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OPTIMIZATION OF CHITINASE PRODUCTION BY BIOTIC AND ABIOTIC FACTORS

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ABSTRACT: Chitin is the naturally occurring polymer and abundant in nature. Chitin is converted into its derivative N-acetyl glucosamine which has high antibacterial, antioxidant, antitumor, ant leukemia and even antiviral property. Due to its cationic property it has many biomedical and industrial applications. Converting this biopolymer into its derivative form using microorganism shows significant yield and purity. In this paper using Pseudomonas aeruginosa the yield of N-acetyl glucosamine is determine by supplying different parameters like pH, temperature, carbon, nitrogen and metal ions.

KEYWORDS: Optimization, biotic, abiotic factors.

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1.INTRODUCTION

Chitin is a nitrogenous insoluble homopolymer which contains β-1, 4 linked N-acetyl glucosamine. Chitin is the second most abundant biopolymer in nature. Chitin is the major component of fungal cell wall, yeast, green algae, in cuticles of insects and also in the exoskeleton of crab and shrimp which is the major source of chitin for industrial purposes [1-3]. Chitin is extracted from the shells of crab and shrimp by acid treatment with HCl to dissolve calcium carbonate in the shells followed by alkali treatment using NaOH to solubilize proteins. Chitosan is obtained from chitin by deacetylation process treated with concentrated NaOH [23,24]. Due to the structural and chemical features of many bioactive natural products marine environment plays an exceptional reservoir.

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Bioactive compounds obtained from marine biota exhibits antitumor, ant leukemia, antibacterial and antiviral activities [13-15]. Disposal of marine biota is a major problem in marine area. Every year the catch for crustacean species for meat increase and the exoskeleton of the crustaceans become major problem in coastal area causing foul smell and slow degradation due to the presence of rich nutrients. Even though it is a major problem the marine waste are not leave as such in the environment [9-12]. For biodegradation using microorganisms is easy way to solve the problem and obtaining the nutrients become high purity. In this present study, *Pseudomonas aeruginosa* to degrade the chitin by obtaining optimum conditions for the microorganisms for enhancing the activity like pH, temperature, carbon, nitrogen and metal ions as sources and the production of N-acetyl glucosamine is estimated by estimating the amount chitinase produced. One unit of chitinase activity is defined as the amount of enzyme required to release 1µmol N-acetyl glucosamine (GlcNAc) in one minute [5-8].

2. MATERIALS AND METHODS

Optimization Parameters in Production of N-acetyl glucosamine

Microorganisms were used to degrade the substrate and the end product N-acetyl glucosamine was estimated by calculating the production of chitinase enzyme and to know the optimum condition we used different sources for their enhancement. Microorganism used in this study was *Pseudomonas aeruginosa* (MTCC 2453).

Biodegradation of chitin in Optimum Substrate Concentration

Minimal media (100 ml) was taken in conical flask with various concentration of substrate chitin powder (0.25, 0.5, 1 and 1.5gms against control) with 5% inoculums at 120rpm in room temperature and the production of chitinase was measured using colorimeter at 540nm for every 24 hours and the optimum production of chitinase was measured and the substrate concentration was optimized.

Optimization of chitinase production at various pH and temperature

Minimal media (100ml) was taken and the chitinase optimization was found at 1g chitin substrate and one gram of chitin powder is taken as substrate and different pH value [6, 7, 8, 9 and 10] was adjusted using HCl and NaOH. 5% of inoculums was added and the production of chitinase was determined in enzyme assay and OD value taken at 540nm for every twenty-four hours. Minimal media 100ml was taken with one gram of chitin powder with 5% inoculums and kept at various temperature ranging from 25°C to 45°C at interval of 5°C variation as (25°C, 30°C, 35°C, 40°C and 45°C) using incubator. The optimized production of chitinase was measured for every twenty-four hours and OD value taken and the values were tabulated.

Optimization of chitinase production at different Carbon sources

Various carbon sources like Starch, Sucrose, Fructose, Mannitol, Lactose were taken as carbon source.100ml of medium was taken in a conical flask with 1% chitin and 5% of inoculums. The flask was kept in shaker for enhancing the growth and the production of chitinase was estimated for

every 24hours and the results were tabulated.

Optimization of chitinase production in different Nitrogen sources

Various nitrogen sources like Yeast Extract, Peptone, Ammonium chloride, Ammonium sulphate and Potassium nitrate were taken. 100ml of minimal media was taken in 250ml conical along with 1% of chitin powder. 5% of inoculums was added to the flask and kept in shaker at optimum temperature and OD values taken for every 24 hours.

Optimization of chitinase production using various metal ions

100ml of minimal media was taken with one ml of colloidal chitin with 5% of inoculums with various metals like Copper, Cobalt, Manganese (Cu, Co, Mn, Fe and Zn) and the production of chitinase was estimated for every 24hours and the values are tabulated.

3. RESULTS AND DISCUSSION

Determination of biodegradation of chitin at different concentration with *Pseudomonas aeruginosa* (MTCC2453)

Optimization of biodegradation with different concentration of chitin such as 0.5%, 1% and 1.5% evaluated with different duration of exposure were depicted in Table 1 and Figure 1. The result revealed maximum biodegradation of chitin by *Pseudomonas aeruginosa* (MTCC2453) were observed at 1% expressed an OD value of 2.38. subsequent increase in substrate concentration lead to decrease activity of 1.24 at 1.5% substrate concentration. Similarly, at low level of substrate concentration of 0.25 revealed moderate activity at 72 hours.

Table 1: To determine the biodegradation of chitosan by *Pseudomonas aeruginosa* at different substrate concentration

S. No	Substrate Concentration (%)	24 hrs	48 hrs	72 hrs	96 hrs
1	0	0.78	0.96	1.14	0.68
2	0.25	0.36	1.21	0.92	0.4
3	0.5	0.92	1.64	1.88	0.76
4	1	0.52	1.08	2.38	0.96
5	1.5	0.30	0.94	1.24	1.18

Optimization parameters for enhanced chitinase activity for *Pseudomonas aeruginosa* (MTCC2453) at different Temperature and pH

In the present study optimization parameters like pH and temperature was studied. Effect of various pH in the range of pH 6, 7, 8, 9 and 10 was read with the fermentation media on the yield of chitinase are presented in Table 2. Present study was conducted at five different temperatures of 25°C, 30°C, 35°C and 40°C and 45°C. Temperature is an important environmental factor which affecting the growth and production by micro-organisms. In the present study the profile of influence of

temperature on chitinase production was detectable between 35°C and 40°C. The optimal temperature for growth and production of chitinase by Pseudomonas aeruginosa was found to be 35° C to the OD value taken and the optimum pH of 8 found to exhibit OD value of 1.49 at 30° C followed by an elevated temperature of 35°C reveal OD value of 1.52. Simultaneously the pH was increased to pH 9 showed an OD of 1.32 and 1.38 recorded at temperature of 30°C and 35°C respectively. Further increase in temperature and pH revealed a decrease chitinase activity.

Table 2: Determination of chitinase by *Pseudomonas aeruginosa* (MTCC2453) **at various temperature and pH**

S. No	pH	25ºC	30ºC	35ºC	40ºC	45ºC
1	pH 6	0.59	0.99	1.04	0.81	0.43
2	pH 7	1.10	1.42	2.26	1.70	0.86
3	pH 8	1.14	1.64	1.98	1.70	0.49
4	pH 9	0.42	0.68	0.97	0.35	0.12
5	pH 10	0.25	0.58	0.41	0.22	0.17

Optimization for the production of chitinase using different carbon sources by *Pseudomonas aeruginosa* (MTCC2453)

All micro-organisms require carbon and energy source for their growth. The range of carbon sources that can be utilized was vast. The results were depicted in Table: 3. The supplementation of additional carbon source (starch, fructose, mannitol, sucrose, lactose) may enhance or suppress when chitin as substrate was employed as energy and substrate source resulted in an enhanced activity with *Pseudomonas aeruginosa* (MTCC2453). In present study fructose as substrate supplemented in media containing *Pseudomonas aeruginosa* (MTCC2453) found to exhibit maximum activity during seventy-two hours of incubation exhibit an OD value of 1.81. Further extended hours of incubation to ninety-six hours revealed reduction in the growth with an OD value of 1.28. Subsequently when sucrose was given as carbon source a gradual increase in activity obtained during an optimum during twenty-four hours followed by forty-eight hours, seventy-two hours and ninety six hours respectively. During seventy-two hours of incubation it was found to be optimum since a maximum activity of 1.21 was noted. However, when mannitol was supplemented as carbon sources a slight decrease during an optimum period of seventy-two hours incubation exhibiting 0.90. Similarly, the other substrate starch and lactose supplemented for enhanced growth of microorganisms along with chitin as substrate revealed little effect with 0.83 and 0.91 of OD value observed during seventy-two hours of incubation. It is interesting to denote that starch was found to be the most effective additional substrate for enhanced growth of *Pseudomonas aeruginosa* (MTCC2453).

Table 3: Determination of chitinase by *Pseudomonas aeruginosa* (MTCC2453) in various carbon sources

S. No	Carbon Sources	24hrs	48hrs	72hrs	96hrs
1	Starch	0.93	0.95	0.83	0.67
2	Fructose	1.16	1.50	1.81	1.28
3	Mannitol	0.97	0.86	0.90	0.71
4	Sucrose	0.95	1.15	1.21	0.90
5	Lactose	0.77	0.93	1.12	0.91

Optimization for the production of chitinase using different Nitrogen sources by *Pseudomonas aeruginosa* (MTCC2453)

Nitrogen is an important component in the growth of microorganisms. Out of the five nitrogen sources tested the additional of the yeast extract enhanced the growth of Pseudomonas aeruginosa during seventy-two hours of incubation with an elevated OD of 2.52. Subsequent increase in ninety-six hours leads to drastic reduction in the activity. In the similar study to optimize peptone as nitrogen source reveal an optimization in seventy-two hours of incubation showed an OD value of 2.39 whereas Ammonium chloride also found to exhibit an OD value of 2.44 during seventy-two hours of incubation. Consequently, added as ammonium sulfate as a nitrogen source lead to a decrease in the growth during seventy-two hours of incubation. Similarly, an addition of Potassium nitrate as nitrogen source revealed a moderate increase with an OD value of 2.40 during seventy-two hours of incubation.

Table 4: Determination of chitinase by *Pseudomonas aeruginosa* (MTCC2453) in various nitrogen sources

S. No	Nitrogen Sources	24hrs	48hrs	72hrs	96hrs
1	Yeast Extract	2.30	2.41	2.52	1.39
2	Peptone	1.99	2.13	2.39	1.20
3	Ammonium Chloride	2.10	2.21	2.44	1.51
4	Ammonium Sulphate	1.51	1.72	1.90	1.23
5	Potassium Nitrate	2.11	2.27	2.40	1.43

Optimization for the production of chitinase using different Metal ions sources by *Pseudomonas aeruginosa* (MTCC2453)

The effect of metal ions or inhibitors on growth activity was tested with Manganese, Copper and Cobalt. The results were depicted in Table:5. Since Manganese is the essential element for the growth of microorganism were tested during different hours of incubation and found to be an optimum incubation during seventy-two hours revealed 2.30. Subsequent analysis of metal ion Copper found to denote a gradual increase from twenty-four to ninety-six hours of incubation.

Consequently, addition of Cobalt reveals a slight decrease in the growth and activity of *Pseudomonas aeruginosa* (MTCC 2453) exhibiting an OD value of 1.53. Subsequent increase during ninety-six hours of incubation the value was found to exhibit gradual reduction.

Table 5: Determination of chitinase by *Pseudomonas aeruginosa* (MTCC2453) in various metal ion sources

S. No	Metal Ions	24hrs	48hrs	72hrs	96hrs
1	Mn	1.92	2.09	2.30	1.40
2	Cu	1.54	1.62	1.79	1.15
3	Co	1.39	1.49	1.53	1.07
4	Fe	0.82	1.77	1.86	0.71
5	Zn	0.42	0.54	0.92	0.51

DISCUSSION

Determine the production of chitinase at different substrate concentration

Biodegradation of chitin by *Pseudomonas aeruginosa* (MTCC 2453), better result was observed in the concentration of 1.5g in seventy-two hours of incubation. Further, the amount of chitinase production reduced randomly but in one g the biodegradation value was gradually increased and shows maximum result in ninety-six hours also. It may be due to the high nutrient present in media after degradation or change in pH and high supplement of substrate. Present study was supported by research groups [17-20].

Determine the effect of pH and temperature

In the present investigation the optimization parameters like temperature pH, Carbon, nitrogen source and metal ions were studied. The effect of temperature on chitinase activity in different range of pH 6-10 was studied. The maximum activity was noted for *Pseudomonas aeruginosa* (MTCC 2453) when the media was adjusted to pH 7 and pH 8. In the chitinase activity declined significantly when the pH was increased or decreased indicating the optimality of the strain *Pseudomonas aeruginosa* (MTCC 2453). Present study, the total agreement with the report of [13, 32] and subsequent analysis is contributed by [21, 13]. Empathized chitinase production in a broad range of *Aeromonas hydrophila*. Simultaneously chitinase activity upon the effect of temperature showed good activity over the temperature at 32ºC to 35ºC temperature affect various biological process. Therefore, the growth of bacteria and enzyme production also affect in the change in incubation time. It is interesting to denote that our study revealed an optimum temperature of 35ºC in accordance to above said result [24,32] reported maximum chitinase production at 35ºC by *Pseudomonas aeruginosa* (MTCC 2453). It is further substantiated that 17-18 observed maximum chitinase production at 35ºC by *Streptomyces.*

Determine the Effect of Carbon source on Chitinase production

The effect of carbon sources tested with *Pseudomonas aeruginosa* (MTCC 2453) on chitinase production revealed starch as suitable carbon source with an OD value of 2.10 at 72 hours of incubation with 1% carbon source, these results were in accordance with other report by [11, 15, 17]. Further it was reported by [16] indicated maximum chitinase production using starch as carbon source exhibited 96.3U/ml. the other carbon sources namely fructose and mannitol exhibited a moderate chitinase production followed by fructose at 72 hours of incubation.

Determine the Effect of Nitrogen source on Chitinase Production

The nutritional and environmental conditions have a great influence on chitinase production, due to the receptor-inducer system. In microorganisms, chitinase production is controlled by a receptor-inducer system; therefore, the composition of the culture medium and fermentation conditions can affect significantly chitinase production [13]. During the last decade, chitinase have drawn much attention in recent years due to their potential biotechnological applications. Besides the enormous applications of chitinase in various fields, their commercial production and scale up of critical importance are noticeably influenced by medium components and environmental factors [17, 26]. For this reason, the medium optimization studies and searching for chitinases production key factors to maximize the production and meet the industrial demands.

Determine the Effect of Nitrogen sources in Chitinase Production

Different nitrogen sources were tested for the suitability to produce chitinase. Yeast extract (0.5%) was the most preferable source yielded the maximum chitinase specific activity. On the other hand, the lower activity was obtained in the presence of peptone as a nitrogen source. Data's were in accordance with other researches [30-32]. The main conclusion obtained from the present study reveals that the optimization of medium composition of nitrogen sources affect significantly chitinase production and may play a pivotal role in cost reduction for the large-scale production. The result suggests that among the 5 nitrogen sources at 1% substrate concentration revealed maximum chitinase production with yeast extract 2.55 at 72 hours of incubation followed by Ammonium chloride, Peptone and Potassium nitrate. It is astonishing to note that substantiate production of chitinase with almost 5 nitrogen sources were studied. The above results coincide with the findings of [29]. In contrast with the present study Ammonium sulfate was found to be effective to enhance the production of chitinase by *Aspergillus* sp. but in our study Ammonium sulfate exhibited moderate chitinase activity. In time with above discussion similar study was reported by [16].

Determine the Effect of Metal ions in Chitinase Production

Metal ions play an important role in maintaining the structure and configuration of enzymes [24-26]. In the present study the effect of various metal ions by *Pseudomonas aeruginosa* (MTCC 2453) was investigated and the results showed that the chitinase production was enhanced by the addition

of Manganese (Mn) followed by Irons (Fe) in the culture media of Pseudomonas aeruginosa (MTCC 2453). However, the enzyme production depressed with the addition of Zinc (Zn). The present study is also support with previous report of chitinase production enhanced by other microorganisms [4, 28].

4. CONCLUSION

Chitin and its derivatives have great economic value in industrial and biomedical field due to their biological property. In the present study the exoskeleton of crab shells were used to derive chitosan, a water soluble heteropolymer contains β (1,4) linked N-acetylglucosamine and D-glucosamine. N-acetylglucosamine from chitin has great economic value in various fields and its preparation by using microorganism *Pseudomonas aeruginosa* (MTCC 2453) leads to production of large amount of N-acetyl glucosamine in low cost and solve environmental problems. The antibacterial activity of N-acetyl glucosamine leads to production of novel drugs for many diseases which is an alternate to chemical drugs. The various parameters were studied to enhance the production of N-acetyl glucosamine using various microorganisms were studied.

CONFLICT OF INTEREST

Authors have no any conflict of interest.

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