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Production, assay, and optimization of Chitinase enzyme produce by bacterial isolates from fish waste dumped soil

K. Shameem Rani
shameemrani@gmail.com
MSS Wakf Board College, KK Nagar, Tamil Nadu

S. Kulandaivel
microskv1970@gmail.com
VPMM College of arts and Science, Krishnankoil, Tamil Nadu

ABSTRACT

The effective chitinase enzyme producing organisms were isolated from prawn shell dumped soil. The isolates were named as FS1 &FS2. The chitinase enzyme was produced only when the organisms was grown on medium containing the prawn shell powder. Optimizations of enzyme production (pH, temparture, substrate concentration) were carried out. In this study both FS1(46.7U/ml) FS2 (77.8U/ml) bacterial strain produced maximum chitinase at pH 7.0. maximum chitinase enzyme was obtained in 0.5% substrate concentration (140U/ml) in 96hrs by FS1 and in 0.8% in substrate concentration (93.3U/ml) in 72 hrs. This enzyme was highly used for antifungal activity.

Keywords: *Chitin, Prawn Shell, N-acetyl-D-glucosamine*

INTRODUCTION

Chitin is a polymer of N-acetyl D-glucosamine residues linked by β-1,4 bonds and it is the second most abundant renewable natural polymer after cellulose(Krithika and Chellaram, 2016). Chitin is Translucent, Pliable, Resilient, quite tough and Insoluble in water. It is widely distributed in nature and almost 10% global landings of aquatic products consist of organisms rich in chitinous materials(Jing Wang *et al*., 2015) The presence of chitinolytic microbes indicates the availability of chitin in the soil (Krithika & Chellaram, 2016). Chitinolytic bacteria can degrade chitin-containing waste into oligo and monomeric components (Kuddus & Ahmad, 2013) by using chitinase enzyme, due to the chitinase enzyme allow conversion the abundant chitin into useful products (Natsir *et al*., 2017) and most of the organisms utilize chitin as a source of nitrogen and carbon including Bacteria (*Serratia, Bacillus, Aeromonas, Vibrio,Pseudomonas, Enterobacter*, *Actinomycetes* etc.,), Fungi (*Trichoderma, Aspergillus* etc.,), viruses, insects, higher plants and animals.

Among the microorganisms 90 – 99% chitinolytic populations are *actinomycetes* (Bansode & Bajekal, 2006), while only a fraction of them arebacterial and less than 1% are fungi (Alexander, 1977).Among the commonly isolated *actinomycetes* genera were *streptosporangium* (47%), *Streptomyces* (30%), *Micromonospora* (15%) and agroup of the allied genera *Actinomadura*, *Microtetraspora*, and *Nonomuraea* (8%).Chitinases are hydrolytic and mycolytic in nature (Park *et al*., 1992). studies with cultures showed that relatively few marine bacteria ranging from 0.4 – 19 % only of the total cultured bacteria degrade chitin (Okutani *et al*., 1975). Screening and isolation of organisms capable of producing chitinase is usually is done on a medium containing chitin. Chitinase activity can be qualitatively assayed by determining the clearance zone developed around the colonies (CZ) to colony size (CS) growing on the chitin agar medium (Cody, 1989;Wirth &Wolf, 1990). This procedure requires longer incubation time for about 5 to6 days and is relatively less sensitive because of the poor visualization of the CZ. Most bacteria isolated express maximum chitinase during the third day of fermentation.

Chitinase producing Fish dumping soil bacteria play an important role in the degradation of chitin. Thus, the present study has been narrowed on Isolation, Screening, Production, Assay and Optimization of chitinase producers from Fish dumping soil sample from Madurai, Tamilnadu, India.

MATERIALS AND METHODS

Collection of Fish dumping soil

The Seafood waste dumping ground soil at the depth of approximately 2- 3cm was collected (from Perungudi, Madurai) in a sterile plastic bag with the help of sterile spatula, the mouth of the bag were tied properly and carried to laboratory for further use. This soil was very rich with chitinous material and hence this source isused for isolating chitinolytic organisms.

Collection of Prawn shell

The Prawn shell wastes were collected from fish market, Therkuvasal, Madurai, Tamilnadu. These wastes were washed with tap water repeatedly and dried oven at 80˚C for 48 hours. After drying, the shells were milled to fine particles. These powdered prawn shell is used as a chitin powder for furtherexperiments.

ISOLATION OF CHITINASE PRODUCERS

From the collected soil sample, 1g of soil sample was added to sterilewater with suitable dilution (10^{-1} to 10^{-10}). From each dilution, 0.1ml was transferred to petriplates containing the chitin agar medium plates by Spread plate technique. The Spread plate method was used to isolate the soil microorganisms. The plates were incubated at 37ºC for 2-3 days. Chitin producers were selected based on the morphology, color and zone formation. Well grown isolated colonies were picked and selected for production of chitinase.

Composition of Chitin Agar medium: K_2HPO_4 – 0.1 g, Nacl – 0.5 g, Chitin powder (Prawn shell powder) – 0.5 g, $MgSo_4.7H_2O$ – 0.004 g, $CaCl_2$ – 0.002 g in 100ml of distilled water (Renwick *et al*., 1991).

SCREENING OF CHITINASE PRODUCING BACTERIA:

From primary screening, two bacterial isolates were selected based on morphologically different and they were streaked on freshly prepared Chitin Agar plates and the plates were incubated at 37ºC for 3-4 days. After incubation the plates were flooded with 0.1% Congo red solution and Destaining with 1% Nacl solution of the plates showed a zone of clearance around the two different colonies (FS1 & FS2) which confirmed chitinolytic activity. These bacteria weresubcultured and maintained in slants at 4ºC.

IDENTIFICATION OF CHITINOLYTIC BACTERIA

The selected chitin degrading bacteria FS1 and FS2 were subjected to morphological and biochemical identification as per Bergey's Manual of systematic bacteriology.

CHITINASE ENZYME PRODUCTION BY SUBMERGED FERMENTATION

For the production of Chitinase enzyme, both FS1 and FS2 culture were inoculated in 250ml conical flask containing the fermentation medium (Chitin Agar medium). These medium contains three different concentration of substrate (Prawn shell powder) such as 0.2%, 0.5% & 0.8%. Then, the flasks were incubatedat 37ºC under shaking condition for 4-6 days. After, every 24 hrs 10ml of culture broth was taken and centrifuged at 3000 rpm for 10mins and the resultant supernatant was used to perform enzyme activity.

PRE-TREATMENT OF PRAWN SHELL POWDER:

0.1g of Prawn shell powder is dissolved in 1.5ml of concentrated Hcl and make up to 100ml using distilled water. It will be used as a substrate stocksolution. This form of colloidal chitin is easily utilized by chitinolytic bacteria for chitinase enzyme production.

ASSAY OF CHITINASE ENZYME

Chitinase production was measured in terms of chitinase activity exhibited by the culture supernatant in the enzyme assay. Chitinase activity was assayed by DNS method (Miller, 1959).

Preparation of standard curve of N-acetyl-D-glucosamine (GlcNAc)

Preparation of standard curve of N-acetyl-D-glucosamine was drawn by measuring the absorbance of solutions containing varied N-acetyl-D-glucosamine levels using 3,5-Dinitrosalicylic acid (DNS) reagent. Standard solution of N- acetyl-D-glucosamine at concentrations of 100 to 1000 µg/ml was prepared using distilled water. Different concentration of test solution make with 1ml of distilled water, 3 ml of DNS reagent was added, and the mixture was incubated in a boiling water bath for 10 mins, instead of test solution, distilled water used as a blank. The color development was measured for the absorbance at wavelength of 540nm usingcolorimeter. A graph was plotted between GlcNAc concentrations against their absorbance value at 540nm (Table 2 and Figure 1).

One Unit (1U) of chitinase was defined as the amount of enzyme that released one µmol of reducing sugar as N-acetyl glucosamine (Glucose) per ml permin under the assay conditions.

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Preparation of DNS Reagent

- Sodium Hydroxide – 8g
- 3,5-Dinitrosalicylic Acid (DNS) – 1g
- Sodium Potassium Tartarate – 30g
- Distilled water - 100 ml

Quantitative Assay for Chitinase

The cell free supernatant was used as an enzyme source. Two sterile test tubes were taken and labeled as Blank and Test. The Test sample consisted of 1ml of 0.1M Sodium Phosphate buffer (pH-7), 1ml of 0.1% Substrate (colloidal chitin) followed by 1ml of Supernatant (enzyme), instead of Substrate, 1ml of distilled water was added as a blank (control assay). Tubes were incubated at 37°C for 1 hr.

After incubation, the reaction was stopped by adding 3 ml of DNS reagent in each tube and heated in boiling water bath for 15mins and cooled rapidly in cold water. Then, the absorption of enzyme activity was measured at 540nm. The reducing sugar was estimated using standard curve of glucose.

OPTIMIZATION OF ENZYME ACTIVITY

The optimization of chitinase enzyme activity was performed to various conditions i.e. different substrate concentration (0.5 to 2.5ml from 0.1%), temperature (35, 37, 39 and 41), pH (5-9) and enzyme concentration (0.5 to 2.5ml). The factors were studied in a sequential manner. One factor was optimizedat a time. The optimal level of this factor was incorporated in the next step.

In this optimization of chitinase production, the maximum chitinase activity of 0.5% (Substrate concentration) of 96th hour FS1 culture was taken and0.8% (Substrate concentration) of 72nd hour FS2 culture was taken as an enzyme source.

Effect of different temperature for chitinase production:

The measurement of the optimum temperature was conducted by placing reaction mixture contains 1ml of Enzyme, 1ml of 0.1% Substrate, 1ml of 0.1M Sodium Phosphate Buffer (pH – 7), instead of substrate, 1ml of distilled water was used as a control (for all test). The tubes were incubated in different temperature ranging from 35ºC, 37ºC, 39ºC and 41ºC for 1hr. After incubation, the enzyme activity was performed to both cultures. The optimum temperature for FS1 culture was 37ºC and FS2 culture was 39ºC.

Effect of different pH for chitinase production:

The optimal pH of the chitinase was determined by varying the pH of0.1M Sodium Phosphate Buffer (5, 6, 7, 8 & 9). These different pH containing Buffer solution was added in a different reaction mixture tubes and the tubes were incubated at 37ºC (FS1 culture) and 39ºC (FS2 culture) for 1 hr. Then, the enzyme activity was performed.

Effect of different Substrate concentration for chitinase production:

The optimum substrate for chitinase production was determined by adding different concentration of 0.1% substrate such as 0.5ml to 2.5ml in each tube containing 1ml of Enzyme and 1ml of 0.1M Sodium Phosphate Buffer (pH – 7 in both cultures). These tubes were incubated at 37ºC (FS1 culture) and 39ºC (FS2culture). The maximum chitinase activity was obtained in 0.5ml of 0.1%substrate in both FS1 and FS2 cultures.

Effect of different Enzyme concentration for Chitinase production:

For optimization of chitinase enzyme was determined by varying the Enzyme concentration by adding 1 ml of Buffer (pH-7), 0.5 ml of Substrate and different concentration of enzymes (0.5 to 2.5 ml) to each tube and incubated both FS1 and FS2 cultures at 37ºC and 39ºC. The maximum enzyme activity was obtained in 2.5ml of enzyme containing tubes i.e the Enzyme activity was increased by increasing of Enzyme concentration.

RESULTS

Isolation and Selection of chitinase producers

Chitinolytic bacteria were isolated from Fish dumping area soil, Madurai, Tamilnadu. The isolates were screened based on zone of clearance. From the primary screening method, two colonies were selected for secondary screening andlabeled as FS1 and FS2. The results were shown in Plate 1.

Identification of chitinolytic bacteria

The isolates were tentatively identified as Actinomycetes (FS1 and FS2)by Morphological and Biochemical test (Kreig,

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1984), according to Bergey's manual of systematic bacteriology. The result was shown in Plate 2 and Table 1.

Production medium for chitinolytic bacteria
Different sets of growth media were formulated by varying the substrates. In each sets of two cultures, substrate concentration only differ such as 0.2 %, 0.5% and 0.8 %. The results were shown in Plate 3, Table 3, Table 4, Figure 2 and Figure 3. The maximum chitinase enzyme activity was observed in FS1 bacteria at theconcentration of 0.5% chitin (140 U/ml) after 96 hrs. The maximum chitinase activity was observed in FS2 bacteria at theconcentration of 0.8% chitin (93.33 U/ml) after 72 hrs.

Optimization of enzyme activity
Effect of temperature for chitinase production
Chitinase production was maximum at 37ºC (46.7 U/ml) by FS1 bacteria and 39ºC (46.7 U/ml) by FS2 bacteria. The results were shown in Table 5, Table 6,Figure 4 and Figure 5.

Effect of pH for chitinase production
The effect of pH of media for the chitinase production, the bacterial cultures were grown at different pH (5 to 9). Among the tested pH, pH – 7 was optimum forboth FS1 (46.7 U/ml) and FS2 (77.8 U/ml) bacteria. The results were shown in Table 7, Table 8 and Figure 6 and Figure 7.

Effect of Substrate concentration for chitinase activity
0.5ml to 2.5ml concentrations of 0.1% substrate was used to elucidate the best concentration for maximum chitinase activity which can be exploited at the industrial level. FS1 and FS2 produced maximum enzyme at 0.5ml of 0.1% stock solution. Accordingly both FS1 and FS2 produced 77.8 U/ml of enzyme, respectively. The results were shown in Table 9, Table 10, Figure 8 and Figure 9.

Effect of Enzyme concentration for chitinase activity
Different concentrations of enzyme were used to elucidate the best concentration for maximum chitinase activity. The maximium enzyme activity wasshown in both FS1 and FS2 at 2.5ml of enzyme concentration. Accordingly, FS1 produced 77.8 U/ml and FS2 bacteria produced 140 U/ml of enzyme, respectively. The results were shown in Table 11, Table 12, Figure 10 and Figure 11.

CONCLUSION

In the present study, the isolated FS1 and FS2 bacteria (Plate 1) showed effective chitinase production with the utilization of chitin from prawn shell waste powder and the isolates were tentatively identified as *Actinomycetes* (FS1 and FS2)by Morphological and Biochemical test (Plate 2, Table 1).

In our studies, Plate 3, Table 3 and Table 4 shows that the substrate concentration in production medium was produced the maximum chitinase activity shown by FS1 was observed in 0.5% (140 U/ml) after 96 hrs and FS2 bacteria was observed in 0.8% (93.33 U/ml) after 72 hrs. Similarly, (Anuradha and Revathi, 2013) reported that produced highest chitinase in *V.aestuaianus* (0.11 U/ml) and *Exiguobacterium* (0.31 U/ml) after 96 hrs of incubation time and 72 hrs in *F.odoratus* (0.18 U/ml) and *S.putrefaciens* (0.29 U/ml). In the case of *K.gibsonii* (Paul, 2012) also reported at 72 hrs of incubation time. Similar observations were also reported by (Saadoun *et al.,* 2009) with *Streptomyces* S_{242} (0.0456 U/ml) and (Shankar Subramaniam *et al*., 2012) also reported *Streptomyces sp,* with 96 hrs of incubation. Although (Mane *et al*., 2018) reported maximum chitinase production at 168 hrs of incubation by *Neisseria sp.* One of the reasons for decreased production may be the lack of nutritions or production of toxic chemicals in the medium results in the inactivation ofsecretary machinery of the enzymes.

The growth of bacteria and enzyme production are also affected with the change in incubation temperature. In our isolated both FS1 and FS2 bacteria were produced maximum chitinase (46.7 U/ml) at 37ºC and 39ºC. The results were shown in Table 5 and Table 6. (Kuddus and Ahmad, 2013; Mane *et al*., 2018; and Bansode and Bajekal, 2006) also reported maximum chitinase production at 37ºC in *A.hydrophila*, HS4 (43.08 U/ml); *A.punctata, HS6* (53.22 U/ml); *Neisseria sp; Bacillus, SB5* and*Streptomyces, SB1*. In the case of *Bacillus licheniformis* (Jholapara *et al*., 2013) and *Streptomyces* S_{242} (saadoun *et al*., 2009); *K.gibsonii* (Paul, 2012) reported at 35ºC. 32ºC in *Streptomyces sp*, (Shankar and Subramaniam, 2012). 30ºC wasreported in *Flavobacterium* (0.05 U/ml) (Anuradha and Revathi, 2013); *S.hygroscopicus* (Haggag and Abdallh, 2012) and T_3 (1.327 U/ml) (Natsir *et al*., 2017). In the case of *Exiguobacterium* (0.04 U/ml) (Anuradha and Revathi, 2013),the optimum temperature for chitinase production was 10ºC. 40ºC was reported in *Vibrio aestuarianus* (0.09 U/ml) and *Shewenella putrefaciens* (0.11 U/ml) (Anuradha and Revathi, 2013). 45ºC in *Nocordia*, SB4 (Bansode and Bajekal, 2006). 50-60ºC in Fungi, VB2 (Bansode and Bajekal, 2006). Bacterial growth was absent in 60ºC.

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In this study, Both FS1 (46.7 U/ml) and FS2 (77.8 U/ml) bacteria Produce maximum chitinase at pH-7. The results were shown in Table 7 and Table 8. Different organisms have different pH optima and decrease or increase in pH on either side of the optimum value results in poor microbial growth. Similar pH optima has been reported in the case of *Bacillus licheniformis* ((Jholapara *et al.,* 2013), was optimum at pH-4, *Streptomyces* sp, (Shankar Subramaniam *et al*.,2012) was optimum at pH-5. In the case of *Vibrio aestuarianus* (0.065 U/ml), *Flavobacterium odoratus* (0.085 U/ml), *Exiguobacterium* (0.075 U/ml) (Anuradha and Revathi, 2013); *Neisseria sp*, (Mane *et al*., 2018); *Streptomyces* SB1 (Bansode and Bajekal, 2006) produced maximum chitinase at pH-6. (Paul, 2012) has been repoted pH-6.5 in *K.gibsonii*. Similarly, the optimum pH-7 was reported in *Streptomyces hygroscopicus* (Haggag and Abdallh, 2012); *Aeromonas punctata*, HS6 (73.43U/ml) (Kuddus and Ahmad, 2013); *Streptonyces* $strain_{242}$ (0.042 U/ml) and Fungi, VB2 (Bansode and Kajekal, 2006). In the case of *Shewenella putrefaciens* (0.05 U/ml) (Anuradha and Revathi, 2013); *Aeromonas hydrophila*, HS4 (93.27 U/ml) (Kuddus and Ahmad, 2013) produced maximum chitinase at pH-8. *Streptomyces*, VB3; *Bacillus*, SB5 and *Nocordia*,SB4 (Bansode and Bajekal,2006) produce maximum chitinase at pH-9.

Table 9 and Table 10, shows that the maximum chitinase production for FS1 and FS2 were observed in 0.5ml of 0.1% substrate (77.8 U/ml). (Natsir *et al*., 2017) observed maximum chitinase in 0.06% (0.343 U/ml) and decreased in 0.08-0.14%. *A.hydrophila*, HS4 (52.8 U/ml) and *A.punctata*, HS6 (43.4 U/ml) were produced maximum chitinase in 0.3% and *K.gibsonii* (Paul, 2012) reported in 0.6%. *Bacillus licheniformis* (Jholapara *et al*., 2013) reported in 1.5%. 1.6% (0.0774 U/ml) was best in *streptomyces sp*, (Saadoun *et al*., 2009) and 2% in *V.aestuarianus* (0.05 U/ml); *F.odoratus* (0.075 U/ml); *S.putrefaciens* (0.04 U/ml) and *Exiguobacterium* (0.039 U/ml) (Anuradha and Revathi, 2013). Table 11 and Table 12 shows that effect of Enzyme concentration for chitinase production. In this study, 0.5 – 2.5 ml of different concentration ofenzymes could be used and they are gradually increased and maximum chitinase observed in 2.5 ml of the enzyme concentration i.e. 77.8 U/ml (FS1) and 140 U/ml (FS2).

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PLATE 1: ISOLATION OF CHITINASE PRODUCING BACTERIA FROM FISH DUMPING SOIL
(FS1 and FS2 bacteria)

PLATE 2: MORPHOLOGY OF ACTINOMYCETES (100X)

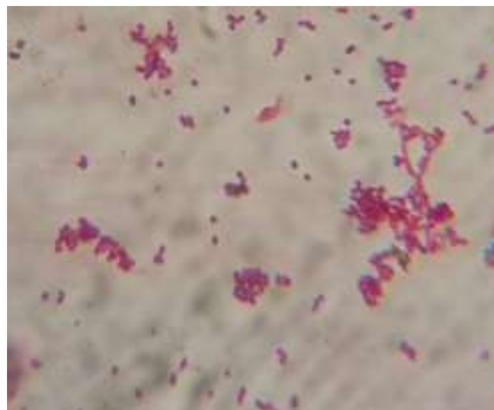

TABLE 1: MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF BACTERIAL ISOLATES

S.No	Characteristics	Bacterial Isolates	
		FS1	**FS2**
1	Gram's staining	(+), Rod shape	(+), Rod shape
2	Starch hydrolysis	Positive	Positive
3	Citrate utilization	Positive	Positive
4	Indole	Negative	Negative
5	Methyl red	Positive	Positive
6	Vogues proskauer	Negative	Negative
7	Catalase test	Negative	Negative

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8	TSI test	Acid butt,Alkaline slant	Alkaline slant

PLATE 3: PRODUCTION OF CHITINASE ENZYME BY THE ISOLATED (FS1 AND FS2)

BACTERIA INSUBMERGED FERMENTATION

TABLE 2: ESTIMATION OF GLUCOSE

Conc.of NAG (µg/ml)	OD value (540 nm)
100	0.09
200	0.18
300	0.19
400	0.28
500	0.33
600	0.39
700	0.45
800	0.50
900	0.55
1000	0.55

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FIGURE 1: STANDARD GRAPH FOR ESTIMATION OF GLUCOSE

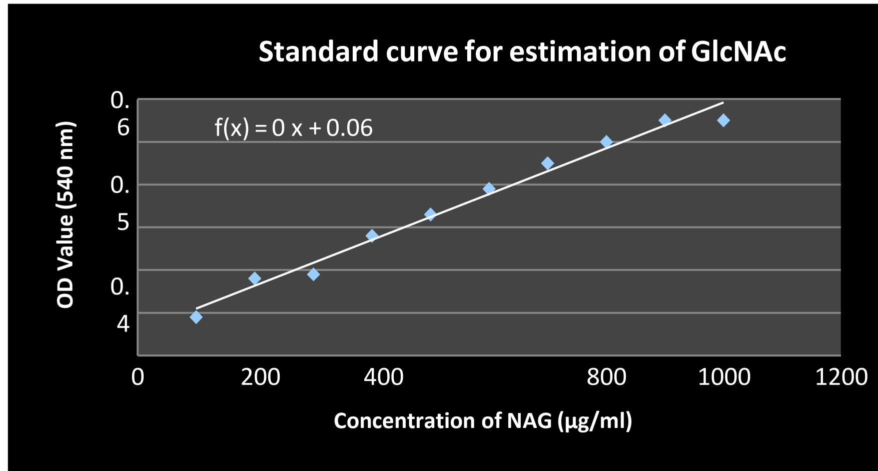

Table 3: Effect of different Substrate concentration for enzyme activity in production medium (FS1)

Effect of Substrate concentration for Enzyme activity (FS1)

Incubation period (hr)	Substrate concentration/ Chitinase Activity (U/ml)		
	0.20%	**0.50%**	**0.80%**
24	62.2	15.6	62.2
48	77.8	46.7	77.8
72	93.3	108.9	77.8
96	124.4	**140.0**	46.7
120	93.3	108.9	31.1
144	62.2	46.7	15.6

160
140
120
100
80
0
24 48 72 96 120 144
0.20%
0.50%
Incubation period (hr)

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FIGURE 2: EFFECT OF DIFFERENT SUBSTRATE CONCENTRATION FOR ENZYME ACTIVITY INPRODUCTION MEDIUM (FS1)

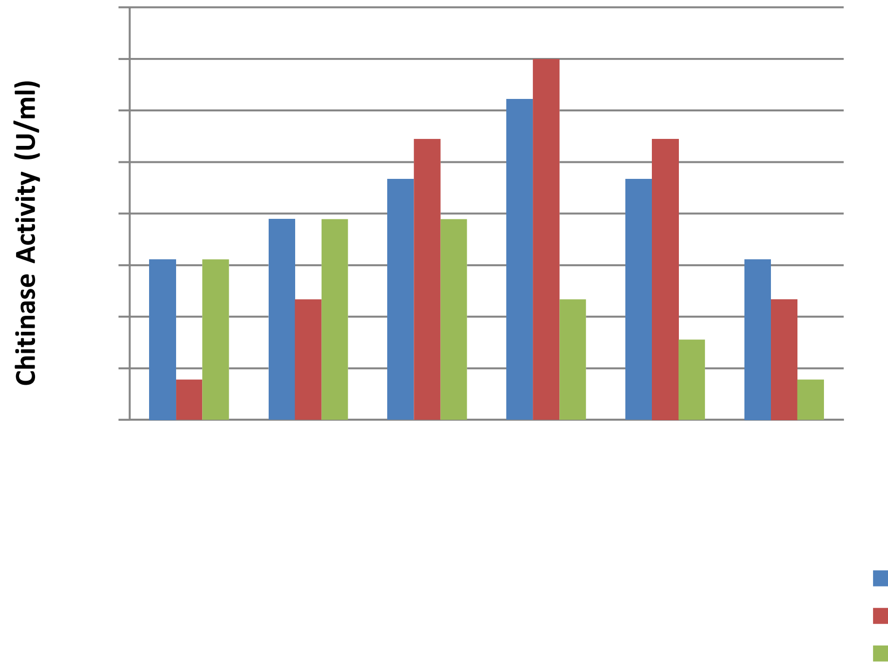

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TABLE 4: EFFECT OF DIFFERENT SUBSTRATE CONCENTRATION FOR ENZYME ACTIVITY INPRODUCTION MEDIUM (FS2)

Incubation period (hr)	**Substrate Concentration/ Chitinase Activity (U/ml)**		
	0.20%	**0.50%**	**0.80%**
24	31.1	62.2	15.6
48	31.1	77.8	93.3
72	46.7	62.2	**93.3**
96	62.2	77.8	46.7
120	31.1	77.8	31.1
144	15.6	62.2	15.6

FIGURE 3: EFFECT OF DIFFERENT SUBSTRATE CONCENTRATION FOR ENZYME ACTIVITY INPRODUCTION MEDIUM (FS2)

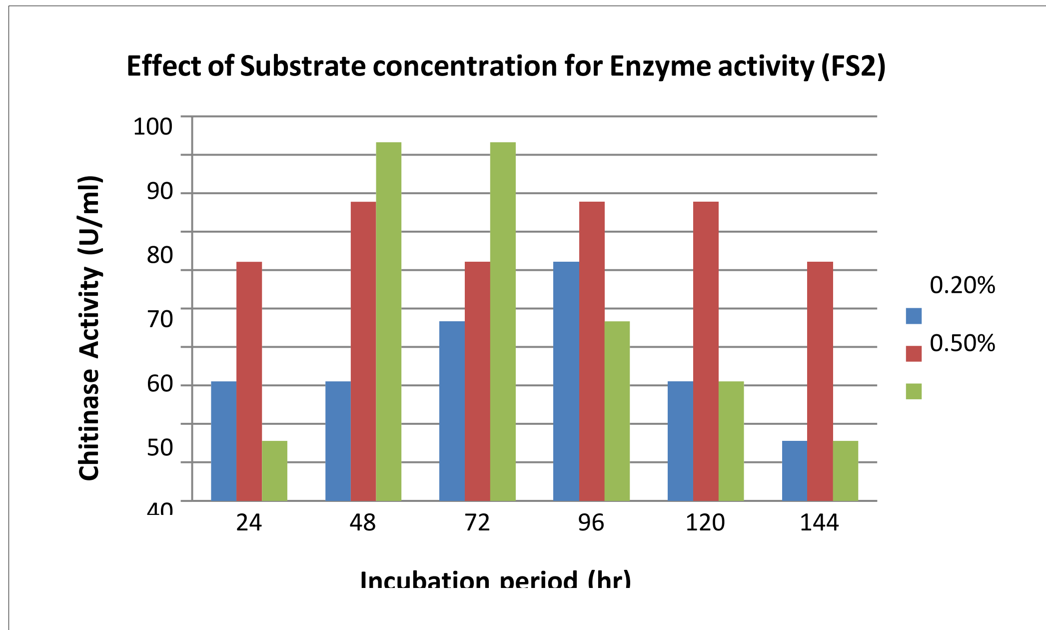

TABLE 5: EFFECT OF DIFFERENT TEMPERATURE FOR CHITINASE ACTIVITY (FS1)

Temperature (˚C)	Chitinase Activity (U/ml)
35	15.6
37	**46.7**
39	15.6
41	15.6

FIGURE 4: EFFECT OF DIFFERENT TEMPERATURE FOR CHITINASE ACTIVITY (FS1)

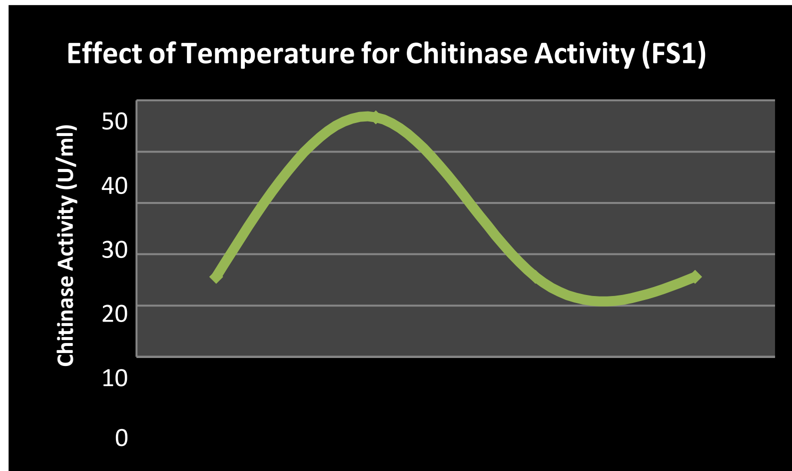

TABLE 6: EFFECT OF DIFFERENT TEMPERATURE FOR CHITINASE ACTIVITY (FS2)

Temperature (˚C)	Chitinase Activity (U/ml)
35	31.1
37	15.6
39	**46.7**
41	31.1

FIGURE 5: EFFECT OF DIFFERENT TEMPERATURE FOR CHITINASE ACTIVITY (FS2)

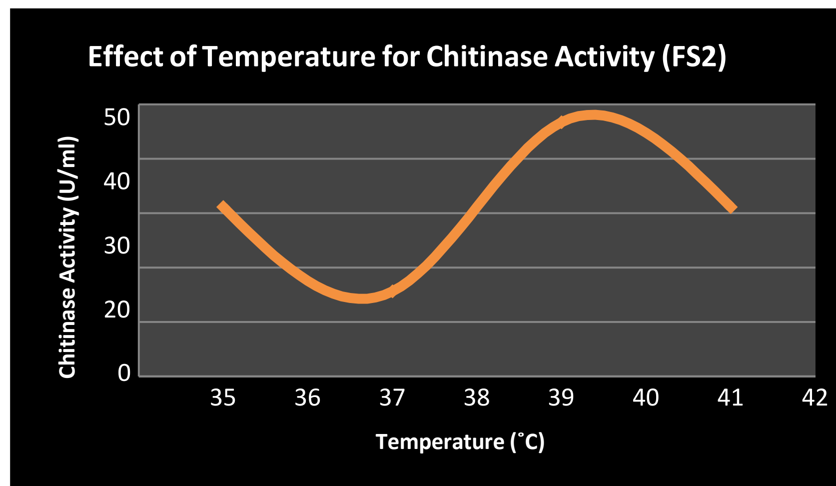

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TABLE 7: EFFECT OF DIFFERENT PH FOR CHITINASE ACTIVITY (FS1)

pH	Chitinase Activity (U/ml)
5	15.6
6	15.6
7	**46.7**
8	31.1
9	15.6

FIGURE 6: EFFECT OF DIFFERENT PH FOR CHITINASE ACTIVITY (FS1)

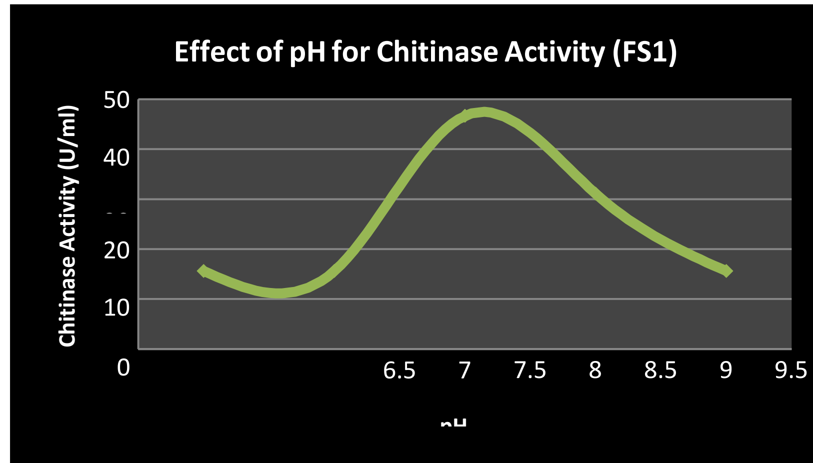

TABLE 8: EFFECT OF DIFFERENT PH FOR CHITINASE ACTIVITY (FS2)

pH	Chitinase Activity (U/ml)
5	31.1
6	15.6
7	**77.8**
8	62.2
9	15.6

FIGURE 7: EFFECT OF DIFFERENT PH FOR CHITINASE ACTIVITY (FS2)

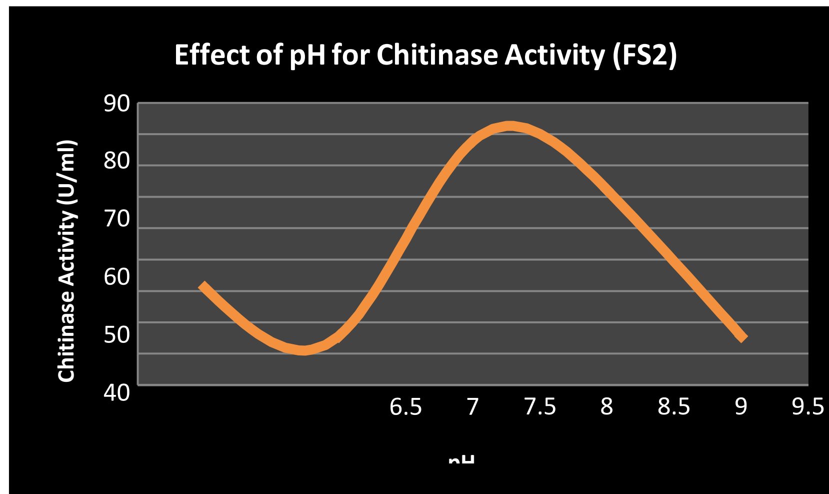

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TABLE 9: EFFECT OF DIFFERENT SUBSTRATE CONCENTRATION FOR CHITINASE ACTIVITY (FS1)

Substrate concentration (ml)	**Chitinase Activity (U/ml)**
0.5	**77.8**
1.0	46.7
1.5	62.2
2.0	15.6
2.5	15.6

FIGURE 8: EFFECT OF DIFFERENT SUBSTRATE CONCENTRATION FOR CHITINASE ACTIVITY (FS1)

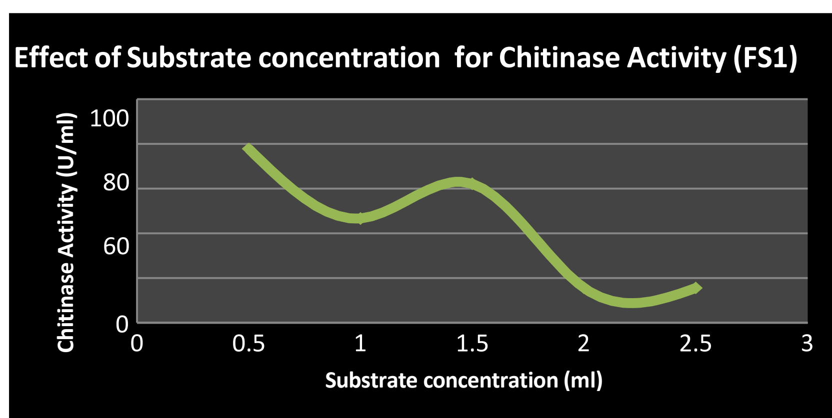


TABLE 10: EFFECT OF DIFFERENT SUBSTRATE CONCENTRATION FOR CHITINASE ACTIVITY (FS2)

Substrate concentration(ml)	**Chitinase Activity(U/ml)**
0.5	**77.8**
1.0	31.1
1.5	15.6
2.0	15.6
2.5	15.6

FIGURE 9: EFFECT OF DIFFERENT SUBSTRATE CONCENTRATION FOR CHITINASE ACTIVITY (FS2)

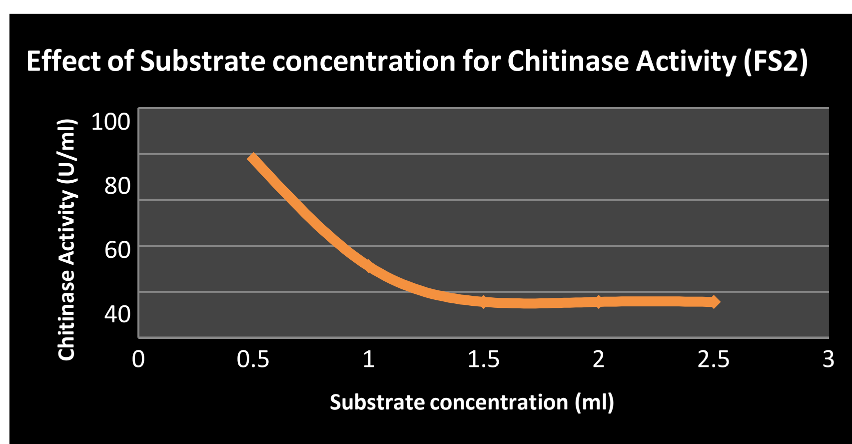


TABLE 11: EFFECT OF DIFFERENT ENZYME CONCENTRATION FOR CHITINASE ACTIVITY (FS1)

Enzyme concentration (ml)	**Chitinase Activity (U/ml)**
0.5	15.6
1	15.6
1.5	46.7
2	46.7
2.5	**77.8**

FIGURE 10: EFFECT OF DIFFERENT ENZYME CONCENTRATION FOR CHITINASE ACTIVITY (FS1)

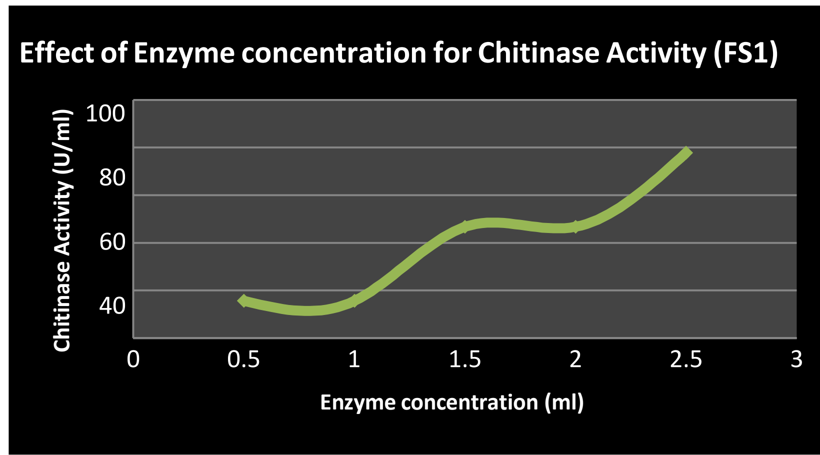
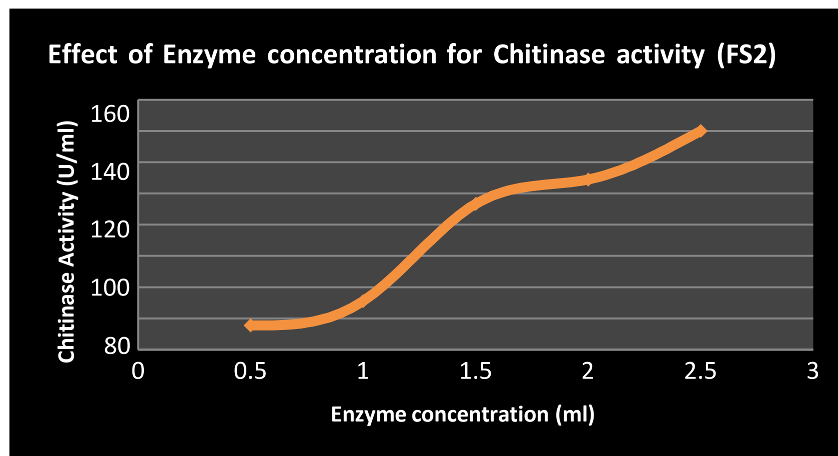


TABLE 12: EFFECT OF DIFFERENT ENZYME CONCENTRATION FOR CHITINASE ACTIVITY (FS2)

Enzyme concentration (ml)	**Chitinase Activity (U/ml)**
0.5	15.6
1.0	31.1
1.5	93.3
2.0	108.9
2.5	**140.0**

FIGURE 11: EFFECT OF DIFFERENT ENZYME CONCENTRATION FOR CHITINASE ACTIVITY (FS2)



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*Dr.K.Shameem Rani, Associate Professor, Department of Zoology,

M.S.S.Wakf Board College,Madurai.

**S.Kulanidaivel, Assistant Professor Department of Microbiology,

VPMM Arts and Science College for Women, Krishnan kovil.

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