THE EFFECTS OF CARBON AND NITROGEN SOURCES FOR THE GROWTH OF SOIL FUNGUS MPF- 7 AND ITS FERMENTATION OPTIMIZATION

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Abstract

In this research work, selected fungal strain collected from Mudon Township. The present study was focused on the utilization of carbon and nitrogen sources for the growth and fermentation conditions of soil fungus MPF-7 on *Escherichia coli*. In the growth effect of the carbon and nitrogen, the excellent growth of MPF-7 was found on glucose and peptone. In the study of fermentation conditions, MPF-7 showed the highest antibacterial activity (22.06 mm) by using 15% seed culture while the best inoculum age was found at 108 hrs on *E. coli*. In addition of glucose as the carbon source and peptone as nitrogen source in the fermentation, MPF-7 showed the highest antibacterial activity (21.05 mm) and (30.78 mm) on *E. coli*. Maximum antibacterial activity was observed at pH 6 (23.36 mm). In the temperature effect, the strong activity was obtained at 25ºC (23.18 mm). In the comparison effect of static and shaking culture of MPF-7, the maximum antibacterial metabolite was observed under shaking condition (20.00 mm) and the static culture of MPF-7 showed the activity (17.87 mm) on *E. coli* respectively.

Keywords: Soil fungi, antimicrobial activity, fermentation optimization

Introduction

The large number of known bioactive compounds (primary and secondary metabolites) of microbial origin are currently produced by fermentation (Gaden, 1959). Therefore the fermentation conditions such as substrates inoculum cultivation and transfer have to optimize for the production of primary and secondary metabolites (Dale, 1984). Carbon and nitrogen sources together with fermentation time have been described to play significant roles in the determination of the final morphology of the culture (Papagianni, 2004). The nature of the nitrogen source has a prominent effect on the production of the antimicrobial metabolite. High nitrogen levels have been noted to repress idiophase production of antibiotics (Spizek J *et al.*, 1995).

Control of ammonia concentration through the mid-cycle was create to be important in the optimization of idiophase secondary metabolite production (Junker *et al*., 1998), though this may reveal the role of nitrogen in growth promotion.

Antibiotic formation usually follows during the late growth phase of the producing microorganism. Microorganisms have developed different mechanisms for uptake and assimilation of mineral and organic forms of N, enabling them to utilize a wide range of organic and mineral compounds (see reviews by Merrick, 1995; Marzluf, 1997).

The production of antimicrobial ingredients be partial by upon the substrate medium for their best growth, temperature, pH and the concentration of nutrients in the medium (Leifert *et al*., 1995). Incubation age and temperature are vital factors that modulate lab growth and significantly affect the amounts of antimicrobial metabolites produced. Production of antibiotics occurs during a distinct idiophase of culture growth phase (Thaer, 2017).

Microbial production of antibiotics is one of the rapidly increasing branches of industrial microbiology. The investigation of new habitats plays a pivotal role in search of new microbes

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possessing potentials to produce novel metabolites, and is urgent to counter the threats posed by the fast developing phenomenon of antibiotic resistance (Shiburaj, 2003).

The aim and objectives of this research were to investigate the utilization of carbon and nitrogen sources of the fungal growth and to optimize the fermentation conditions.

Materials and Methods

Preliminary study for antimicrobial activity

The selected fungus (MPF- 7) was grown of BMEA medium for 3 days. The selected fungus was inoculated into 25 mL seed medium and incubated at room temperature. After 3 days, 25 mL seed culture was transferred into the 25 mL of fermentation medium and incubated at room temperature. Fermentation was carried out for 3-7 days.

Medium used in Antimicrobial activity test (Ando *et al,* 2004)

(**Seed medium** Glucose 20.0 g, Sucrose 3.0 g, Yeast Extract 3.0 g, $KNO_3$1.0 g, K_2HPO_4 0.1 g, Distilled Water 1000 mL, pH 6.5), (**Fermentation medium** (Glucose 20.0 g, Yeast Extract 3.0 g, K_2HPO_4 0.01g, $MgSO_4$. H_2O 0.01 g, $CaCO_3$ 1.0 g, Distilled Water 1000mL, pH 7) and (**Assay (GYP) medium** Glucose 50.0 g, Yeast Extract 30.0 g, Peptone 30.0 g, Agar 14.0 g, Distilled Water1000 mL, pH 6.5).

Agar well Method (Collins, 1965)

Selected strain was tested by agar well method for the antimicrobial activities. One day old culture test (0.1 mL) was added to 100 mL of assay medium and thoroughly mixed and poured into plate. After solidification, cork borer was left to set. Cork borer was used to make the wells (8 mm in diameter) in the autoclaved basal antimicrobial test-medium. Wells impregnated with 3-7 days old culture fermented broth (20 µL) was carefully added into the wells and incubated at room temperature for 24 to 48 hours. Therefore, the diameter of the zones had been observed as potent activity as shown by respective strain. Clear zones surrounding the wells indicated the presence of antimicrobial activities which inhibit the growth of the test organisms selectively.

Table 1 Test Organism used for Antimicrobial Activity

Test No	Test Organism	Diseases
1	*Escherichia coli* AHU5436	Diarrhea, pneumonia, abdominal pain

The Utilization of Carbon and Nitrogen Sources for the Fungal Growth

The optimal fermentations are very important for maximal productivity metabolites. To determine the effect of carbon sources on antimicrobial metabolite production from MPF-7, different carbon sources such as glucose, fructose, lactose, xylose, potato, soluble starch, sucrose, corn and tapioca were used. Nitrogen sources such as peptone, polypeptone, casein, yeast, gelatin, malt, potassium nitrate, sodium nitrate, ammonium nitrate, ammonium sulfate, ammonium chloride and fish cake were also used.

Study on the effects of age and size of inoculum for the fermentation

The proper cultivation (age) and transfer (size) of the inoculum is crucial for the production of metabolites (Crueger & Crueger, 1989; Emily, 2009). In these studies, the seed culture - 5%, 10%, 15%, 20%, 25% and 30% were employed for the fermentation and age of seed culture was employed at 72, 84, 96, 108, 120 and 132 hrs.

Study on different carbon sources utilization for the fermentation

Carbon sources (each 1.0 g or 1.0 mL) such as glucose, fructose, lactose, xylose, potato, soluble starch, sucrose, corn and tapioca were used. Fermentations were incubated at 25ºC for 6 days.

Study on different nitrogen sources utilization for the fermentation

Nitrogen sources (each 1.0 g or 1.0 mL) such as peptone, polypeptone, casein, yeast, gelatin, malt, potassium nitrate, sodium nitrate, ammonium nitrate, ammonium sulfate, ammonium chloride and fish cake were used. Fermentations were incubated at 25ºC for 6 days.

Effect of pH

The optimum pH of the fermentation medium for antibacterial metabolite production was done by carried out the fermentation at seven different pH values such as 4, 5, 6, 7, 8, 9 and 10. For each pH values, 25 mL of fermentation medium (adjusted to desired pH by using either 1 N NaOH or 0.1 N HCl) was taken in 100 mL conical flasks and autoclaved at 121ºC for 45 minutes. The inoculated flasks were incubated at 25ºC.

Effect of temperature

The optimization temperature for antibacterial metabolite production was undertaken. MPF-7 was incubated at five different temperatures 20, 25, 30, 35, 40 and 45ºC. The fermented broths were tested by using ager well diffusion assay for antibacterial activity against *E.coli*.

Effect of agitation

The antibacterial activity of MPF-7 was studied at two conditions such as agitation (shaking) and stationary (static) conditions.

Results

In the present study, the growth of soil fungus MPF -7 was studied on various carbon and nitrogen sources. Among the carbon sources, the excellent growth of MPF -7 was found on glucose (47.57-49.19 mm) followed by fructose (41.50-43.34 mm) and potato (39.89-41.56 mm). Among the carbon sources, the excellent growth of MPF -7 was found on peptone (49.67-51.98 mm) followed by polypeptone (47.45-49.34 mm), fish cake (45.34-47.37 mm), KNO_3 (41.54-43.86 mm) and Malt extract (37.56-40.32 mm) respectively.

In the size of inoculum, MPF-7 showed the highest antibacterial activity showed at 15% seed culture followed by 10% seed culture (20.79 mm) In the age of inoculum, MPF-7 showed the best activity (18.64 mm) at 108 hrs on *E. coli*. In addition of glucose as the carbon source, MPF-7 showed the highest antibacterial activity (21.05 mm*)* followed by fructose, lactose and soluble starch.

The effect of nitrogen sources were also used and the best result was found in peptone (30.78 mm). Maximum antibacterial activity was observed at pH 6 (23.36 mm). In the temperature effect, the strong activity was obtained at 25ºC (23.18 m) on *E. coli*. In the comparison effect of static and shaking culture of MPF-7, the maximum activity was observed under shaking condition (20.00 mm) and the static culture of MPF-7 showed activity (17.87 mm) on *E. coli* respectively.

Table 2 Colony character and growth of MPF-7 on various carbon sources

Sr. No	Carbon sources	Surface colour	Reverse colour	Size (mm)	Growth
1	Glucose	Brownish green	Brownish cream	**47.57-49.19**	**Excellent**
2	Lactose	Brownish green	Brown	37.35-39.49	Good
3	Sucrose	Pale green	Cream	35.34-37.39	Good
4	Xylose	Brownish green	Brownish cream	37.56-39.23	Good
5	Fructose	Brownish green	Brownish cream	41.50-43.34	Excellent
6	Soluble starch	Brownish green	Brownish cream	37.35-39.37	Good
7	Potato	Brownish green	Brownish cream	39.89-41.56	Excellent
8	Tapioca	Brownish green	Brownish cream	36.56-38.34	Good
9	Corn	Green	Cream	35.50-37.34	Good

20-30 mm = Moderate growth, 30-40 mm = Good, 40 to above = Excellent

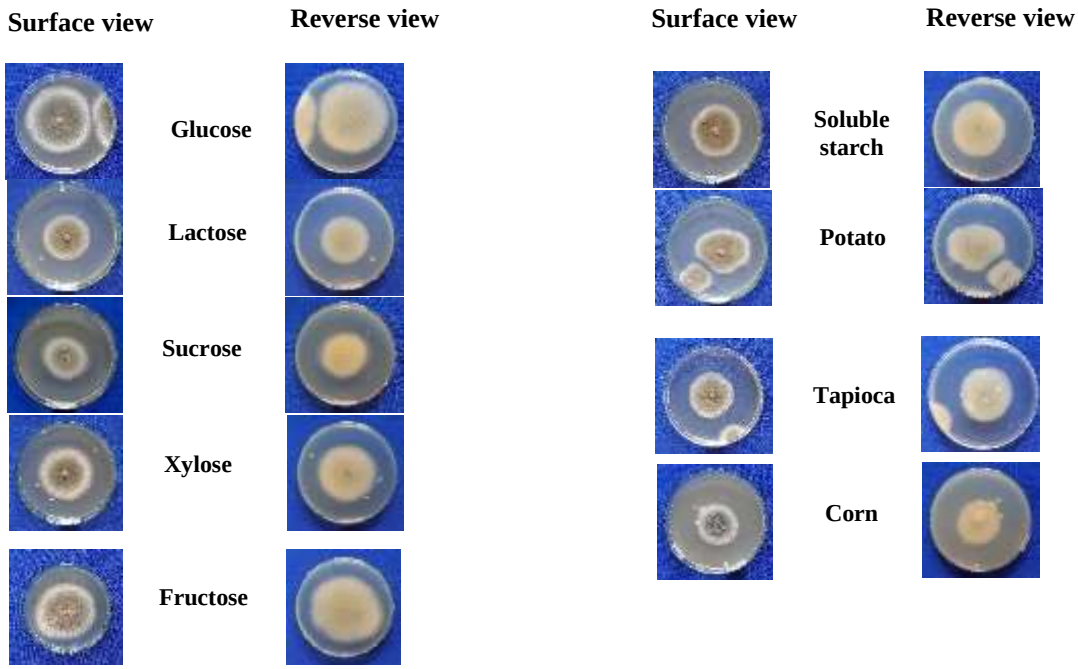

Figure 1 Colony character and growth of MPF-7 on various carbon sources

Table 3 Colony character and growth of MPF-7 on various nitrogen sources

Sr. No	Nitrogen sources	Surface colour	Reverse colour	Size (mm)	Growth
1	Peptone	Brownish cream	Greenish cream	**49.67-51.98**	**Excellent**
2	Polypeptone	Brownish cream	Greenish cream	47.45-49.34	Excellent
3	Casein	Greenish cream	Cream	37.56-39.67	Good
4	Yeast	Cream	cream	35.12-37.56	Good
5	Gelatin	Brownish cream	cream	29.56-33.13	Good
6	Malt	Pale green	Cream	37.56-40.32	Excellent
7	KNO_3	Brownish cream	Greenish cream	41.54-43.86	Excellent
8	$NaNO_3$	Brownish cream	cream	25.21-27.98	Moderate
9	NH_4NO_3	Brownish cream	cream	25.67-26.87	Moderate
10	$(NH4)_2SO_4$	Cream	White	23.34-25.67	Moderate
11	NH_4Cl	White	White	21.66-25.98	Moderate
12	Fish cake	Brownish green	Green cream	45.34-47.37	Excellent

20-30 mm = Moderate growth, 30-40 mm = Good, 40 to above= excellent

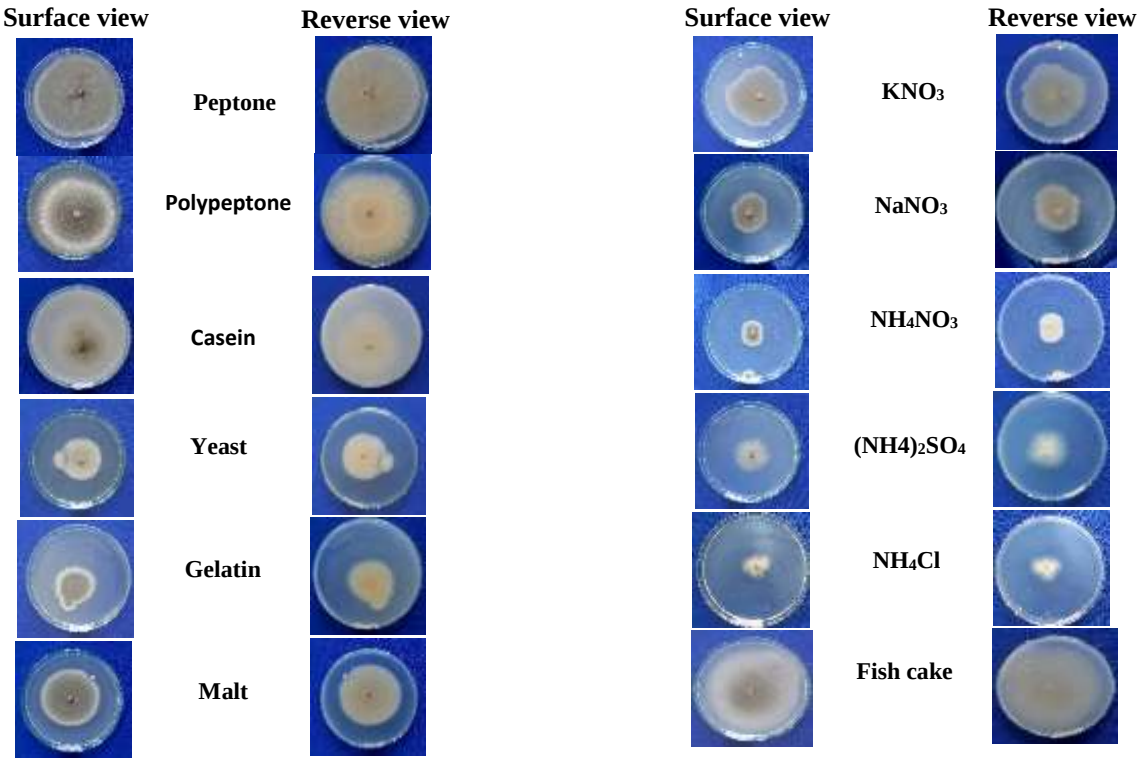

Figure 2 Colony character and growth of MPF-7 on various nitrogen sources

Table 4 The effect of sizes of inoculums of MPF-7 against *E. coli*

Sr. No	Sizes of inoculums (%)	Test organism and Inhibition Zone (mm) *E. coli*
1	5	15.54
2	10	20.79
3	**15**	**22.06**
4	20	21.71
5	25	20.15
6	30	17.12

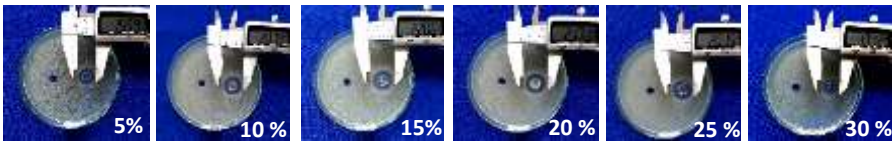

Figure 3 The effect of sizes of inoculums of MPF-7 against *E. coli*

Table 5 The effects of ages of inoculum MPF-7 against *E. coli*

Sr. No	Age of culture (hrs)	Test organism and Inhibition Zone (mm) *E. coli*
1	72	**14.34**
2	84	15.25
3	96	15.53
4	**108**	**18.64**
5	120	16.58
6	132	15.30

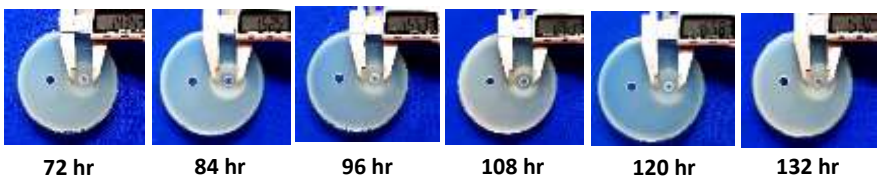

Figure 4 The effect of ages of inoculums of MPF- 7 against *E. coli*

Table 6 Effect of carbon sources on the fermentation of selected MPF- 7 against *E. coli*

Sr. No	Carbon source	Test organism and Inhibition Zone (mm) *E. coli*
1	**Glucose**	**21.05**
2	Fructose	20.50
3	Lactose	20.36
4	Soluble Starch	20.16
5	Sucrose	19.39
6	Xylose	17.99
7	Tapioca	17.54
8	Corn	17.34
9	Potato	15.96

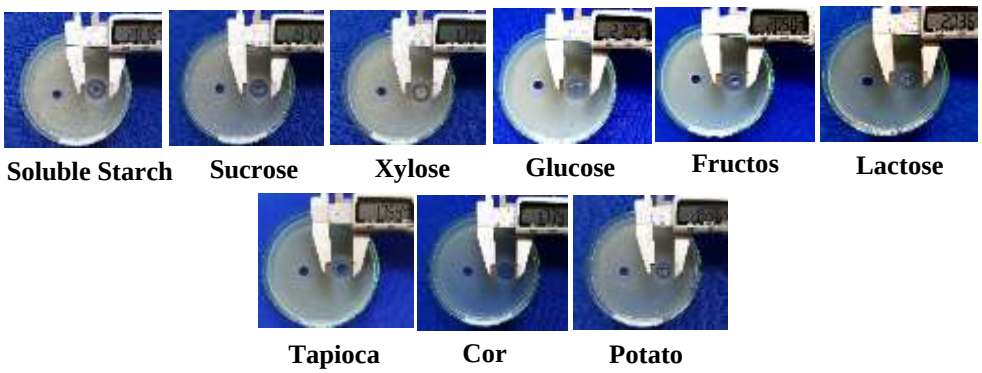

Figure 5 The effect of carbon sources of MPF-7 against *E. coli*

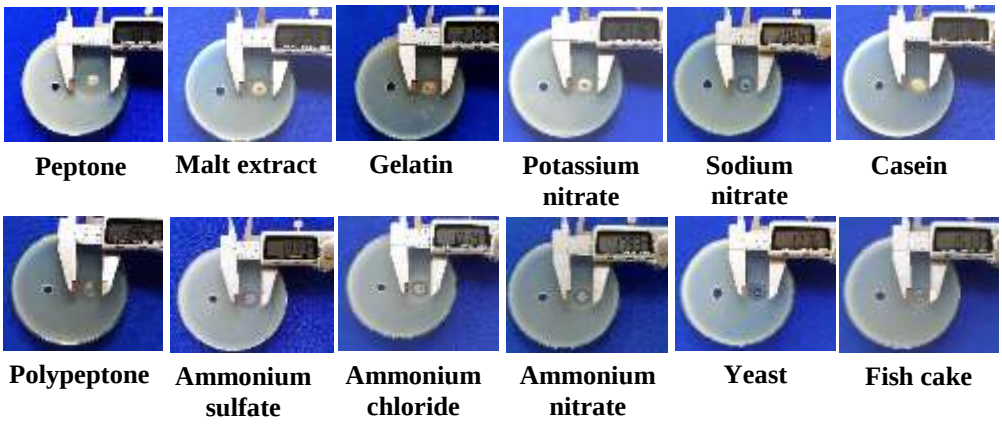

Figure 6 The effect of nitrogen sources of MPF-7 against *E. coli*

Table 7 Effect of nitrogen sources on the fermentation of selected fungi MPF- 7 against *E. coli*

Sr. No	Nitrogen sources	Test organism and Inhibition Zone (mm) *E. coli*
1	**Peptone**	**30.78**
2	Malt extract	22.98
3	Gelatin	21.39
4	Potassium nitrate	21.16
5	Sodium nitrate	20.64
6	Casein	20.18
7	Polypeptone	19.62
8	Ammonium sulfate	19.33
9	Ammonium chloride	18.14
10	Ammonium nitrate	17.93
11	Yeast	17.76
12	Fish cake	15.13

Table 8 Effect of pH on the fermentation of selected fungus MPF-7 against *E. coli*

Sr. No	pH	Test organism and Inhibition Zone (mm) *E. coli*
1	4	20.78
2	5	20.91
3	**6**	**23.36**
4	7	20.53
5	8	20.34
6	9	19.84
7	10	17.90

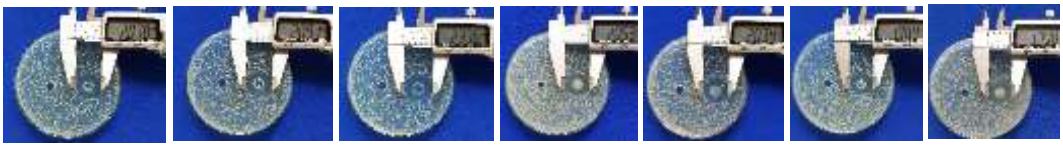

Figure 7 The effect of pH on the fermentation of MPF-7 against *E. coli*

Table 9 Effect of temperature on the fermentation of selected fungus MPF-7 against *E. coli*

Sr. No	Temperature (ºC)	Test organism and Inhibition Zone (mm) *E. coli*
1	20	22.02
2	**25**	**23.18**
3	30	22.81
4	35	18.91
5	40	18.88
6	45	16.22

Figure 8 The effect of temperature on the fermentation of MPF-7 against *E. coli*

Table 10 Effect of agitation on the fermentation of selected fungus MPF-7 against *E. coli*

Sr. No	Agitation	Test organism and Inhibition Zone (mm) *E. coli*
1	**Shaking**	**20.00**
2	Static	17.87

Figure 9 The effect of agitation on the fermentation of MPF-7 against *E. coli*

Discussion and Conclusion

In current research work, the growth effect and optimum fermentation of soil fungus MPF-7 was observed on *E. coli*.

While the effect of sizes of inoculums were considered by using 5%, 10%, 15%, 20 %, 25 % and 30 % inoculums, and the best antibacterial activity (22.06 mm*)* was perceived at 15% seed culture. Consistent with this study my research similar has shown to be similar with the research conducted by Tomita (1988).

In the study of age of inoculum, the best antibacterial activities were gained at 108 hrs. This result campare with Reddy *et al*., 1985 discovered that maximal production of antibiotic substances occurred after 96 hrs.

In this investigation work, the various carbon sources were used for the growth morphology of MPF-7. MPF-7 was excellent growth on glucose,

potato and fructose. Besides the various nitrogen sources were also used and excellent growth was found on peptone, polypeptone, malt, KNO_3 and fish cake.

Furthermore glucose as the carbon source, MPF-7 showed the highest activity (21.05 mm*)* whereas use as nitrogen sources. This results are in agree with (Buchanan *et al*., 1984) and (Calvo *et al.,* 2002). The best results was found in peptone (30.78 mm) on *E. coli*.

El-Tayeb *et al*., 2004; Rizk *et al.,* 2007 have been studied on different aspects of microbial media for example carbon and nitrogen sources, minimal salts, trace elements, vitamins and pH.

And then, maximum antibacterial activity was observed at pH 6 (23.36 mm) on *E. coli*. pH 6.0 is the greatest for the production of antimicrobial metabolite by *A. terreus*. Like result had been stated previous by Nishihara *et al.* (2001) during the production of FR198248, a new anti-influenza agent at pH value among 6.3 to 6.4 from *A. terreus*.

In the current study, the temperature effect, the strong activity was gained at 25ºC (23.18 mm on *E. coli*). Rizk *et al*., 2007 described that physical features such as incubation temperature can apply diverse effects on the growth and production phases of secondary metabolism. Pandey *et al*., 2005 also described that temperature is an important parameter that controls the overall growth and development of the microorganisms.

The fermentation broth was planned at two conditions such as agitation (shaking) and stationary (static) conditions. In the contrast effect of static and shaking culture of MPF-7. The maximum of antibacterial metabolite was detected under shaking condition (20.00 mm) and the static culture (17.87 mm) on *E. coli* singly. Tani *et al*, 2004 described well produce of antibiotics in shake culture fermentation condition.

It can be concluded that the optimal fermentation conditions necessary for further research plan and current detail characterization of bioactive compounds.

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