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Production of Chitinase by Solid State Fermentation

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Abstract: The goal of this research project is to find out the novel Chitinases producing bacteria. The soil samples were collected from the Miraj and Osmanabad fish market and enriched in MS medium containing chitin as the sole source of carbon. 10 Bacterial species were isolated from soil samples by serial dilution technique and spread plate technique on nutrient agar and potato dextrose agar medium. The isolated bacterial species were screened for Chitinase production by primary screening on Colloidal chitin agar medium. Those Bacteria gives highest Chitinase hydrolysis zone used for Chitinase production by Solid State fermentation (SSF). The extracted Chitinase enzyme further used for Chitinase activity by DNS method in that highest Chitinase activity was 11.46µg/ml/min. on 11th day of fermentation.

Key words: Chitin, Chitinase, SSF, DNS.

Introduction:

Chitin (the Greek word for "envelope") is one of the most predominant polysaccharide in nature. After cellulose, it is on the second place in biological turnover and it is important component of many organisms from different taxonomic groups (Gooday *et al.*, 1990). Chitin is well-known as an insoluble structural polysaccharide that occurs in the exoskeleton and gut linings of many insects, invertebrates such as crustaceans, protozoa, fungi and diatoms which could be hydrolyzed by chitin degrading enzymes such as Chitinases (Kramer *et al.*, 1986). Chitin has not been identified in prokaryotes (exception is *Streptomyces* spore walls), plants and vertebrates (Agullo *et al*., 2003).

Chitin is a linear β-1, 4-N-acetylglucosamine polysaccharide (Cabib *et al.*, 1987) is the most abundant renewable natural resource after cellulose (Deshpande *et al*., 1986). Approximately 75% of the total weight of shellfish, such as shrimp, crabs and krill are considered as waste, and comprises 20 - 58% of the dry weight of the said waste (Wang *et al.*, 1997). Biocontrol of pathogenic fungi depends upon antibiosis, competition and lysis (Dhar *et al.*, 2012).

The **Chitinases** producing organisms have been isolated from a number of sources such as air, water, soil, marine water etc. (Wang *et al*., 1997) Chitinases have been isolated from the stomach of certain mammals including humans. Although mammals do not produces chitin; they have two functional Chitinases – chitoriosidase – CHT1 and acidic mammalian Chitinase – AM Case that high sequences similarity but lack Chitinase activity (Gooday *et al.*, 1990).

Chitinases have many industrial and agricultural applications such as fungisides, bacteriosides, biopesticides, against mosquitoes, SCP etc.

Materials and Methods

Materials

Chitin shrimp shell, 3, 5-dinitrosalycilic acid (DNSA) and other materials were used from microbiology department of Dr. BAMU, sub-campus Osmanabad, India. All other chemicals used were analytical grade.

Methods

Different methods and materials have been used in this current work during the isolation, screening, production, Chitinase activity by DNS method.

Sample collection

Soil samples were collected from the Miraj and Osmanabad (India) fish market. The soil samples were taken from 4-5 cm depth with the help of sterile spatula and kept in sterile plastic bag which was brought to laboratory and were kept in refrigerator at 4^{0}C till further processing.

Preparation of colloidal chitin

Colloidal chitin was prepared from the shrimp well chitin flakes by Hsu and Lockwood method (1975). In this method, the chitin flakes (40 g) were slowly added to 600 ml of conc. HCl and kept at 30°C for 60 min. with vigorous stirring. Chitin flakes was precipitated as a colloidal suspension by adding it slowly to 20 ml of distilled water at 4- 10°C. The suspension was collected by filtration and washed by suspending it in about 51 ml of distilled water. Washing was repeated 3 times until pH of the suspension was 3.5 and used as a substrate.

Enrichment of Chitinase producers

5 grams of the soil samples were enriched in 100 ml of Minimal Salts Medium (MSM) containing chitin as the sole source of carbon. The enrichment was carried out at 25°c with 150 rev /min. in incubator shaker and viable count was studied.

Isolation of the Chitinase producers

Serial dilution technique and Spread plate technique was used for the isolation of Chitinase producers. Serially diluted enriched soil samples were spread on sterile nutrient agar media plates. The plates were incubated at 37°C for 24 -48hrs. Well grown isolated colonies were picked and sub-cultured on sterile nutrient agar media plates to obtain pure culture and stored NA slants at 4°C as stock cultures.

Screening of Chitinase producers

Primary Screening

Well grown isolated bacterial colonies were spot inoculated on sterile colloidal chitin agar media and were incubated at 37°C for 24 -48hrs. After incubation the plates were flooded with 2% Congo red and were examined for the formation of a clear zone i.e. zone of hydrolysis around the colonies. On the basis of zone of hydrolysis Chitinase producers was selected and used for further processing.

Secondary Screening

Solid state fermentation:

Solid-state fermentation was employed for production of Chitinase. 10 g of Rice bran were transferred to the seven individual 250 ml cotton plugged Erlenmeyer flasks. The flasks were autoclaved at 15 lb/inch2 pressure and 121ºC for 15 min., and cooled at room temperature. The Chitinase positive were inoculated in cotton plugged Erlenmeyer flasks for SSF and incubated for 14 days for Chitinase production.

Enzyme Extraction

After 7 days of incubation period, 30 ml of 0.05 M Phosphate buffer (pH 6.0) was added to the fermented substrate in first flask. The contents of the flask were crushed with the help of a glass rod and flasks were rotated on a rotary shaker at 120 rpm for 1 h at 30ºC afterwards the fermented medium filtered through whatman filter no. 1 filter paper. After filtration, contents were centrifuged at 5,000 rpm for 10 min at 4^{o}C and clear supernatant from each of the tubes was collected for further studies and tubes was stored at 4°C until used. These whole procedures were repeated for remaining six SSF flasks.

Chitinase Enzyme activity

Chitinase enzyme activity was assayed by using DNS method to measure the amount of reducing sugar liberated from chitin. The crude enzyme protein was used for measuring the activity at pH 6 (phosphate buffer 0.5) at 37^{0}c for 30 min.

Chitinase enzyme unit activity

According to the International Union of Biochemistry 1 enzyme International unit has been defined as amount of enzyme required to release 1µmol of reducing sugar in 1 min. at 40°c and at atmospheric pressure.

Result and discussion

Isolation of Chitinase producers

10 isolates were obtained. They were labelled as RT1, RT2 up to RT10 and were subculture on sterile nutrient agar slants in duplicates and stored at 4˚C. The results were shown in Table I

Table1: Colony size and zone of chitin hydrolysis shown by isolates in primary screening

Isolate No.	Colony size (Cm)	Zone of chitin hydrolysis(Cm)	Isolate No.	Colony size(Cm)	Zone of chitin hydrolysis(Cm)
RT1	0.2	0.6	RT 6	2.2	3.6
RT2	0.3	0.7	RT 7	2	4
RT3	0.3	1	RT 8	2.2	4.2
RT4	0.3	0.9	RT 9	1.5	2.8
RT5	0.2	0.8	RT10	1.1	2.6

Fig: I Isolation and screening of Chitinases producing microorganisms from soil sample.

Table II: Selection of the isolates for secondary screening on the basis of colony size and zone of chitin hydrolysis

Isolate no.	RT 2	RT 5	RT 6	RT 7
Colony Size.(cm)	0.4	2.2	2.2	0.3
Zone of hydrolysis(cm)	1.8	3.6	4.6	2.6

Primary Screening

All 10 isolates were screened for Chitinases production on sterile colloidal chitin medium. Among the 10 isolates only 5 isolates were showing maximum chitin hydrolysis zone. The results were shown in figure I and Table II.

Secondary Screening and Chitinase activity by DNS method

RT 6 shows highest zone of hydrolysis in primary screening that's why RT 6 was selected for secondary screening by using SSF. After 7 days of incubation period Chitinases activity were determined up to 14 days by DNS method and the results were shown in figure II.

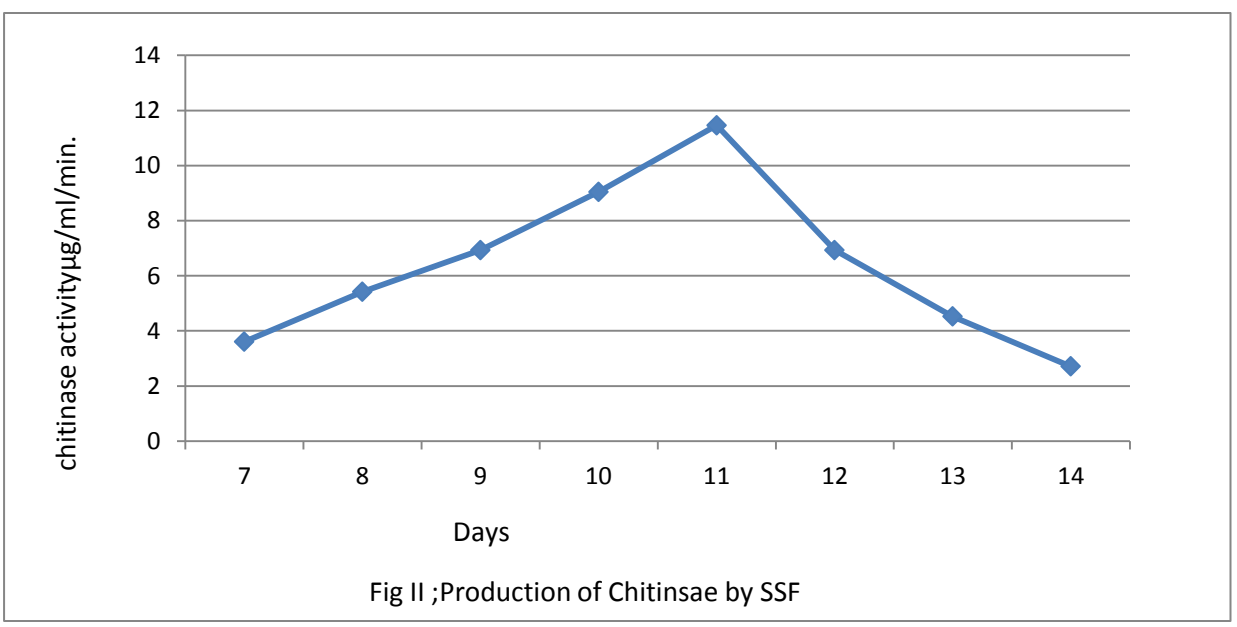

Fig II ;Production of Chitinsae by SSF

Discussion

According to Park et., al. 1992 Numerous studies have investigated sevaral aspects of chitin degradation on nematicidal activity by using bacterial chitinase which are mainly isolated from the soil samples were collected from the fish market, waste water from different industries, dead insects samples etc. In the present research study, by taking reference of park et., al. 1992. We collected soil samples from fish markets from different areas and dead insects. The color of the soil samples was brown to blackish and moist nature. From these samples different chitinase producers were isolated on colloidal chitin agar medium. This isolated result was similar to those shown by Mahmood *et al*., 2007. Chitin acts as a sole carbon source for highest chitinase production said by Dhar *et al.*, 2012. Isolation of the chitinolytic microorganisms from the soil samples was initially carried out by using serial dilution and spread plate method on colloidal chitin agar medium. The results were similar with Shanmugaiah *et al*., 2008. In the Primary Screening plates containing isolated bacterial organisms were flooded with 2% congo red to check hydrolysis of chitin. These hydrolysis results were similar to those shown by Bansode V.B. and Bajekal S.S. 2006. After hydrolysis only 10 bacterial isolates were confirmed as Chitinase producers. In the Secondary screening Chitinase positive bacterial species were confirmed by SSF. After fermentation the crude Chitinase activity was estimated by DNS method gave by Miller; 1959. Highest Chitinase activity of 11.46µg/ml/min. on 11th day of incubation period was shown by isolate RT 6. These results are in accordance with those shown by Sudhakar *et al;* 2011 because they also got highest Chitinase activity on 11th day.

Conclusion

This study concludes that Chitinases would be effective against different fungal pathogens. Chitin is one of the underutilized bio resources in the world. Even though it is available on large scale especially from the marine waste with novel advances in scientific research. The production of chitinases widely applied in the field of chemistry, biomedical, biotechnological, agricultural and environmental protection. Wide scope for extensive research to achieve industrial scale production from the chitinases by SSF. Isolated strain produces novel chitinases by using Rice bran as substrate so process is not costly.

References

Agullo E, Rodriguez MS, Ramos V, Albertengo L. 2003. Present and future role of chitin and chitosan in food. *Macromol Biosci* 3: 521-530. .

Bansode, V. B. and Bajekal, S. S. (2006) Characterization of chitinase from microorganisms isolated from Lonar lake. Indian Jour. of Biotech. 5: 357-363.

Cabib, E. (1987)The synthesis and degradation of shellfish chitin wastes process and selection of microorganisms .J. Food .Sci., **43:1158**-1161.

Deshpande, M.V (1986). Enzymatic degradation of chitin and its biological applications J. FoodSci. Ind.Res. **45:**273-281.

Dhar, P. and Kaur, G. (2010) Effects of carbon and nitrogen sources on the induction and repression of chitinase from *Beauveriabssiana isolates*, Afr.J.Biotech. **9:**8098-8099.

Gooday, G.W. (1990) The ecology of chitin degradation : K.C.Marshall (Ed.),Advance in microbial ecology.

Kramer, K.J. Muthukrishnan, S.(1997) Insect chitinases molecular biology and potential use as biopestcides insect Biochem.mol.Biol.**27(11)**:887-990.

Mahmood, M E. (2007)." Production of chitinase from Serratia marcescens by using solid and liquid fermentation state and its Role in Biological treatment". Thesis MD ,College of Science/ University of Baghdad,Iraq.

Park R. D, Kim K.m. cho.M.J.(1992).Purification and characterization of ethylene induced β-1,3-glucanase from soyabean(Glycins max c.)leaves.kor.J.Biochem.25:597-603.

Shanmugaiah V, Mathivanan N, Balasubramanian N,Manoharan P.T (2008).Optimization of cultural conditions for isolated from production of chitinase by Bacillus laterosprous mmL2270isolated from rice Rhizosphere soil. African J. of Biot. **7(15):** 2562-68.

Sudhakar, P. and Nagarajan, P. (2011) Production of chitinase by solid state fermentation from *Serratia marcescens*, Int J Chem. Tech Res. **3(2):**590-598.

Wang, SL and Chang, WT (1997). Purification and characterization of two bifunctional chitinase by *Psudomonas aeruginos*a K-187 in a shrimp and crab shell powder medium. Appl. Envion. Microbiol. **63:** 380-386.