GROWTH CONDITIONS AND OPTIMIZATION PARAMETERS OF FERMENTATION OF SELECTED SOIL BACTERIUM KM-39

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Abstract

The present study was focused by the growth conditions and fermentation optimization of selected soil bacterium KM-39 against *Candida albicans.* In the growth of KM-39, carbon and nitrogen sources were used and the excellent growth were found on lactose and yeast extract. The highest antifungal activity of KM-39 was found by the fermentation conditions such as 2 days old culture period, 20% inoculum size, 72 hours age of inoculum, FM-5 at pH 8 and temperature 40ºC. The maximum antifungal activity was obtained by using the maltose in the carbon source and potassium nitrate in the nitrogen source. In the comparison of static culture and shaking culture of KM-39, the antifungal activity of shaking culture was more higher than the static culture on *Candida albicans*.

Keywords: Fermentation conditions, growth of bacteria, antimicrobial activity

Introduction

Bacteria are single-cell organisms and the most numerous denizens of agriculture, with populations ranging from 100 million to 3 billion in a gram.They are capable of very rapid reproduction by binary fission (diving into two) in favorable condition.One bacterium is capable of producing 16 million more in just 24 hours (Soltner *et al*, 2003).

The secondary metabolite is obtained by fermentative process.Fermentation is a complex process, it not only depends on the performance and fermentation medium, also requires the suitable environmental conditions such as inoculation volume, medium capacity, fermentation time, temperature, agitation rate and initial pH. These factors may affect the antibiotics production (Martin et al., 1980).

Thus soil is comples product of parental material, or geology, topography, climate time and biological activity on anthropogenic activity (Graffin, 1972).

The nature and concentration of nutrients in the medium such as carbon, nitrogen, phosphorus and minerals as well as the essential substances for biosynthetic pathway can promote the production of larger quantities of one or more compound inferring directly on its performance (Gupte and Pulkarni, 2002).

The *Candida albicans*is human-pathogenic fungus whose control is one of the most problems in today's medical field. It is part of the normal microflora of the mouth, vagina and gastrointestinal tract. Among the various *Candida albicans*infections that can occur under these conditions is a vaginitis (inflammation of the vagina) characterized by a thick, cheeselike discharge. Another condition caused by *Candida albicans*urethritis in both women and man (Michael, 1993).

This research work was carried out by the optimum fermentation conditions of selected bacterium (KM-39).

The aim and objectives of this research were to investigate the utilization of carbon and nitrogen sources of the selected bacterial growth and to optimize the fermentation condition of selected bacterium (KM-39).

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Materials and Methods

The effects of fermentation period, size and age of inoculum for fermentation of KM-39

The fermentation period (24, 48, 72, 96 and 120 hrs) were employed for the production of antimicrobial metabolite.

In the investigation of sizes of inoculum (5%, 10%, 15%, 20%, 25% and 30%) were used for the antimicrobial activity of KM-39. Seed culture was inoculated at room temperature. In the study of ages of inoculum, the incubation of seed culture times (24, 36, 48, 60, 72, 84, 96, 108, 120, 132, 144 and 156 hrs) were used and transferred into the fermentation media. Fermentation were carried out for 7 days and antimicrobial activity was tested by agar well diffusion method.

Preparation of Agar Well Method

Isolated strains were subjected with antimicrobial activities by agar well method. Cork borer was used to make the wells (8 mm in diameter) in the autoclaved basal antimicrobial test-medium. Well impregnated with 24, 48 and 72 hours fermented broth (20 µL) were incubated at room temperature for 24-48 hours. After 24-48 hours of incubation, the clear zones were measured.

Therefore, the diameter of clear zones has observed as potent activity shown by respective strain. Clear zones surrounding the test wells were indication of the presence of antimicrobial activities which inhibit the growth of the test organisms selectively (Collins, 1965).

Test organisms

Table 1 Test Organism Used for Antifungal Activity

Test No	Test Organism	Diseases
1	NITE 09542	Alimentary tract, skin infection

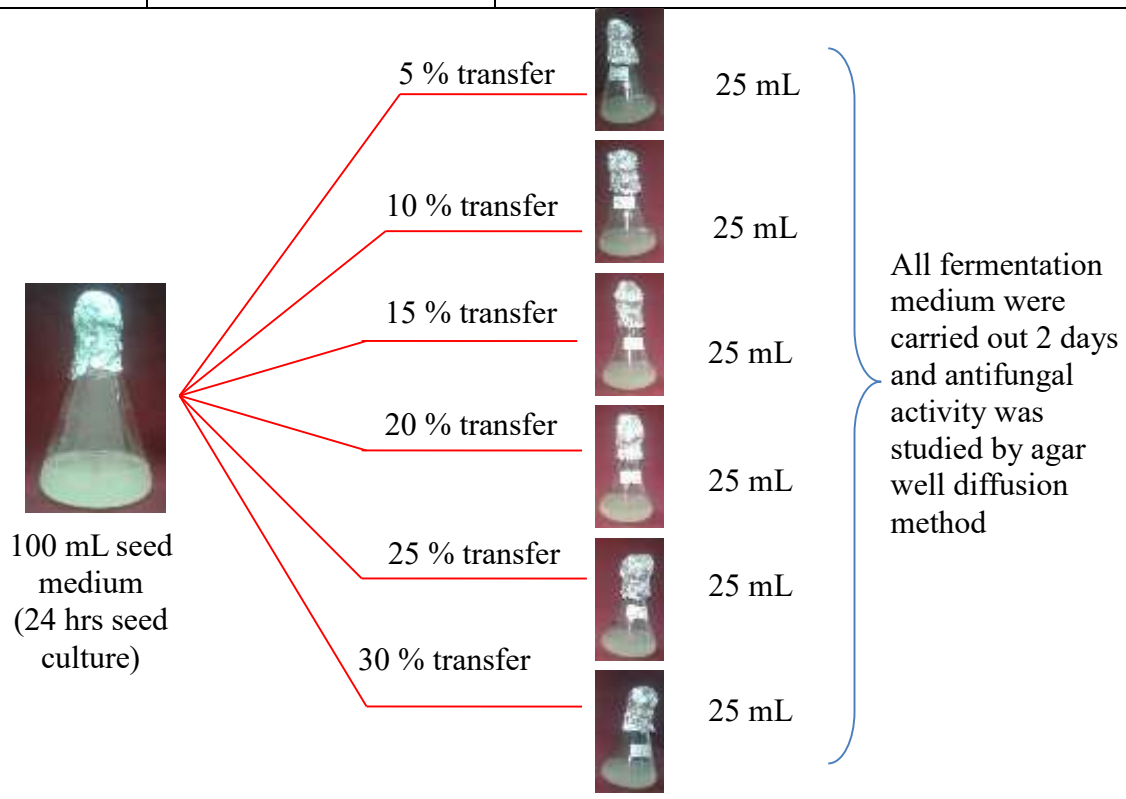


Figure 1 Study on the effects of sizes of inoculum in the fermentation

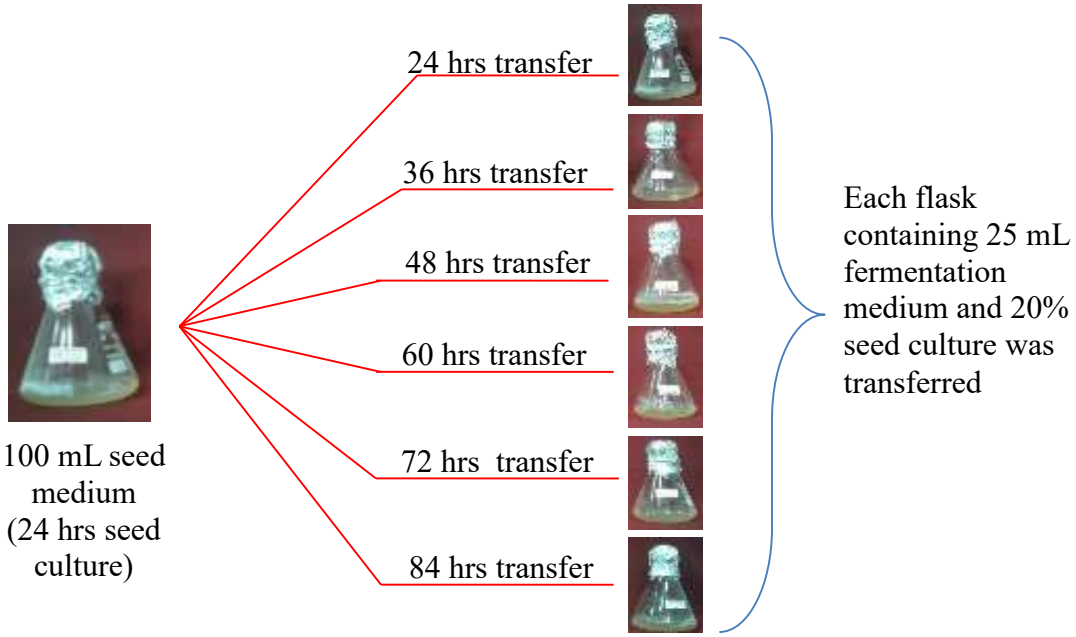


Figure 2 Study on the effects of ages of inoculum in the fermentation

Carbon and Nitrogen Utilization

Optimal fermentation conditions are very important for maximal productivity of metabolites. In this study, carbon and nitrogen sources were employed in the fermentation for the production of antimicrobial metabolites. Carbon sources such as arabinose, dextrose, fructose, galactose, lactose, maltose, sucrose, xylose, glycerol, mannitol and soluble starch were used. Nitrogen sources such as asparagine, casein, gelatin, peptone, urea, yeast extract, ammonium chloride, ammonium sulphate, ammonium nitrate, potassium nitrate, sodium nitrate and malt extract were also used.

Media used in fermentation study (NITE, 2005)

Fermentation was undertaken with suitable conditions of 20% sizes and 48 hrs ages of inoculum with twelve different media. Fermentation was carried out 5 days and antimicrobial activity test was carried out every 24 hrs.

Effect of incubation pH and temperature on KM-39

Effects of different pH were used for antimicrobial activity of pH 4, 5, 6, 7, 8, 9 and 10. These different pH were adjusted by NaOH and HCl. The selected bacterium KM-39 was inoculated and incubated at five different temperatures by using 25ºC, 30ºC, 35ºC, 40ºC and 45ºC.

Effect of aeration upon the secondary metabolite

100 mL conical flask containing 50 mL of the best fermentation medium was incubated on the shaker (100 rpm) for 2 days. At the same time, another those fermentation medium was incubated under static condition without shaking. These shaking culture and static culture were compared by using agar well diffusion assay method.

Results

The effects of carbon sources for the growth of soil bacterium KM-39

In the present study, the effects of different carbon sources were observed for the growth rate of KM-39. KM-39 showed the excellent growth by the addition of arabinose, dextrose, lactose, maltose, sucrose, xylose, glycerol and mannitol and good results on fructose, galactose and soluble starch.

Table 2 Growth morphology of selected bacterium KM-39 on various carbon sources

No.	Carbon sources	Growth result	Growth mm
1	Arabinose	Excellent	11.78
2	Dextrose	Excellent	10.51
3	Fructose	Good	5.10
4	Galactose	Good	6.23
5	Lactose	Excellent	21.17
6	Maltose	Excellent	7.81
7	Sucrose	Excellent	8.79
8	Xylose	Excellent	9.21
9	Glycerol	Excellent	7.91
10	Mannitol	Excellent	9.35
11	Soluble starch	Good	5.94

1-2 mm = poor 3-4 mm = moderate 5-6 mm = good 7> = excelle

Figure 3 Growth morphology of selected bacterium KM-39 on carbon sources (2 days old culture)

The effects of nitrogen sources for the growth of soil bacterium KM-39

In this study, excellent growth of KM-39 was found on asparagine, gelatin, urea, yeast extract, ammonium chloride, ammonium sulphate and sodium nitrate, moderate growth on casein and showed good results on other sources peptone, ammonium nitrate, potassium nitrate and malt extract.

Table 3 Growth morphology of selected bacterium KM-39 on various nitrogen sources

No.	Nitrogen sources	Growth result	Growth mm
1	Asparagine	Excellent	7.99
2	Casein	Moderate	4.97
3	Gelatin	Excellent	7.40
4	Peptone	Good	6.57
5	Urea	Excellent	7.12
6	Yeast extract	Excellent	16.78
7	Ammonium chloride	Excellent	7.34
8	Ammonium sulphate	Excellent	7.10
9	Ammonium nitrate	Good	6.08
10	Potassium nitrate	Good	6.93
11	Sodium nitrate	Excellent	7.06
12	Malt extract	Good	5.65

1-2 mm = poor 3-4 mm = moderate 5-6 mm = good 7> = excellent

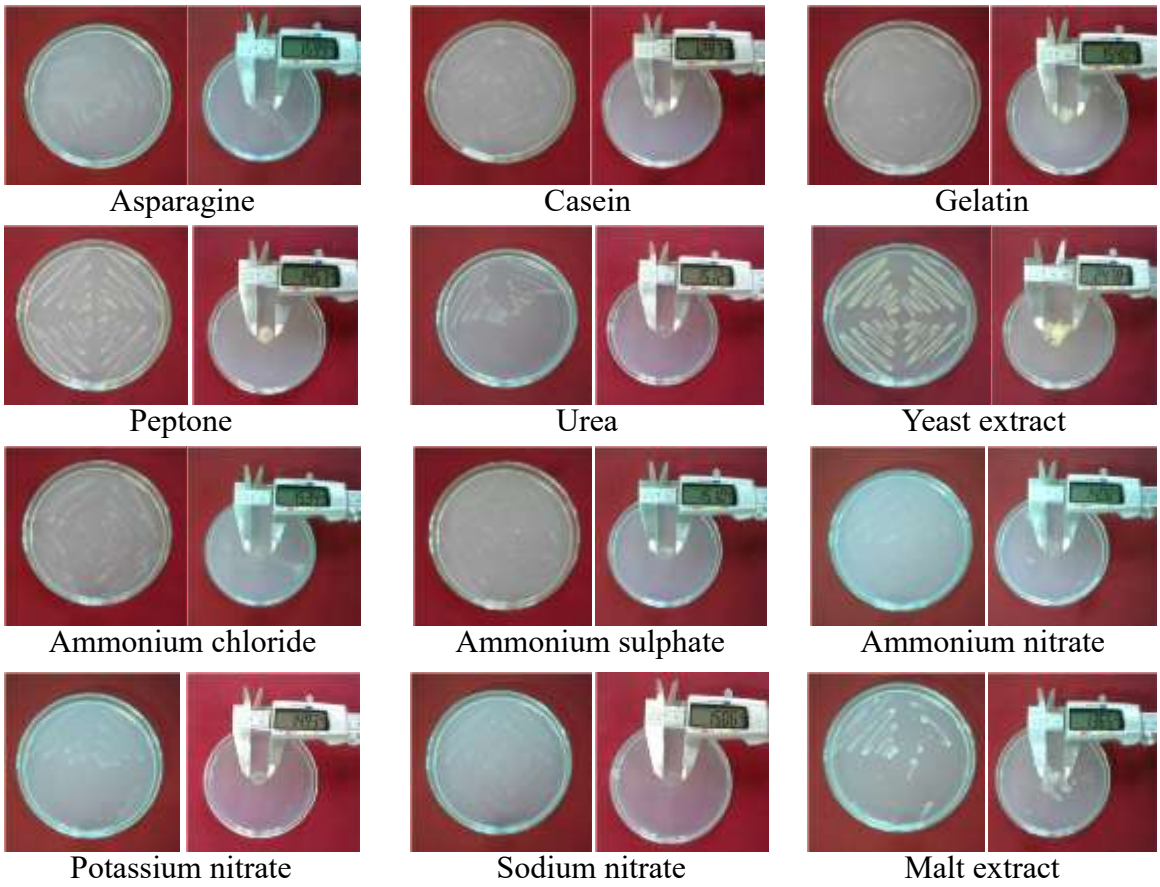


Figure 4 Growth morphology of selected bacterium KM-39 on nitrogen sources (2 days old culture)

Table 4 Antifungal activity on fermentation period of selected bacterium KM-39 against *C. albicans*

Fermentation period (day)	Antimicrobial activity (mm)
1 day	28.74
2 days	**32.35**
3 days	24.79
4 days	22.12
5 days	20.96

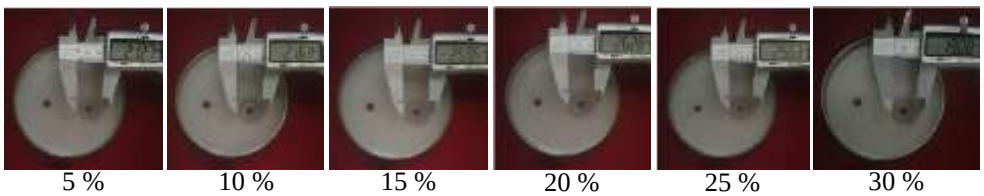
1 day 2 days 3 days 4 days 5 days

Figure 5 Antifungal activity of selected bacterium KM-39 against *C. albicans*

Table 5 Effect of size of inoculum for selected bacterium KM-39

Size (%)	Inhibitory zone (mm)
5%	21.27
10%	21.64
15%	25.95
20%	**26.28**
25%	25.53
30%	19.08

Test organism was *C. albicans*(fermentation 2 days)



5 % 10 % 15 % 20 % 25 % 30 %

Figure 6 The effects of sizes of inoculum on *C. albicans*for KM-39

Table 6 The effect of ages of inoculum of the fermentation against *C. albicans*

Age of Inoculum (hrs)	Antifungal activity inhibitory zone (mm)
24 hrs	22.79
36 hrs	25.98
48 hrs	29.53
60 hrs	29.61
72 hrs	**31.93**
84 hrs	28.69
96 hrs	27.37
108 hrs	27.29
120 hrs	25.88
132 hrs	25.80
144 hrs	25.49
156 hrs	21.75

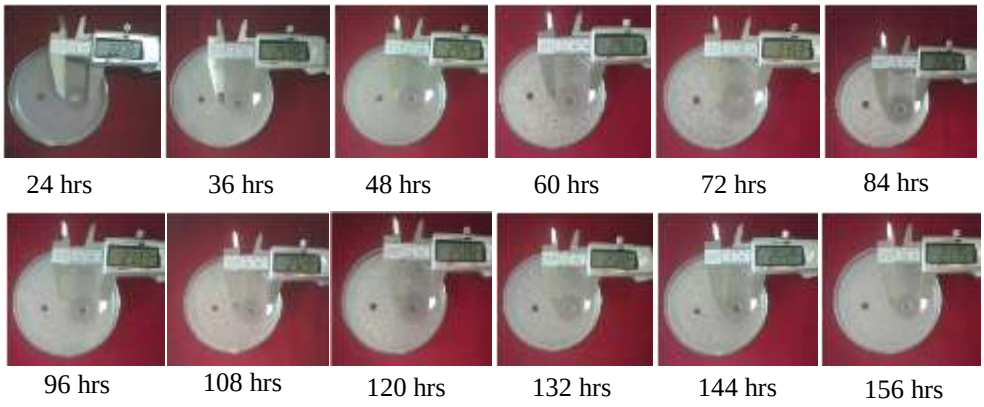


Figure 7 The effects of age of inoculum on *C. albicans*for KM-39

Investigation of carbon sources utilization

The effects of different carbon sources were observed for the growth rate and maximum antimicrobial metabolites production. The addition of arabinose, dextrose, lactose, maltose, sucrose, xylose, glycerol and mannitol were excellent growth, fructose, galactose and soluble starch showed good results. In the antifungal activity, the highest activity of KM-39 was obtained by using the maltose (23.08 mm) on *C. albicans.*

Table 7 Antifungal activity on different carbon sources of selected bacterium KM-39

No.	Carbon sources	Inhibitory zone (mm)
1	Arabinose	21.18
2	Dextrose	20.34
3	Fructose	20.87
4	Lactose	21.78
5	Galactose	20.34
6	Xylose	21.50
7	Sucrose	21.28
8	**Maltose**	**23.08**
9	Glycerol	19.76
10	Mannitol	19.64
11	Soluble starch	20.93

Agar well = 8 mm

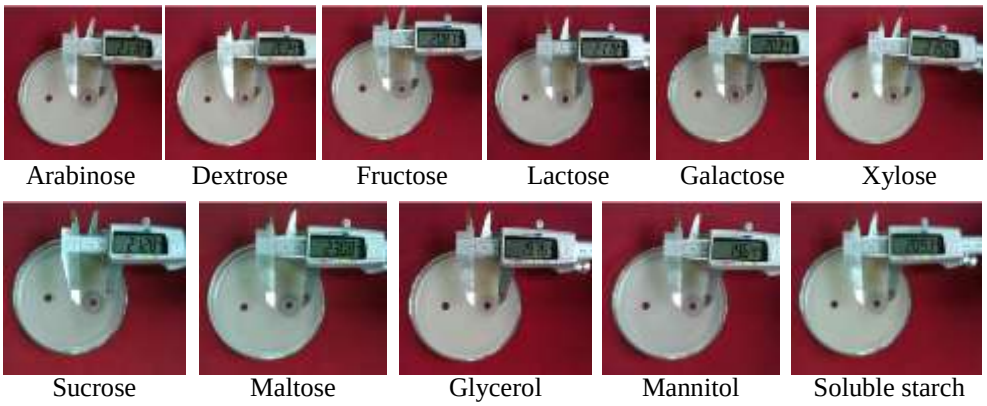


Figure 8 Effects of carbon utilization of fermentation against *C. albicans*

Investigation of nitrogen sources utilization

In this study, excellent growth of KM-39 was found on asparagine, gelatin, urea, yeast extract, ammonium chloride, ammonium sulphate and sodium nitrate, moderate growth on casein

and other peptone, ammonium nitrate, potassium nitrate and malt extract showed good results. The highest antifungal activity was obtained by using potassium nitrate (38.04mm) on *C. albicans.*

Table 8 Antifungal activity on different nitrogen sources of selected bacterium KM-39

No.	Nitrogen sources	Inhibitory zone (mm)
1	Asparagine	20.36
2	Casein	18.61
3	Gelatin	18.54
4	Peptone	19.32
5	Urea	17.61
6	Yeast extract	21.67
7	Ammonium chloride	17.64
8	Ammonium sulphate	12.63
9	Ammonium nitrate	27.35
10	**Potassium nitrate**	**38.04**
11	Sodium nitrate	31.66
12	Malt extract	19.21

Agar well = 8 mm

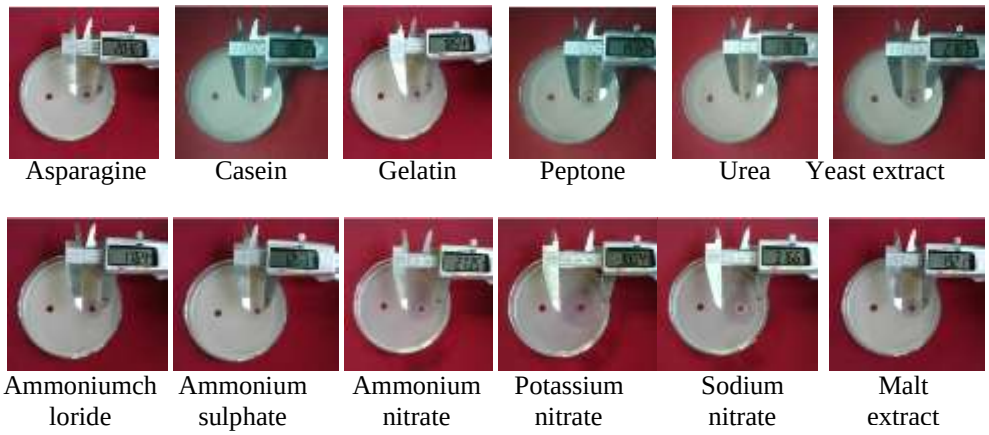


Figure 9 Antifungal activity on different nitrogen sources of selected bacterium KM-39

Table 9 Effects on pH utilization of KM-39 against *C. albicans*

pH range	Inhibitory zone (mm)
4	29.84
5	30.11
6	30.25
7	31.66
8	**32.00**
9	30.48
10	29.15

Figure 10 Effects of pH on the fermentation of KM-39 on *C. albicans*

Table 10 Effect of different temperature utilization of fermentation against *C. albicans*

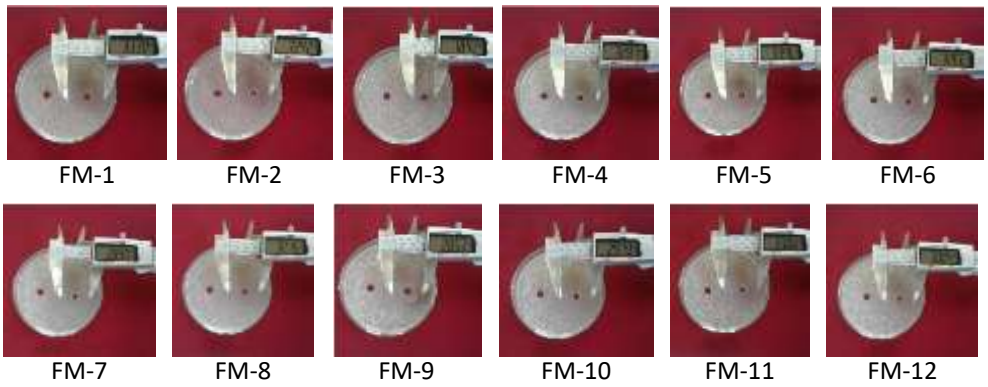
Temperature range	Inhibitory zone (mm)
25ºC	16.69
30ºC	21.41
35ºC	22.07
40ºC	**23.17**
45ºC	-

25ºC 30ºC 35ºC 40ºC

Figure 11 Effects of different temperature utilization of fermentation against *C. albicans*

Table 11 Selection of fermentation medium based on the results of antifungal activity of KM-39

Fermentation media	Antifungal activity (mm)
FM-1	33.30
FM-2	32.42
FM-3	18.42
FM-4	32.87
FM-5	**33.46**
FM-6	32.66
FM-7	28.40
FM-8	32.16
FM-9	28.53
FM-10	29.19
FM-11	33.06
FM-12	30.04



FM-1 FM-2 FM-3 FM-4 FM-5 FM-6

FM-7 FM-8 FM-9 FM-10 FM-11 FM-12

Figure 12 Selection of fermentation medium based on the results of antifungal activity of KM-39

Comparison between shaking culture and static culture

In the comparison between shaking culture and static culture, the shaking culture of KM-39 showed the inhibitory zone (25.22 mm) and the static culture showed (22.00 mm) for 2 days fermentation against *C. albicans.*

Shaking culture on *C. albicans*

Static culture on *C. albicans*

Figure 13 Comparison of shaking culture and static culture against*C. albicans*

Discussion and Conclusion

In the present study, the fermentation period of selected bacterium KM-39 showed the highest antifungal activity(32.35mm) on *C.albicans.*In the investigation to optimize the fermentation, it was found that 72 hrs age of inoculum and 20% of size of inoculum were suitable for fermentation.

Mansi and Charlie, 2003 reported that 72 hrs ages of inoculum was suitable for fermentation.The addition of lactose as a carbon source resulted the excellent growth(21.17mm) and yeast extract as nitrogen source also showed the excellent growth of (16.78mm) in KM-39.

Moreover maltose as the carbon source, KM-39 showed the highest activity(23.08mm) while potassium nitrate (38.04mm) showed the best results in nitrogen sources.The carbon substrate has a dual role in biosynthesis and energy generation, with carbohydrates being the usual carbon source for microbial fermentation processes(ward 1991; Stanbury *et al*. 1995).

In the comparison of shaking culture and static culture, the diameter of inhibitory zone was more higher activity (25.22 mm) on *C. albicans*than the static culture.

Mansi and Charlie, 2003 also reported that shake-flask cultures are better in an incubator shaker to produce large volumes of culture for analysis or for use as an inoculum of a fermenter.

It was concluded that the present study, the optimal fermentation conditions are very important for maximum productivity metabolites and further work will be studied detail characterization of bioactive compounds.

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