Fermentation Optimization of *Aspergillus foetidus* Against *Escherichia coli*

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Abstract

In the research, the utilization of carbon and nitrogen sources for the *Aspergillus foetidus* and fermentation optimization were undertaken on *Escherichia coli*. In the study of age and size of inoculum, 72 hrs is the best for fermentation while 5% seed culture was observed. Among them, the best antimicrobial activities were obtained in 144 hrs (23.41 mm *E. coli*) on test organism. In addition of glucose as the carbon source, *Aspergillus foetidus* showed the highest antimicrobial activity (25.86 mm *E. coli*). In the investigation on effect of nitrogen sources, the best result was found in peptone (31.65 mm on *E. coli*). Maximum antimicrobial activity was observed at pH 6 (25.50 mm on *E. coli*). In the temperature effect, the strong activity was obtained at 25ºC (23.63 mm on *E. coli*). In the comparison effect of static and shaking culture of *Aspergillus foetidus*, the maximum of antimicrobial metabolite was observed under shaking condition (22.46 mm on *E. coli*) and the static culture of *Aspergillus foetidus* showed activity (23.93 mm on *E. coli*) respectively.

Keywords: fermentation, antimicrobial activity

Introduction

Microorganisms are a virtually unlimited source of novel chemical structures with many potential therapeutic applications (Behal V. 2000). One of the most important factors determining microbial and biochemical soil activity is temperature (Singh *et al*., 2010). Jarvan *et al*. (2014) suggest that temperature is one of the most important factors influencing the count and occurrence of microorganisms and is also a determinant of the activity of intracellular and extracellular enzymes. The large number of known bioactive compounds (primary and secondary metabolites) of microbial origin are currently produced by fermentation. Variations in the fermentation environment often result in an alteration in antibiotic production (Gaden, 1959). Therefore, the fermentation conditions such as substrates inoculum cultivation and transfer have to optimized for the production of primary and secondary metabolites (Dale, 1984). Carbon and nitrogen sources together with fermentation time have been reported to play significant roles in the determination of the final morphology of the culture (Papagianni, 2004). The choice of carbon sources greatly influenced secondary metabolism and therefore antibiotic production. (Martin JE *et al.*, 1978 and EI- Benna N *et al.,* 2006).

Materials and Methods

In the previous study, *Aspergillus foetidus* was observed for the further investigations due to its highest antimicrobial activity against test organism. Therefore, in the study, the utilization of carbon and nitrogen sources for the growth and fermentation optimization were undertaken.

Agar well method (Collins, 1965)

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Aspergillus foetidus 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 organism selectively.

Table 1. Test Organism used for Antimicrobial Activity

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

Growth of *Aspergillus foetidus* on various carbon and nitrogen sources

The optimal fermentations are very important for maximal productivity metabolites. To determine the effect of carbon sources on antimicrobial metabolite production from *Aspergillus foetidus*, different carbon sources such as Fructose, Glucose, Lactose, Xylose, Maltose, Potato, Wheat, Rice, Soluble Starch, Sucrose and Tapioca were used. Nitrogen sources such as Casein, Fish cake, Gelatin, Malt extract, Peanut, Peptone, Polypeptone, Potassium nitrate, Sodium nitrate, Ammonium nitrate, Ammonium sulfate, Ammonium chloride and yeast extract were also used.

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 this studies, age of seed culture was employed as 36, 48, 60, 72 and 84 hrs with 5% seed culture. The seed culture, 5%, 10%, 15%, 20%, 25% and 30% using 72 hrs age were employed for the fermentation.

Study on different carbon sources utilization for the fermentation

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

Effect of pH

The optimization of pH of the fermentation medium for antimicrobial 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 antimicrobial metabolite production was undertaken. *Aspergillus foetidus* was carried out at five different incubation temperatures 20, 25, 30, 35, 40 and 45ºC. 25 mL of fermentation broth was taken in 100 mL conical flasks and autoclaved at 121ºC for 45 minutes. The inoculated flasks

were incubated at the six incubation temperatures. The fermented broths were tested for antimicrobial activity against test pathogens by using ager well diffusion assay.

Effect of agitation

The fermentation broth was studied at two conditions such as agitation (shaking) and stationary (static) conditions.

Results

In present study, *Aspergillus foetidus* was tested for antimicrobial activities with *Escherichia coli* by using agar well diffusion method. The fungal strains exhibited antimicrobial activities. And then, the utilization of carbon and nitrogen sources for the growth and fermentation optimization were undertaken. In the study of age and size of inoculum, 72 hrs is the best for fermentation while 5% seed culture was observed. Among them, the best antimicrobial activities were obtained in 144 hrs (23.41 mm on *E. coli*) on test organism as shown in table 11 and Figure 10.

In addition of glucose as the carbon source, *Aspergillus foetidus* showed the highest antimicrobial activity (25.86 mm on *E. coli*) as shown in table 6 and figure 5. In the investigation on effect of nitrogen sources were also used and the best results were found in peptone (31.65 mm on *E. coli*) as shown in table 7 and figure 6.

Maximum antimicrobial activity was observed at pH 6 (25.50 mm on *E. coli*) as shown in table 8 and figure 7. In the temperature effect, the strong activity was obtained at 25ºC (23.63 mm on *E. coli*) as shown in table 9 and figure 8. In the comparison effect of static and shaking culture of *Aspergillus foetidus*, the maximum of antimicrobial metabolite was observed under shaking condition (22.46 mm on *E. coli*) as shown in table 10 and figure 9 and the static culture of *Aspergillus foetidus* showed activity (23.93 mm *E. coli*) as shown in table 10 and figure 9 respectively.

Colony character and growth of *Aspergillus foetidus* on various carbon source

In this research work, the various carbon sources were used for the growth morphology of *Aspergillus foetidus*.

Table 2. Colony character and growth of *Aspergillus foetidus* on various carbon sources

No	Carbon sources	Surface colour	Reverse colour	Size (mm)	Growth
1	Corn	coffee black	cream	47.56-47.89	Excellent
2	Wheat	Black	cream	30.34-33.45	Good
3	Rice	coffee black	coffee cream	47.34-48.35	Excellent
4	Fructose	coffee black	coffee cream	45.56-47.23	Excellent
5	Glucose	coffee black	coffee cream	35.50-39.34	Good
6	Lactose	coffee black	coffee cream	30.35-35.37	Good
7	Potato	Brown	cream	28.89-35.56	Moderate
8	Soluble starch	coffee black	coffee cream	23.56-29.34	Moderate
9	Xylose	coffee cream	cream	47.50-49.34	Excellent
10	Sucrose	coffee cream	cream	34.45-40.34	Good
11	Tapioca	coffee cream	cream	23.08-30.12	Moderate

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

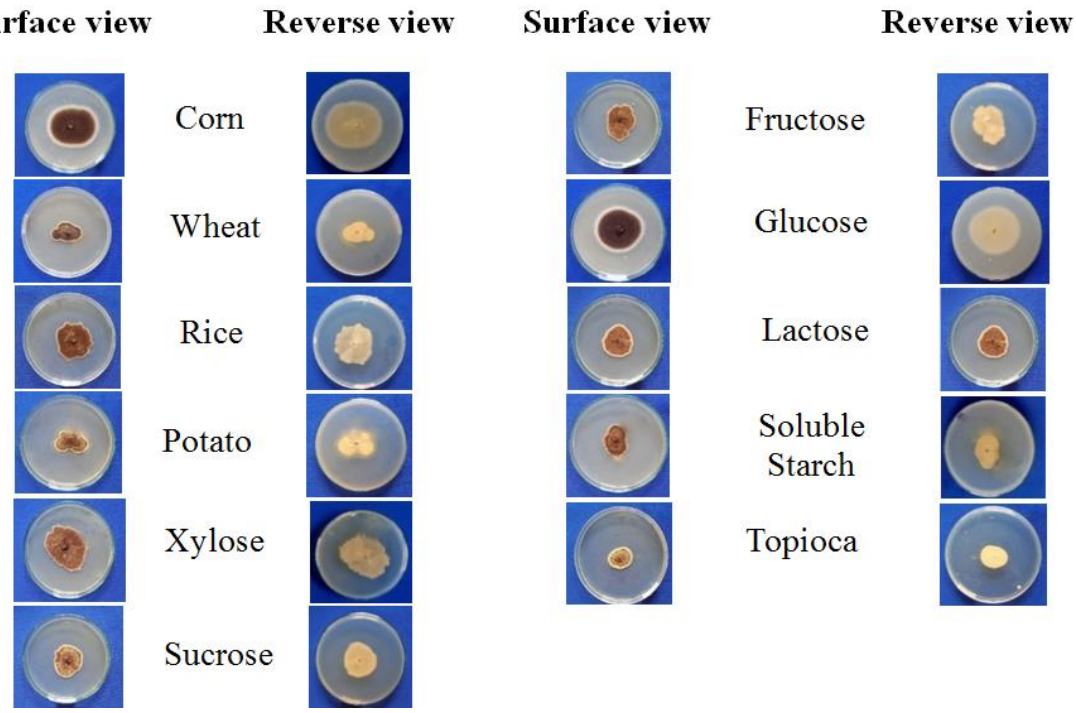


Fig.1.Colony character and growth of *Aspergillus foetidus* on various carbon sources

Colony character and growth of *Aspergillus foetidus* on various nitrogen sources

In this research work, the various carbon sources were used for the growth morphology of *Aspergillus foetidus*.

Table 3. Colony character and growth of *Aspergillus foetidus* on various nitrogen sources

No	Nitrogen sources	Surface colour	Reverse colour	Size (mm)	Growth
1	Casein	black	Brownish cream	49.67-49.98	Excellent
2	Fish cake	Black	Brownish black	46.45-47.34	Excellent
3	Gelatin	Black	Brown	48.56-49.67	Excellent
4	Malt extract	Brownish black	Brownish cream	49.12-51.56	Excellent
5	Peanut	Black	Brownish cream	45.56-46.13	Excellent
6	Peptone	Black with white	Cream	38.56-39.32	Good
7	Polypeptone	Black with white	Brownish cream	49.54-51.86	Excellent
8	KNO_3	Black with white	Cream	20.21-21.98	Good
9	$NaNO_3$	Brownish white	White	25.67-26.87	Good
10	NH_4NO_3	Brownish white	Cream	21.34-21.67	Good
11	$(NH4)_2SO_4$	Brownish black	Cream	27.66-27.98	Good
12	NH_4Cl	Brownish white	Cream	20.34-21.37	Good
13	Yeast extract	Cream	Yellowish cream	37.78-38.79	Excellent

20-30 mm = Moderate growth 2.30-40 mm = Good 3. 40 to above= excellent

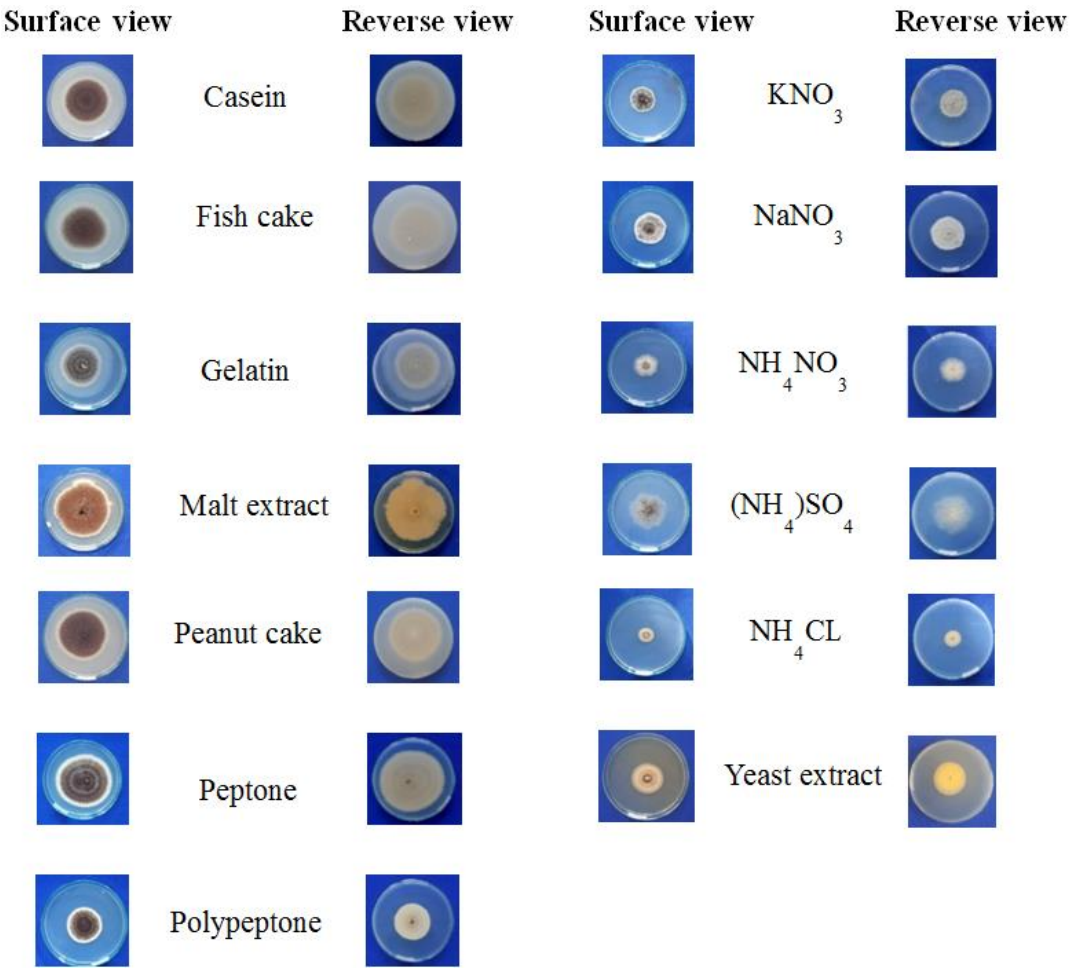


Figure 2. Colony character and growth of *Aspergillus foetidus* on various nitrogen sources

The effects of ages of inoculums of *Aspergillus foetidus* against *E. coli*

Table 4. The effects of ages of inoculums of *Aspergillus foetidus* against *E. coli*

Age of culture (hrs)	Activity (mm) on *E. coli*
36	22.32
48	23.45
60	24.00
72	**27.35**
84	24.09

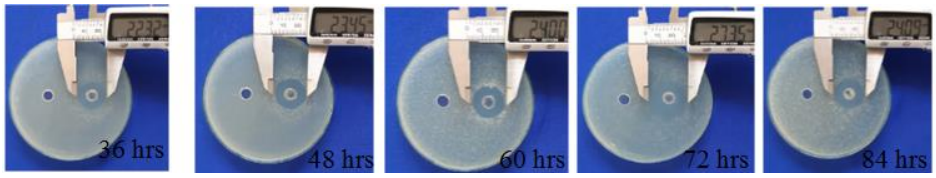


Figure 3. The effects of age of inoculum for the fermentation against *E. coli*

The effect of sizes of inoculums of *Aspergillus foetidus* against *E. coli*

Table 5. The effect of sizes of inoculums of *Aspergillus foetidus* against *E. coli*

Sizes of inoculums (%)	*E. coli* and Inhibition Zone (mm)	Sizes of inoculums (%)
1	**5**	**21.88**
2	10	17.44
3	15	21.20
4	20	17.03
5	25	17.78
6	30	20.34

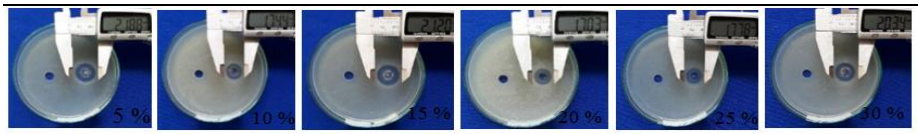


Figure. 4. The effect of sizes of inoculums of *Aspergillus foetidus* against *E. coli*

Effect of carbon sources on the fermentation of selected fungus *Aspergillus foetidus* against *E. coli*

Table 6. Effect of carbon sources on the fermentation of selected fungus *Aspergillus foetidus* against *E. coli*

No	Carbon source	Test organism and Inhibition Zone (mm)
1	**Glucose**	**25.86**
2	Fructose	25.19
3	Lactose	22.24
4	Soluble Starch	21.50
5	Sucrose	25.54
6	Xylose	23.35
7	Tapioca Powder	23.36
8	Corn	22.24
9	Potato	23.25
10	Wheat	21.95
11	Rice	17.56

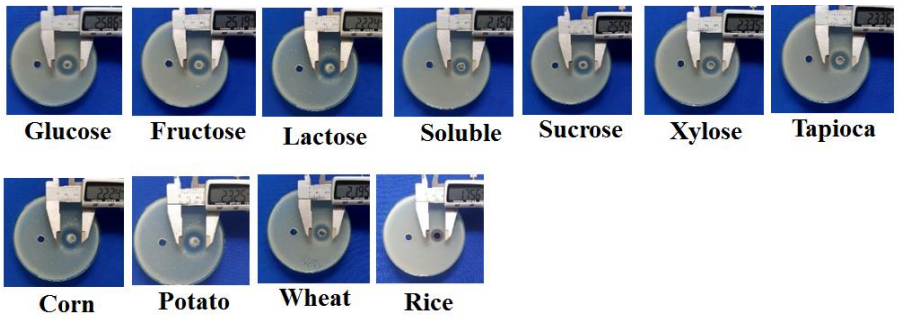


Figure 5. The effect of carbon sources of *Aspergillus foetidus* against *E. coli*

Effect of nitrogen sources on the fermentation of selected fungus *Aspergillus foetidus* against *E. coli*

Table 7. Effect of nitrogen sources on the fermentation of selected fungus *Aspergillus foetidus* against *E. coli*

No	Nitrogen sources	Test organism and Inhibition Zone (mm)
		E. coli
1	**Peptone**	**31.65**
2	Malt extract	30.07
3	Gelatin	27.54
4	Potassium nitrate	26.78
5	Sodium nitrate	26.56
6	Casein	25.84
7	Polypeptone	25.77
8	Ammonium sulfate	23.66
9	Ammonium chloride	23.45
10	Ammonium nitrate	23.21
11	Yeast	23.04
12	Fish cake	22.29
13	Peanut cake	18.70

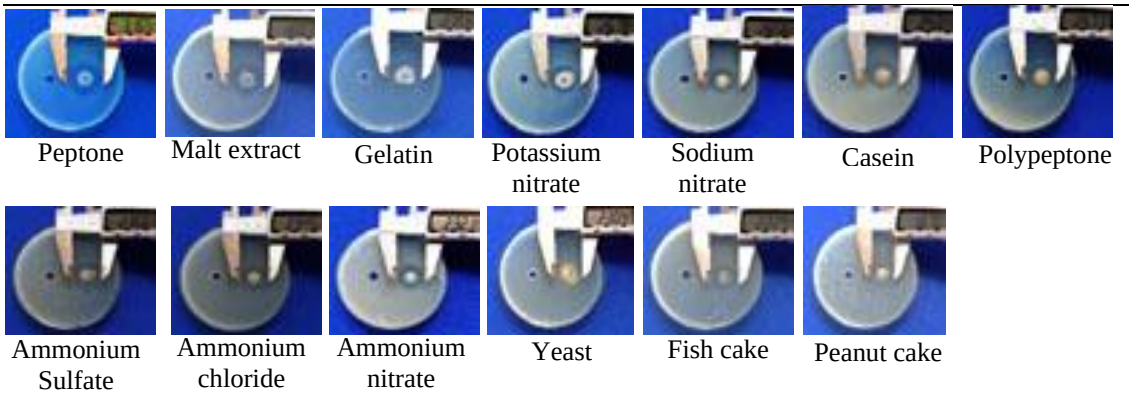
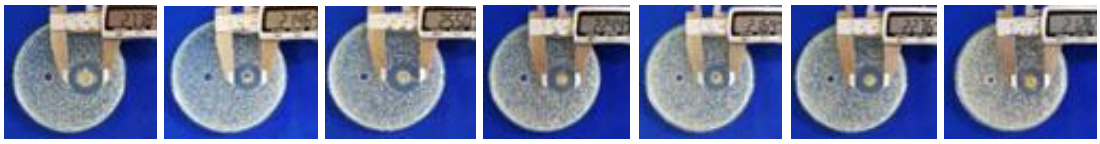


Figure 6. Antimicrobial activity of *Aspergillus foetidus* on nitrogen sources

Effect of pH on the fermentation of selected fungus *Aspergillus foetidus* against *E. coli*

Table 8. Effect of pH on the fermentation of selected fungus *Aspergillus foetidus* against *E. coli*

No.	pH	Test organism and Inhibition Zone (mm)
1	4	27.78
2	5	21.46
3	**6**	**25.50**
4	7	22.44
5	8	21.64
6	9	22.76
7	10	21.26



pH 4 pH 5 pH 6 pH 7 pH 8 pH 9 pH 10

Figure 7. The effect of pH on the fermentation of*Aspergillus foetidus* against *E. coli*

Effect of temperature on the fermentation of selected fungus *Aspergillus foetidus* against *E. coli*

Table 9. Effect of temperature on the fermentation of selected fungus *Aspergillus foetidus* against *E. coli*

Sr. No	Temperature (ºC)	Test organism and Inhibition Zone (mm)
		E. coli
1	20	22.43
2	**25**	**23.63**
3	30	22.95
4	35	21.64
5	40	21.28
6	45	20.69

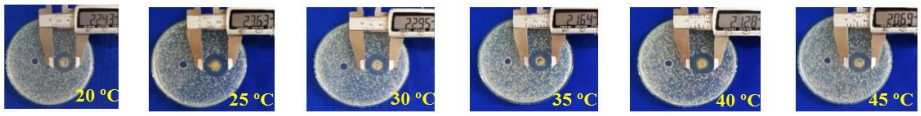


Figure 8. The effect of temperature on the fermentation of *Aspergillus foetidus* against *E. coli*

Effect of agitation on the fermentation of selected fungus *Aspergillus foetidus* against *E. coli*

Table 10. Effect of agitation on the fermentation of selected fungus *Aspergillus foetidus* against *E. coli*

No	Agitation	Test organism and Inhibition Zone (mm)
1	Shaking	22.46
2	**Static**	**23.93**

Shaking Static

Figure 9. The effect of agitation on the fermentation of *Aspergillus foetidus* against *E. coli*

Time Course of Fermentation by *Aspergillus foetidus* against *E. coli*

Table 11. Time Course of Fermentation by *Aspergillus foetidus* against *E. coli*

No	Fermentationperiod (hrs)	Test organism and Inhibition Zone (mm)
1	48	15. 08
2	60	18.09
3	72	15.79
4	84	21.36
5	96	18.13
6	108	18.87
7	120	22.30
8	132	22.02
9	**144**	**23.41**
10	156	20.93

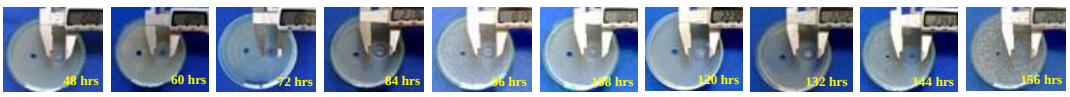


Fig. 10. Time Course of Fermentation by *Aspergillus foetidus* against *E. coli*

Discussion and conclusion

In present research work, *Aspergillus foetidus* were tested for antimicrobial activities with by using agar well diffusion method. According to these results, isolated fungus *Aspergillus foetidus* was selected for antimicrobial metabolite production.

Rizk *et al*., 2007 described physical factors such as incubation temperature, can exert different effects on the growth and production phases of secondary metabolism. In the study on the time course of fermentation, it was found that 144

ages of inoculums gave the highest activities (23.41 mm) against *E. coli* and followed 132 hrs (22.02 mm), 120 hrs (22.30 mm).

In this research work, the various carbon sources were used for the growth morphology of *Aspergillus foetidus*. *Aspergillus foetidus* was great growth on fructose, lactose, soluble starch and sucrose. In addition of glucose as the carbon source showed the highest antimicrobial activity (25.86 mm *E. coli*) where as use as nitrogen sources, the best results were found in peptone (31.65 mm). pH 6.0 was the best in this study. In the present study, the production of antimicrobial metabolite in *Aspergillus foetidus* has been found at 25ºC (23.63 mm).

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