebrates and plants, through complex, tropic interactions. They are very important functional group of soil organisms (Abigail *et al.*, 2005). One gram of soil may harbor up to 10billion microorganisms of possible thousands of different species. Soil and marine environments contain thousands of unknown microbial species, many of them fungi. Soil microorganism are involved in many biogeochemical processes (Arnold, 2001). Fermentation procedure must be developed for the cultivation of microorganisms under optimal conditions and for the production of desired metabolites or enzymes by the microorganisms (Yamane and shimizu, 1984). The aim of the present investigation was to find out the effects of age and size of inoculums, carbon and nitrogen sources for the production of antibacterial metabolites.

Materials and Methods

Effect of Carbon and Nitrogen Sources for the Growth of Fungus KFO-06, *Penicillium rugulosum*

Carbon sources (each 1.0g) such as glucose, sucrose, glycerol, soluble starch, fructose, molasses, potato powder and tapioca powder were used. Above carbon sources with basal medium was Yeast extract 0.2 g, Polypeptone 0.3 g, K_2HPO_4 0.001g, $MgSO_4$.$7H_2O$ 0.001g, $CaCO_3$ 0.02g, DW 100mL, pH 6.5.

Nitrogen sources (each 0.5g) such as Yeast extract, NZ amine type A, Polypeptone, Meat extract, KNO_3, Rice bran, Peanut and Fish cake were utilized. Above nitrogen sources with basal medium was Glucose 1.5g, Glycerol 0.5g, K_2HPO_4 0.001g, $MgSO_4$ $7H_2O$0.001g, $CaCO_3$ 0.02g, DW 100mL, pH 6.5.

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The Microbial Growth Kinetic of Fungus KFO-06

Microbial growth kinetic of fungus KFO-06 was investigated by the methods of Crueger and Crueger, 1989; Phay, 1997; Theobald *et al*., 2000; Singh, 2008. Seven days old culture (agar plate) was inoculated into the seed culture 100 ml medium (Glucose 2.0%, Yeast extract 0.5%, Polypeptone 0.5%, pH 6.5). The cultured was performed for 120 hrs at 25·C. Dried Cell Weight (DCW%) was measured 12 hrs intervals (Omura,1985; Phay,1997; Theobald *et al*., 2000; Singh, 2008).

Effect of Ages of Inoculums for the Fermentation

The fungus 7 days old culture was transferred into seed medium. Seed cultures of 66 hrs, 72 hrs, 78 hrs, 84 hrs, 90 hrs and 96 hrs incubation was inoculated into the conical flasks containing fermentation medium. The procedure for the study on the effects of ages inoculums was shown in figure 1. After that, seed culture was checked in 6 hours intervals for the ages.

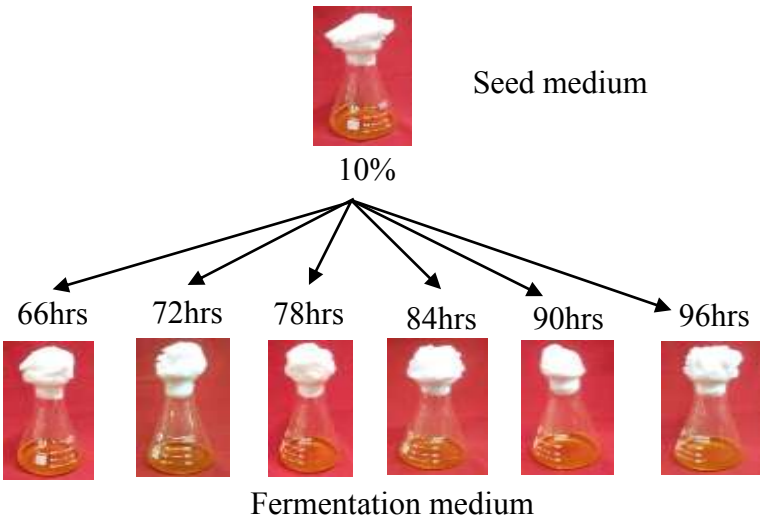


Figure 1 Procedure for the effects of ages of inoculums for the fermentation

Effect of Sizes of Inoculums for the Fermentation

In the study of sizes of inoculums, 5%, 10%, 15%, 20%, 25%, 30% of 72hrs seed culture were utilized for the fermentation. Fermentation was carried out 8 days and antibacterial activity was tested by paper disc diffusion assay (Omura,1985, Crueger and Crueger 1989). Figure 2.

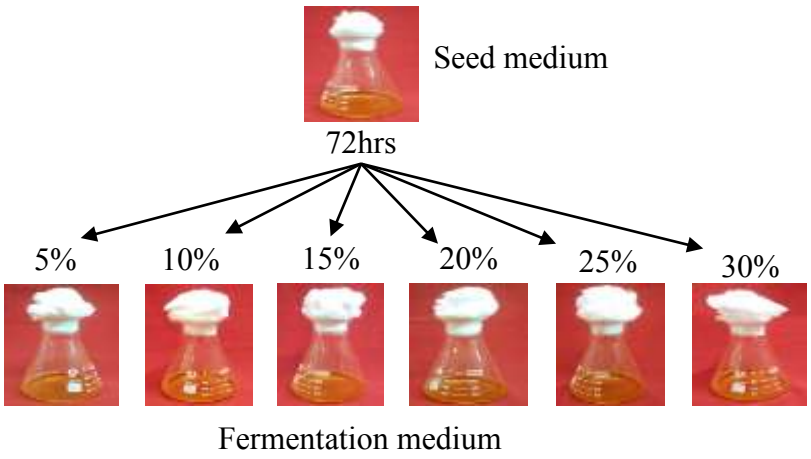


Figure 2 Procedure for the effects of sizes of inoculums for the fermentation

Effects of Carbon and Nitrogen Sources for the Fermentation

In the study of the effects of carbon and nitrogen sources for the fermentation. Carbon sources with basal medium Yeast extract 1.0%, Polypeptone 0.7%, $K_2HPO_47H_2O$ 0.001g, $FeSO_4$ 0.001g, $CaCO_3$ 0.03g, DW 100ml, pH 6.0 and nitrogen sources with basal medium Glucose 2.0g, Glycerol 1.0g, $K_2HPO_47H_2O$ 0.001g, $FeSO_4$ 0.001g, $CaCO_3$ 0.03g, DW 100ml, pH 6.0 were utilized with 72 hrs. age and 15% size of seed culture at 25·C for 8 days.

Medium Optimization for Fermentation and Production of Antibacterial Substance

In the investigation of the effects of fermentation medium for the production of metabolites six kinds of media were utilized with 72 hrs age and 15% size of seed culture at 25·C for 8 days depending on the utilization of carbon and nitrogen sources.

NITE (2004)

Seed Medium		**Assay Medium**		**Fermentation Medium**	
Glucose	1.5 g	Glucose	1.0 g	Glucose	1.0 g
Glycerol	0.5 mL	Yeast extract	0.3 g	Glycerol	1.5 mL
Yeast extract	0.8 g	Agar	1.8 g	Malt extract	0.4 g
Polypeptone	1.0 g	DW	100 mL	Yeast extract	1.0 g
K_2HPO_4	0.001 g	pH	6.5	Polypeptone	0.6 g
$MgSO_4.7H_2O$	0.001 g			MgSO4.7H2O	0.001 g
DW	100 mL			K2HPO4	0.001 g
pH	7			CaCO3	0.1 g
				DW	100 mL
				pH	6.5

Fermentation Media (NITE, 2005)

FM-1		**FM-2**		**FM-3**	
Glucose	1.0 g				
Glycerol	1.0 mL	Glucose	1.0 g	Glycerol	1.0 mL
Yeast extract	0.8 g	Sucrose	0.5 g	Yeast extract	0.8 g
Polypeptone	0.8 g	Yeast extract	1 .0 g	Peptone	0.8 g
K_2HPO_4	0.001 g	K2HPO4	0.001 g	K2HPO4	0.001 g
DW	100 mL	DW	100 mL	DW	100 mL
pH	6.5	pH	6.5	pH	6.5

FM-4		**FM-5**		**FM-6**	
Tapioca power	1.0 g	Glucose	0.5 g	Glycerol	1.0 mL
Glycerol	1.0 g	Molasses	1.5 g	Soluble starch	1.0 g
Sucrose	0.5 g	KNO3	0.5 g	Yeast extract	0.8 g
Fish cake	1.0 g	Yeast extract	1.0 g	Polypeptone	1.0 g
K_2HPO_4	0.001 g	K_2HPO_4	0.00 1g	K_2HPO_4	0.001 g
DW	100 m	DW	100 mL	DW	100 mL
pH	6.5	pH	6.5	pH	6.5

Results

Effects of Carbon and Nitrogen Sources for the Growth of Fungus KFO-06, *Penicillium rugulosum*

In the investigation, the selected fungus KFO-06 were used carbon source such as, Sucrose, Glycerol, Tapioca powder, Potato powder, Glucose, Soluble starch, Molasses and Fructose. Nitrogen sources such as KNO_3 Fish cake, Yeast extract, Meat extract, Polypetone, NZ amine type A, Peanut cake and Rice bran were utilized. The excellent growth of the fungus was found on carbon sources such as Tapioca powder, nitrogen sources such as Peanut cake. It was found that the good growth of KFO-06 on carbon source was sucrose, Glycerol, Glucose, Soluble starch, Molasses and Fructose and nitrogen source was KNO_3 Fish cake, Yeast extract, Meat extract, Polypetone, NZ amine type A and Rice bran. Carbon source of Potato powder gave poor growth for fungus KFO-06. These results are shown in Tables 1 and 2, Figures 3 and 4.

Table 1 Morphological Characters of Fungus KFO-06 on Various Carbon Sources

Carbon sources	Growth
Sucrose	Good
Glycerol	Good
Tapioca powder	Excellent
Potato powder	Poor
Glucose	Good
Soluble starch	Good
Molasses	Good
Fructose	Good

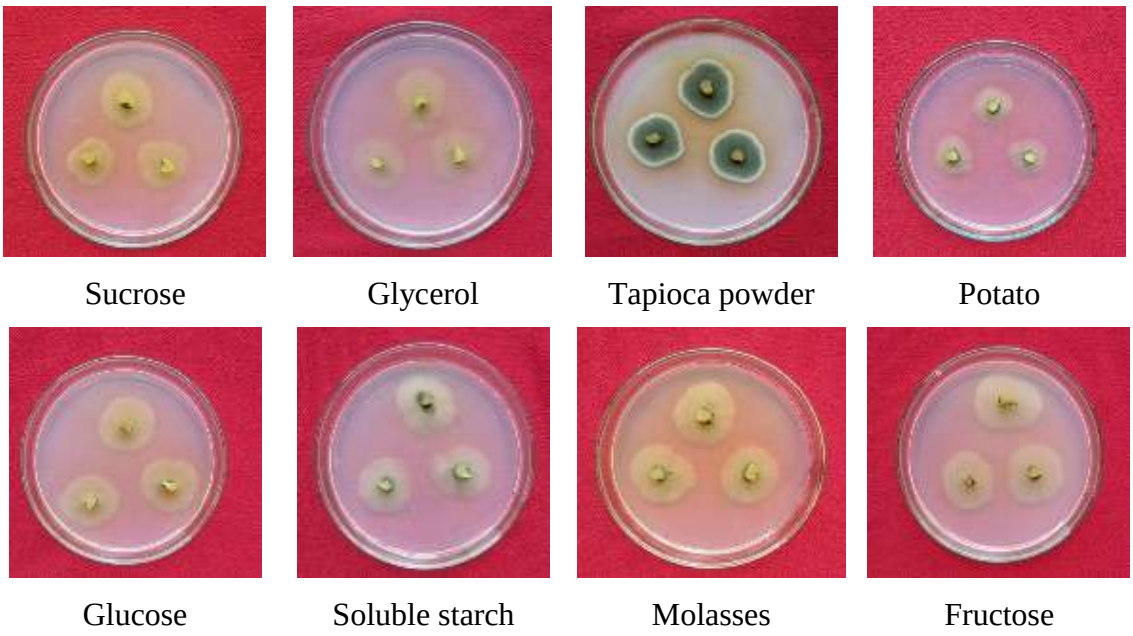


Figure 3 Morphological characters of fungus KFO-06 on various carbon sources (7-days old culture)

Table 2 Morphological Characters of Fungus KFO-06 on Various Nitrogen Sources

Nitrogen sources	Growth
KNO_3	Good
Fish cake	Good
Yeast extract	Good
Meat extract	Good
Polypeptone	Good
NZ amine type A	Good
Peanut cake	Excellent
Rice Bran	Good

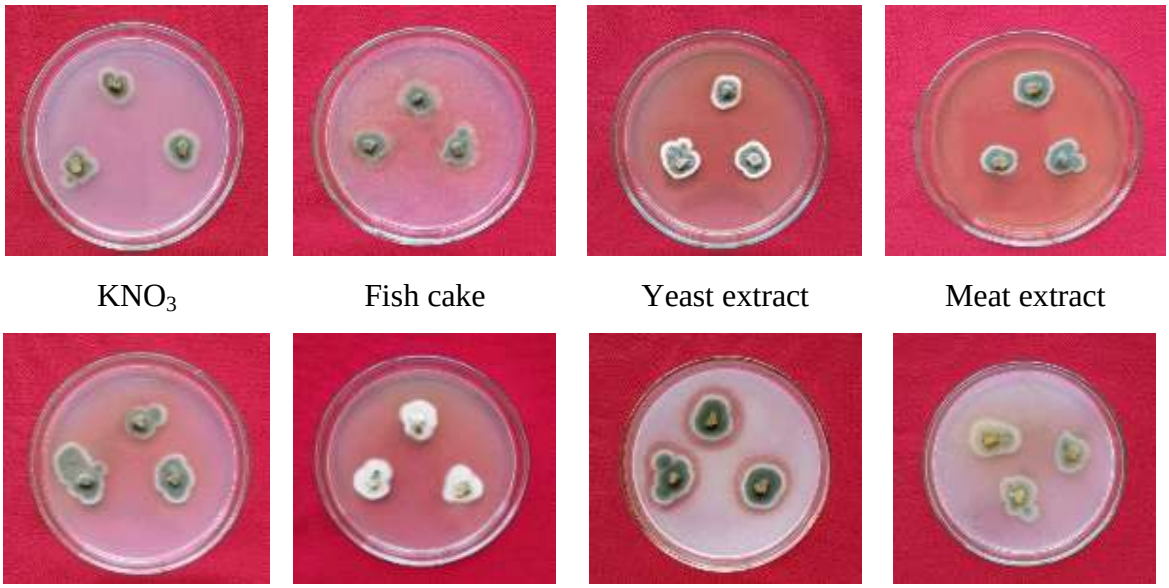


Figure 4 Morphological characters of fungus KFO-06 on various nitrogen sources (7-days old culture)

The Microbial Growth Kinetics of *Penicillium rugulosum*

In the microbial growth kinetics study, as shown in Figure 5, it was found that the lag phase was between 48 hrs and 66 hrs. Growth phase was between 66 hrs and 96 hrs. It was considered that growth phase (66 hrs to 96 hrs) is to be conducted to optimize the age of inoculum for the fermentation. Therefore, the effect of age of inoculums (66 hrs to 96 hrs) was continuously investigated for the fermentation.

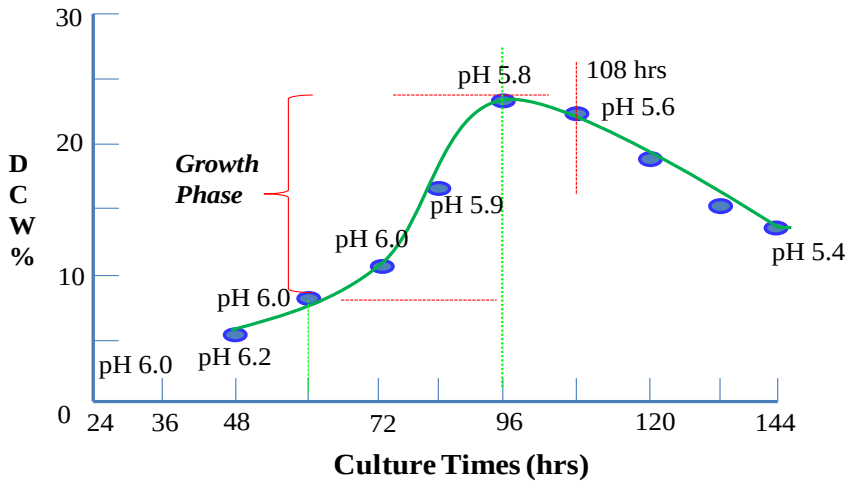


Figure 5 Microbial growth kinetics of *Penicillium rugulosum*

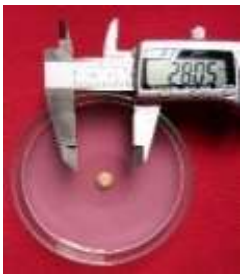
Ages of Inoculums for the Fermentation

In the investigation of the age of inoculums, six different hours of 66 hrs, 72 hrs, 78hrs, 84 hrs, 90 hrs and 96 hrs were used and the results showed that the inhibitory zone of 23.15mm, 28.05mm, 24.03mm, 22.31mm, 20.75mm and 19.18 mm, respectively against *Escherichia coli*. It was observed that 72hrs seed culture showed the best activity on *Escherichia coli* than others seed culture. Therefore, 72 hrs seed culture was selected for the fermentation as shown in Table 3 and Figure 6.

Table 3 The Effects of Ages of Inoculum for KFO-06 against *Escherichia coli*

Culture time (hrs)	Activity (Clear zone, mm)
66	23.15
72	28.05
78	24.03
84	22.31
90	20.75
96	19.18

66 hrs



72 hrs

78 hrs



84 hrs **90 hrs** **96 hrs**

Figure 6 The effects of ages of inoculum on the fermentation of fungus KFO-06

Based on the result, 72 hrs age of seed culture showed the inhibitory zone 28.05mm on *Escherichia coli.*

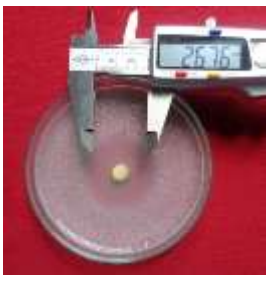
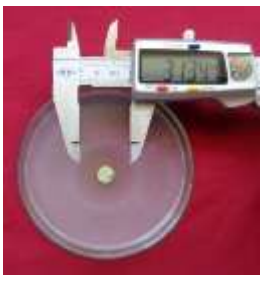
Sizes of Inoculums for the Fermentation

In the study, seed culture 5%, 10%, 15%, 20%, 25% and 30% six conditions (six flasks) were employed to determine the size of inoculums for the fermentation. The results showed the inhibitory zone of 19.16mm, 26.76mm, 31.34mm, 27.00mm, 22.42mm and 18.46mm, respectively against *Escherichia coli.* It was found that 15% size of inoculums gave the best activity on *Escherichia coli.* (Table 4, Figure 7). According to the results, it was determined that 15% inoculum was proper condition to carry out the fermentation.

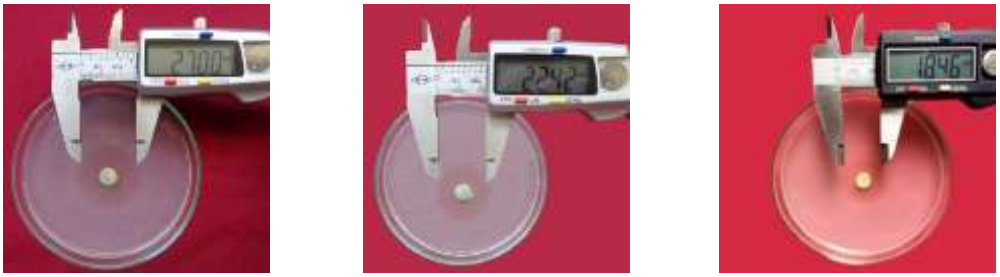
Table 4 The Effects of Sizes of Inoculum for KF-06 against *Escherichia coli* (activity at 5 days fermentation)

Sizes of Culture, %	Antibacterial activity (Clear zone, mm)
5 %	19.16
10 %	26.76
15 %	31.34
20 %	27.00
25%	22.42
30%	18.46

According to the results, 15% size of inoculum was selected for fermentation.

5% **10%** **15%**



20% **25%** **30%**

Figure 7 The effects of sizes of inoculum for KFO-06 against *E.coli*

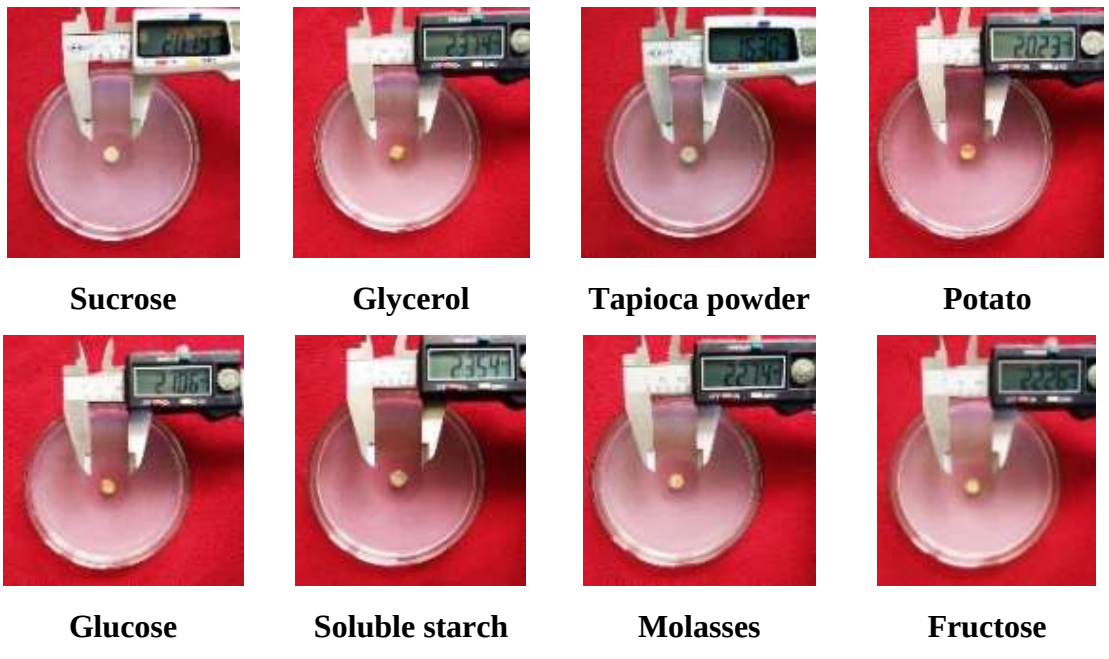
According to the result, 15% size of inoculum gave the best activity on *Escherichia coli*.

The Effects of Carbon and Nitrogen Sources for the Fermentation

The effects of carbon and nitrogen sources for the fermentation study were shown in (Table 5 and Figure 8) and (Table 6, Figure 9), respectively.

Table 5 The Effects of Carbon Sources on the Fermentation

Carbon sources	Inhibitory zone (mm)
Sucrose	20.79
Glycerol	**23.74**
Tapioca powder	16.30
Potato powder	20.23
Glucose	21.06
Soluble starch	23.54
Molasses	22.74
Fructose	22.26



Sucrose **Glycerol** **Tapioca powder** **Potato**

Glucose **Soluble starch** **Molasses** **Fructose**

Figure 8 Antibacterial activity of KFO- 06 on various carbon sources

Table 6 The Effects of Nitrogen Sources on the Fermentation

Nitrogen sources	Inhibitory zone (mm)
KNO_3	10.16
Fish cake	11.33
Yeast extract	**22.31**
Meat extract	18.62
Polypeptone	17.00
NZ amine type A	18.46
Peanut cake	12.56
Rice Bran	9.86

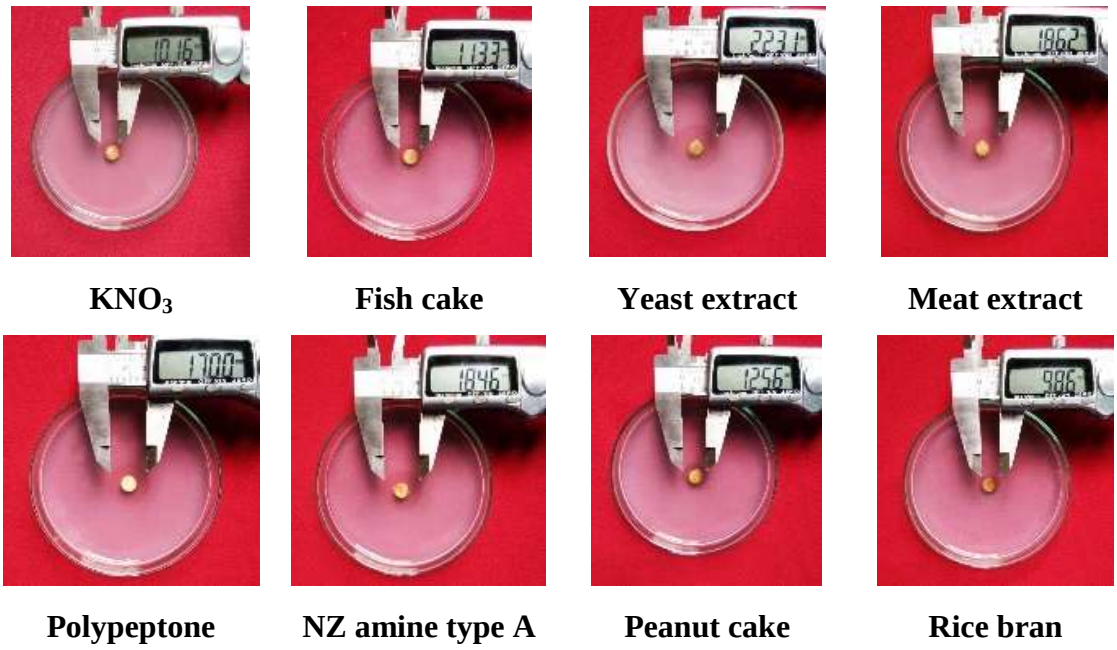


Figure 9 Antibacterial activity of KFO- 06 on various nitrogen sources

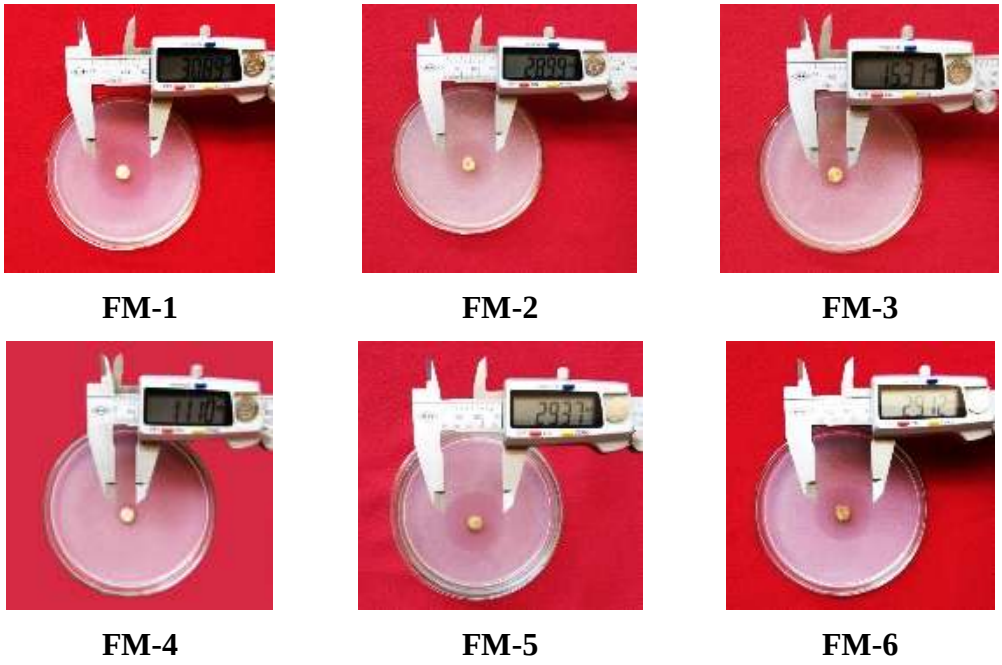
Medium Optimization for Fermentation and Production of Antibacterial Metabolites

In the study of medium optimization six kinds of fermentation media were used. It was observed that in the six kinds of fermentation of the inhibitory zone of FM-1showed 30.89 mm, FM-2, 28.99 mm, FM-3, 15.31mm, FM-4, 11.10 mm, FM-5, 29.37mm and FM-6 29.12mm inhibitory zone respectively. It was determined that FM-1 showed the best activity on *Escherichia coli* (Table 7 and Figure 10). Therefore, FM-1 was selected for the production of antibacterial metabolite.

Table 7 Selection of Medium Based on the Results of Antibacterial Activity

Fermentation media	Inhibitory zone (mm)
FM-1	**30.89**
FM-2	28.99
FM-3	15.31
FM-4	11.10
FM-5	29.37
FM-6	29.12

According to the results, FM-1 is the highest activity on *E. coli.*



FM-1 **FM-2** **FM-3**

FM-4 **FM-5** **FM-6**

Figure 10 Antibacterial activity of KFO- 06 on various fermentations

Based on the results, FM-1 gave the best activity on *E. coli* than other medium.

Discussion

The balance of nutrients (carbon and nitrogen sources) are crucial for the fermentation to produce the metabolite (Omura, 1985). Fermentation medium is also important for the production of metabolite (Crueger and Crueger, 1989). In the study of KFO-06 carbon and nitrogen containing media were investigated, tapioca powder was excellent; glucose, glycerol, sucrose, soluble starch, molasses and fructose were good and potato powder was poor for carbon source. Satish, 2008 reported that glucose was found to be the suitable carbon source for growth of microorganisms. NZ amine type A, yeast extract, KNO_3, fish cake, polypeptone, rice bran and meat extract were good and peanut cake was excellent for nitrogen sources.

In the microbial growth kinetic study, it was found that the lag phage was between 48 hrs and 66 hrs. Growth phase was between 66 hrs and 96 hrs. It was considered that growth phase (66 hrs to 96 hrs) is to be conducted to optimize the age of inoculum for the fermentation.

Therefore, the effect of age of inoculums (66 hrs to 96 hrs) was continuously investigated for the fermentation.

The fermentation studies, the growth phase was between 66 hrs to 96 hrs. It was observed that the growth of fungus KFO-06 declined after 96 hrs. The results obtained in this study show that organic nitrogen sources such as yeast extract support rapid growth and antibacterial productions. Several authors suggested that yeast extract is a good substrate for many microorganisms (Smith *et al.*, 1975). In the studies of the effects of ages and sizes of inoculum for fermentation, it was observed that 72 hr ages and 15% sizes of seed culture were suitable for the fermentation. Six kinds of fermentation media were used for the antibacterial activity of selected fungus KFO-06. According to the results of antibacterial activity, fermentation medium FM-1 was selected for the production of antibacterial metabolite. It was concluded that according to the result, FM-1 was the optimum fermentation medium for fungus KF-06 against *Escherichia coli.* Senthi, 2017 reported that the isolated soil fungi showed maximum antibacterial activity against *Escherichia coli.* It is thus indispensable that optimal effect of fermentation conditions can only produce the best result in metabolite production.

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