CHAPTER THREE

Results

3.1 Preliminary screening for chitinase enzyme

The result of the preliminary screening for chitinase test of six strains of *Geomyces* spp. used in this study is shown in Figure 3.1. It reflects the relative enzyme activities (RA) of extracellular chitinase enzyme for each of the utilized fungal organisms.

1) The replicate values of colony meters and their corresponding RAs for each of the strains is shown in Appendix 1 and 2, whereas each RA was calculated the following formula:

$$RA = \frac{\text{Clear zone diameter} - \text{Colony diameter}}{\text{Colony diameter}}$$

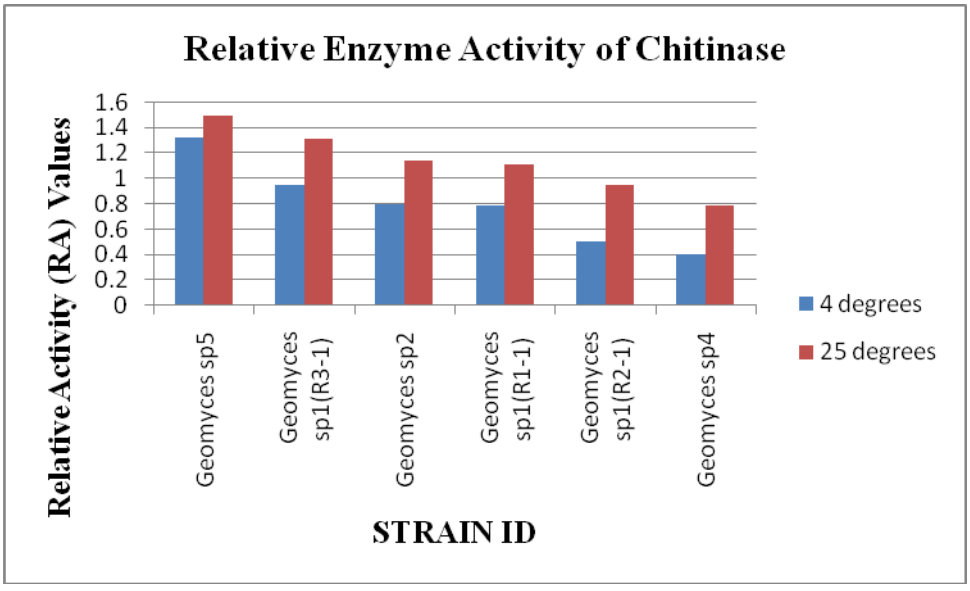

Figure 3.1 The relativity activity of chitinase on the *Geomyces* strains.

Each of the strains replicated at 25 ^{0}C was measured for average RA in order to ascertain those with good chitinase activity (Appendix 1.0). *Geomyces* sp. 5 (AK07KGI102 R1-4) showed chitinase activity with average RA value of 1.49 cm. For first replicate, the clear zone diameter was 3.85 cm and colony diameter was 1.45 cm. This therefore generated R1=3.85-1.45/1.45 =1.65 cm while second the plate was 3.25-1.15/1.15 =1.82 cm (Figure 3.2). Similarly the results of the third replicate showed R3 =2.95-1.05/1.05 =1.80 cm, the fourth replicate, R4 =2.15-0.9/0.9 = 1.38 cm while the last replicate, R5= 1.9-1.05/1.05 = 0.80 cm.

Figure 3.2 Photographs of chitinase activity from *Geomyces* sp. 5 (AK07KGI102 R1-4) on colloid chitin agar. **a)** at 25˚C. **b)** at 4˚C.

Geomyces sp. 1 (AK07KGI601 R3-1) being the second strain recorded an average RA of 1.32 cm, which showed significant enzyme activity from 5 replications (Figure A3.1). First replicate had the clear zone diameter of 2.2 cm while the colony zone diameter was 0.85 cm. After calculation, RA was 1.58 cm. Moreover, RA2 =1.4 cm, RA3 =1.44 cm, RA4 = 1.52 cm and RA5 was 0.63 cm.

Also, both strains of *Geomyces* sp. 2 (AK07KGI902 R1-1) and *Geomyces* sp. 1 (AK07KGI402 R1-1) showed significant enzyme activity, the average activity value

from both strains were 1.14 cm and 1.11 cm, respectively (Figure A3.2; Figure A3.3). On the other hand, *Geomyces* sp. 1 (AK07KGI501 R2-1) RA was 0.95 cm and *Geomyces* sp. 4 (AK07KGI902 R1-1) showed 0.79 cm for RA, both strains showed low significant activity (Figure A3.4; Figure A3.5).

Furthermore, the chitinase activity for each of the strains were carried out in triplicates at 4 ^{0}C (Appendix 2.0). Therefore, *Geomyces* sp. 5 (AK07KGI102 R1-4) still showed relatively good chitinase activity as shown in (Figure 3.1). The clear zone diameter of the first plate was 1.75 cm with a colony diameter of 0.85 cm; hence an RA of 1.05 cm. Second plate showed a clear zone diameter of 2.45 cm while the colony diameter was 0.9 cm, which gave RA 1.72 cm. The third plate had clear zone diameter of 1.9 cm and colony diameter of 0.85 cm which resulted into RA value of 1.2 cm. Therefore, this strain recorded an average RA of 1.32 cm at 4 ^{0}C (Figure 3.2).

Geomyces sp. 1 (AK07KGI601 R3-1) at 4 ^{0} C showed low significant activity, had an average RA of 0.95 cm. For this strain, the first plate had a clear zone diameter of 1.75 cm and colony zone diameter of 1.15 cm that gave RA 0.52 cm. The RA values for the second and third plates were 0.91 cm and 1.44 cm respectively.

Measured relative activities between strains *Geomyces* sp. 2 (AK07KGI902 R1-1) and *Geomyces* sp. 1 (AK07KGI402 R1-1) were 0.79 cm and 0.78 cm respectively that showed low significant activity. Also, *Geomyces* sp. 1 (AK07KGI501 R2-1) and *Geomyces* sp. 4 (AK07KGI902 R1-1), at 4 ^{0}C showed low significant activity, as their chitinase relative activity was 0.5 cm and 0.40 cm, respectively.

Clear zone was observed from day six for different fungi isolates depending on their ability to consume chitin. Diameter of clear zones ranged from 0.8-3.85 cm from different temperature. *Geomyces* sp. 5 (AK07KGI102 R1-4) showed the biggest clear zone as compared to other strains. Generally, the majority of the strains showed

significant enzyme activity at 25˚C while only *Geomyces* sp. 5 (AK07KGI102 R1-4) showed significant enzyme activity at 4˚C. From this point, the fungal cultivation using for chitinase production by colloid chitin broth was done at 25˚C. Based on these results, strains selected in the preliminary screening were *Geomyces* sp. 5 (AK07KGI102 R1-4) and *Geomyces* sp. 1 (AK07KGI601 R3-1) to quantify chitinase enzyme

3.2 Shake flask culture for chitinase production

After ten days of inoculation, the culture broth has been observed pellets formation having pale-yellow coloration (Figure 3.3; Appendix 4.0).

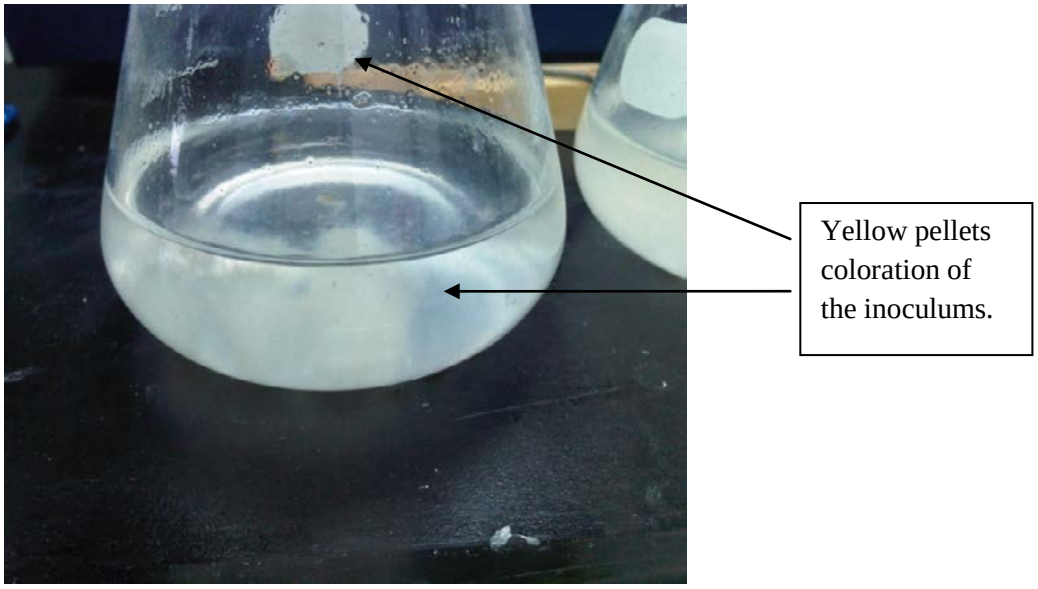

Figure 3.3 Culture indicating yellow pellets after 10 days of inoculation and shaking.

3.3 Quantification of protein-Bradford assay

3.3.1 Construction of standard curve for protein determination

Absorbance at 595 nm of the corresponding known concentrations of bovine serum albumin (BSA) were measured in triplicate values as shown in Appendix 5.0. A

standard curve plot of the BSA concentrations against corresponding absorbance gave a linear equation Y= 9.6371x + 0.0024 within Beer's Law.

3.3.2 Concentration of protein on samples-Bradford method

Table 3.1 and Table 3.2 showed the absorbance reading at 595 nm and the protein concentration readings from *Geomyces* sp. 5 (AK07KGI102 R1-4) and *Geomyces* sp. 1 (AK07KGI601 R3-1) in triplicates values, respectively. The concentration of the protein was calculated based on the formula from standard curve Y= 9.6371x + 0.0024; hence 0.0028 mg/ml and 0.0020 mg/ml of protein were obtained respectively for the organisms.

Table 3.1 Protein concentration from *Geomyces* sp. 5.

R1	R2	R3	Average of absorbance at 595 nm	Concentration of protein (mg/ml)	Average of protein on sample (mg/ml)
0.022	0.04	0.03	0.030	0.0029	
0.022	0.037	0.031	0.03	0.0029	0.0028
0.020	0.028	0.04	0.029	0.0028	

Table 3.2 Protein concentration from *Geomyces* sp. 1.

R1	R2	R3	Average of absorbance at 595 nm	Concentration of protein (mg/ml)	Average of protein on sample (mg/ml)
0.019	0.022	0.02	0.020	0.0018	
0.029	0.026	0.022	0.025	0.0023	0.0020
0.021	0.024	0.029	0.024	0.0022	

3.4 Chitinase Enzyme Assay-Sugar Reductions

3.4.1 Construction of standard curve for Sugar Reduction

Series of NAG dilution (8 times) was done to obtain the standard curve at room temperature. Appendix 6.0 showed the result of the average reading of absorbance at 535 nm from each concentration of NAG (mg/ml) in triplicates values. The graph of standard curve used to obtain relationship in order to generate the concentration of NAG from two strains of *Geomyces*.

3.4.2 Production rate of NAG from samples

Based on formula from standard curve, (y =0.645x), *Geomyces* sp. 5 is relativity high than *Geomyces* sp. 1. Production rate of NAG from *Geomyces* sp. 5 was 0.673 µmol/ml/hr (Table3.3) while production rate of NAG from *Geomyces* sp. 1 was 0.554 µmol/ml/hr (Table 3.4). The enzyme activity was calculated according to Rojas-avelizapa *et al.*, (1999) a chitinase activity unit (CU) was defined as the amount of enzyme required to produce 1 µmol of NAG in 1 hr. Chitinase activity from *Geomyces* sp. 5 was 0.67 U/ml while chitinase activity from *Geomyces* sp. 1 was 0.55 U/ml.

Table 3.3 Production rate of NAG from *Geomyces* sp. 5.

R1	R2	R3	**Average of Absorbance at 535 nm**	Production rate of NAG (mg/ml/hr)	Production rate of NAG(µmol/ml/hr)	**Enzyme activity (U/ml)**	**Std. Error Mean**	**Std. Deviation**
0.088	0.090	0.104	**0.094**	0.146	0.660			
0.086	0.090	0.118	**0.098**	0.152	0.687	**0.673**	**0.008**	**0.014**
0.103	0.095	0.090	**0.096**	0.149	0.673			

Table 3.4 Production rate of NAG from *Geomyces* sp. 1.

R1	R2	R3	**Average of absorbance at 535 nm**	Production rate of NAG (mg/ml/hr)	Production rate of NAG (mol/ml/hr)	Production rate of NAG (µmol/ml/hr)	**Enzyme activity (U/ml)**
0.073	0.074	0.079	**0.075**	0.116279	$5.24X\ 10^{-7}$	0.524	
0.074	0.089	0.90	**0.084**	0.130233	$5.87X\ 10^{-7}$	0.587	**0.554**
0.079	0.087	0.071	**0.079**	0.122481	$5.51X\ 10^{-7}$	0.551	

3.5 Effect of pH and temperature on enzyme activity

The optimal pH for chitinase activity produced by *Geomyces* sp. 5 was examined. From study conducted, it was found that the optimal pH value was at pH 6.5. Figure 3.4 illustrates the effect of different pH values on chitinase activity.

The optimum temperature for chitinase activity was detected at 37 ºC. Figure 3.5 showed the effect of different temperatures on chitinase activity. Appendix 7.0 and Appendix 8.0 show the effect of different pH values and temperatures towards chitinase activity.

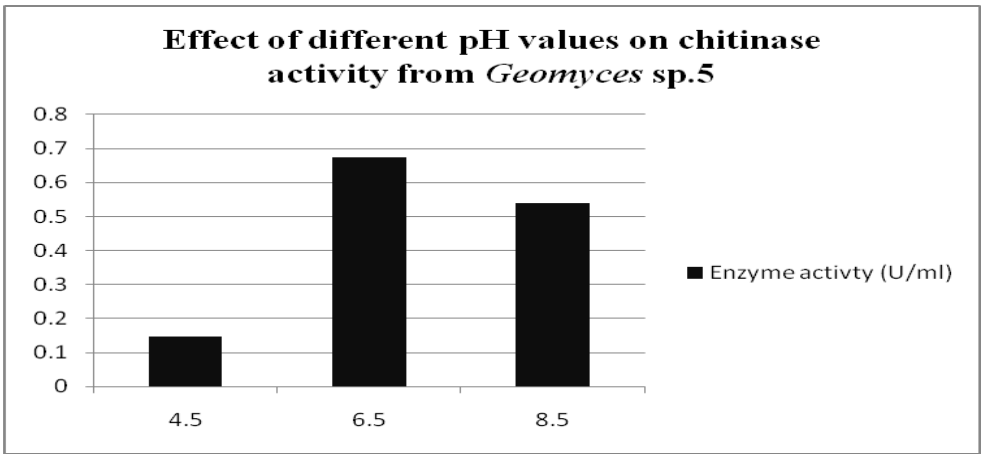

Figure 3.4 Effect of different pH values on chitinase activity from *Geomyces* sp.5.

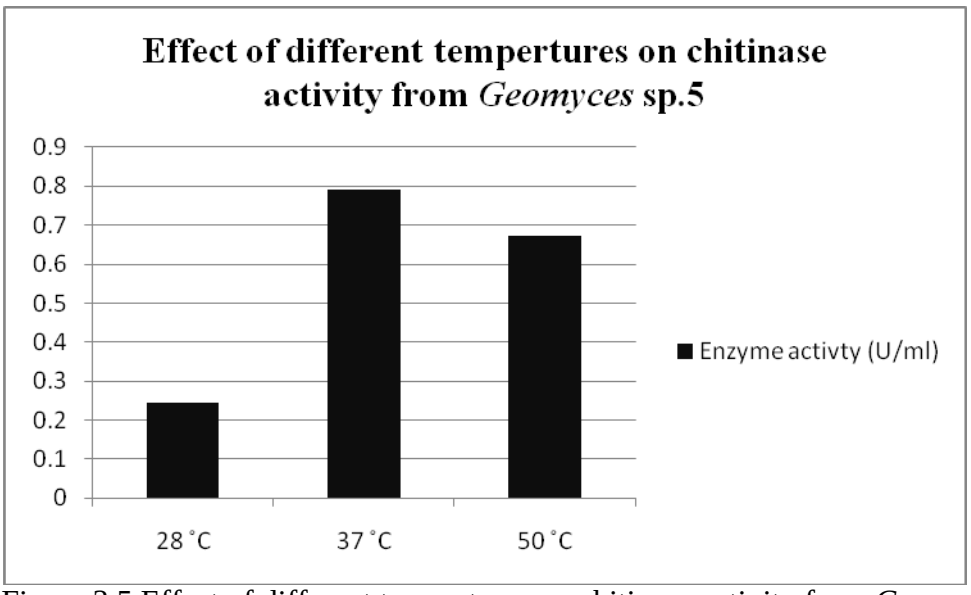

Figure 3.5 Effect of different tempertures on chitinase activity from *Geomyces* sp.5.