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Study And Isolation Of Tannase Enzyme Production Bacteria From Tea Waste Dump Soil Site.

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Abstract

Tannase (Tannin acylhydrolase EC.3.1.1.20) is an enzyme which catalyses the hydrolysis of tannic acid. It also acts on esters of gallicacid commonly found in tea extracts. The hydrolysis of these esters enhances the solubility of tea in cold water, in present work screening of tannase production bacteria from tea waste dump site and identification of hydrolytic tannase producing organisms. Production of crude extra cellular hydrolytic tannase from the bacterial isolate by submerged fermentation and also study optimization of factor affecting tannase activity. It is based on the spectrophotometric determination of gallic acid formed by the enzymatic hydrolysis of tannic acid. The kinetic parameters of the enzymatic reaction have been studied and an assay procedure has been formulated

Keywords: Tannase enzyme producer, submerged fermentation, tea dump soil waste.

1. Introduction

Hydrolysable tannins such as gallotannin and ellagitannin are widely distributed in the plant kingdom. these tannins bind readily with proteins and other macromolecules to form indigestible or in soluble complexes, thereby not only reducing nutritional value of animal feed[(1)]Tannase (tannin acylhydrolase)break specifically the ester bonds to diminis the tannins' binding property.it has long been known that several fungi species such as aspergillus spp. and penicillin spp. are capable of producing a larger amount of this enzyme For industrial use, the enzyme is invariably of fungal origin[(2)]Over the past decade ,many bacterial species have also been reported to produce tannase, the include streptococcus gallolyticus[(3)].Bacillus lichenifomis and several lactobacilli species[(4)].in the course of further characterizing tannase-producing bacteria ,we have noticed appreciable variation in size of the clear zone among the isolates grown on tannin treated plate[(3)-(4)].Tannase or tannin acyl hydrolase catalyses the hydrolysis of the ester bonds present in the hydrolysable tannin and gallic acid ester ,the main commercial application of tannase are in the preparation of instant tea and manufacture acron liquor. Tannase is also used for the production of gallic acid, which is an impartment intermediately compound in the synthesis of anti bacterial drugs. Beside tannase is used as clarifying agent in some wines, fruit juices and coffee flavored soft drinks. Tannase is an extracellular, inducible enzyme produced in the presence of tannic acid by fungi, bacteria and yeast [(5)].The first step in the development of microbial enzyme production is the lineage selection. Extra cellular enzymes were preferred because they are easily extracted and do not require expensive extraction methods. Studies on the production of tannase using solid, liquid and submersed fermentation have been reported ductionprocesses [(6)-(7)]. the fermentation broth can use by-products such as wheat bran, rice bran, sugar beet pulp, fruit pulps, banana waste, cassava waste and coffee residues, adding tannic acid. The use of by products or residues rich in sources of carbon for fermentation purposes is an alternative way of solving pollution problems that can be caused by incorrect disposal in the environment In the present work isolation and screening of tannase producing bacteria, from waste tea dump site that check its activity at different pH and salt concentration .bacterial tannase produce by submerged fermentation process and verify it's active by enzyme assay.

2. Materials & Methods

2. 1 Screening and isolation:

Various tea waste dump site soil sample ware collected and enrichment culture method was used for tannase production. Enrichment culture was spread on tannin agar plate and screening was done after incubation at 37 C for 48 hours. Then after colonies were isolated [(8)]. Enrichment culture broth composition: tannin broth (in 100 ml).

Yeast extracts	1.o% gm
Nacl	0.5%gm
Tannin	0.5%gm
Sucrose	1.0%gm

Tannin agar plat: (in 100 ml)

Yeast extracts	1.o% gm
Nacl	0.5%gm
Tannin	0.5%gm
Sucrose	1.0%gm
Agar-Agar	2.0%gm

2.2 Identification:

Based on cultural and morphological characters, the isolate were identified as gram negative rods and named as LS 9.

2.3 Production of enzyme:

Tannase producing isolate were incubation in to 50ml of tannin broth in a 250ml Erlenmeyer flask and incubated at 37 c for 24 hours .the culture was filtered and the filtrate was assayed for the tannase activity.(fig:1)

2.4 Effect of temperature on enzyme activity:

The filtrate was use for study of enzyme active at various temperature, range of temperature were 25°c to 50°c, check maximum active of enzyme at particular temperature. (fig: 2)

2.5 Effect of pH on enzyme activity:

Enzyme active at different pH range 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 studied and graphical presentation is done.(fig:3)

2.6 Enzyme assay:

- The degree of hydrolysis of ester bond in tannic acid is estimated by spectrophotometrically measuring the disappearance of absorbance at 340 nm.
- The reaction mixture consisted of 1 ml of 0.35% tannic acid in citrate buffer (pH5.5), 0.25ml crude enzyme solution.
- The reaction mixture incubated at 30 c for 15 mins.
- The reaction is stopped by adding 5ml of 90% ethanol solution.
- Take O.D at 340nm.
- One unit (U) is defined as the amount of enzyme which hydrolyzes 1 micro mol of the ester bond in tannic acid per min at 30c and pH 5.5(fig:5)

2.7Kinetic study of enzyme:

The effect of various factor like substrate concentration, enzyme concentration, pH and temperature on tannase enzyme activity are studied .the result obtained are graphically represented.(fig:4)

3. Results and Discussion

In this work, sample was collected from tea dump site and isolated organism by design tannin enrichment broth provide proper incubation, organisms isolated base on tannin degradation on tannin agar plate, there were two organisms and different fungi was isolated but above this only one organisms selected and check its active by production tannase enzyme concentration and 2.0 ml con.of tannin broth provide good enzyme producing activity (Figure: 1) than study of effect on different pH and temperature of enzyme active result given below(figure:2&3)

Figure: 1 study enzyme concentration activity (U/gm/min).

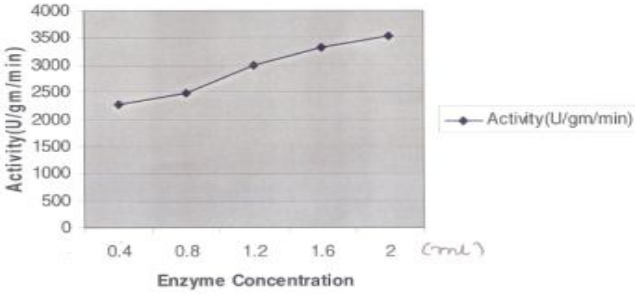


Figure: 2 effect of temperature on enzyme active. (U/gm/min)

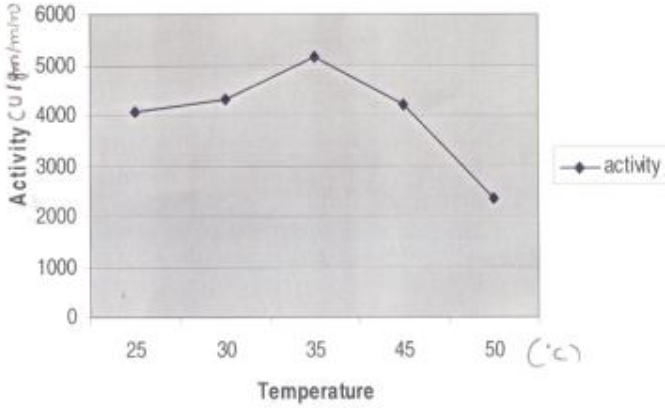


Figure: 3 effect of pH on enzyme active. (U/gm/min)

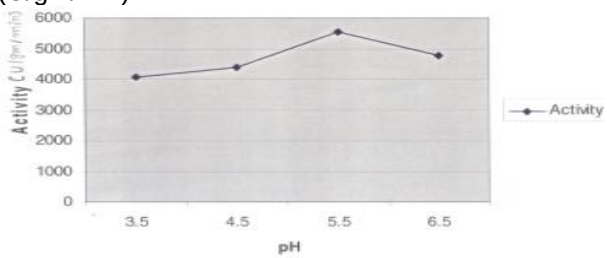


Figure: 4 effect of substrate concentration on enzyme active, (U/gm/min).

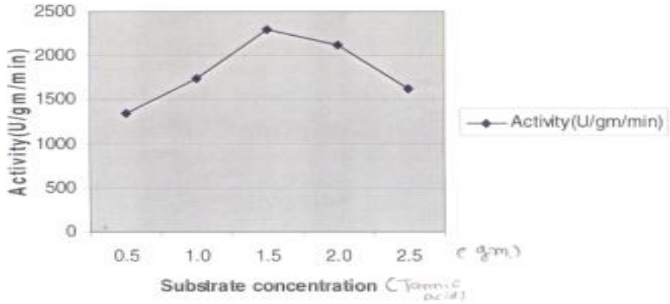
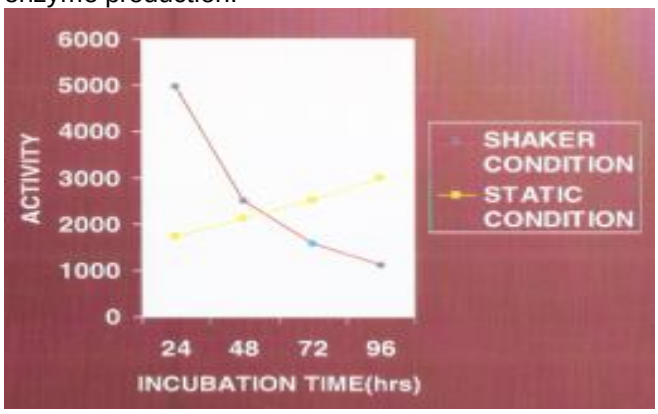


Figure: 5 submerge culture fermentation process on enzyme production.



Its indicate production of tannase enzyme at various pH and temperature range. Studies submerge culture fermentation process (shaker condition and static conviction) on enzyme production and maximum enzyme production was at 24 hrs in shaking condition. (Figure: 4) and checked tannase production activity at different tannin substrate concentration. (Figure: 5)

4. Conclusions

The bacterial isolated LS 9 was isolated from tea waste dump site soil and found to be an extra cellular hydrolytic tannase producer. Maximum enzyme production was detected containing 15miligram concentration of tannin. Optimum activity of tannase was detected at pH5.5 and temperature was 35 degree Celsius. The bacterial isolated LS 9 produce tannase only in presence of tannin indicates its inducible nature

5. Acknowledgements

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References

1. McLeod m.n., plant tannin-their roll in forage quality nutr.abst.rew44, 803-815, 1974
2. Lekha, p.k. and lonsane, b.k. production and application of tannin acyl hydrolsase: state of the art. *advances in applied microbiology*, vol. 44, 1997, p. 215-260
3. Osawa, fujsawa, t., sly.l.l.1995a .streptococcus galloluticus sp.nou, gallate degrading organism formerly assigned to streptococcus bovis,system appl.microbial.18.74-78
4. Osawa.r, kuroisok.goto.s.shimizu.a. Isolation of tannin degrading lactobacilli from human fermented food. Apply .environment. Microbial. 18. 368-373, 2000
5. Aguilar, c.n. and Gutiérrez-sanchez, g.review: sources, properties, applications and potential uses of tannin acyl hydrolase. *Food science and technology international*, vol. 7, no. 5, p. 373-382, October 2001
6. Battestin, vania; macedo, Gabriela and pastore, gl'aucia. optimizing the fermentation broth for tannase production by a new isolated strain *paecilomyces variotii.journal of technology*, vol. 118, p. s49-s49, august 2005,
7. lekha.p.k.lonsane, b.k.production and application of tannin acyl hydrolase state of the art .appl.microbial 44,215-260, 1997
8. Van de lagemaat, j. and Pyle, d.l. solid-state fermentation and bioremediation: development of continuous process for the production of fungal tannase. *chemical engineering journal*, October 2001, vol. 84, no.2, p. 115-123
9. Rajkumar, g.s, Nandy, S.C., 1983.isolation purification and some property of panicillium chysogenum tannase.appi.environment microbial 46.525-527.
10. Rhoddes.d.f.cates,Rs.,1976,a general theory of plant anti-herbivore chemistry, resent adv.phytochem 10,168-213
11. Banerjeed, mondal, k.c.2001.production and characterization of extra cellular and intracellular tannase from newly aspergillumsauleatusdbt.g.j.basicmicrobial 41.313-318