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Fungal Production of Tannase:A Review

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Abstract:

Tannin acyl hydrolase (E.C.3.1.1.20) is commonly referred as tannase, hydrolyses ester and depside bonds of hydrolysable tannins to produce gallic acid, glucose and galloyl esters. Tannase has many industrial applications. The enzyme has potential uses in the treatment of tannery effluents. A lot of research is done on production aspects of tannase, but is still considered as one of the costly industrial enzymes. This is due to less yields and long fermentation time of the processes. In view of the growing demand, it is important to isolate high productive strains and develop economically feasible processes. This study reviews the fungal sources, screening methods, modes of production, temperature and pH of fermentation of tannase enzyme.

Keywords : tannase, enzyme, hydrolysable, fermentation

1. Introduction

Tannase or tannin acyl hydrolase (E.C.3.1.1.20) is an inducible enzyme produced by variety of microorganisms such as fungi, bacteria and yeast (Aguilar and Gutie´rrez-Sanchez, 2001).It catalyses the hydrolysis of tannins. Tannins are the phenolic compound with molecular weight of 500-3000 dalton.Hydrolysable tannin such as tannic acid consists of carbohydrate core, usually glucose to which no. of gallic acid molecules are esterified (Skene & Brooker, 1995). Tannase hydrolyses the ester and depside bonds in hydrolysable tannins, releasing glucose and gallic acid .Gallic acid has significance in food and pharmaceutical industries [Pourrat Het al. 1985]. The enzyme has also been used in the prevention of phenol-induced mediarization in wine [Koichi Y et al. 1972], coffee-flavored soft drinks manufacturing [Suzuki S, 1973], clarification of beer and fruit juices [Massechelin CA, 1981], stabilization of malt polyphenol [Giovanelli G et al., 1989] and as a sensitive analytical probe for determining the structure of naturally occurring gallic acid esters [Haslam E et al. 1970]. Garcia-Conesa et al. (2001) reported its application in plant cell wall digestibility by breakage of polyphenolics such as dehydrodimer cross links present in the cell wall. Tannase can be obtained from plant, animal and microbial sources. From plant sources, the enzyme is present in tannin rich vegetables mainly in their fruits, leaves, branches and barks of trees like konnam, mirobolano and badul (Madhavakrishnaet al., 1960). The most important source to obtain the enzyme is by microbial way, because the produced enzymes are more stable than similar ones obtained from other sources (Lekha and Lonsane, 1997). Tannase from fungal sources are reported to be active in a wide range of pH and temperature. Tannase are the Extracellular enzymes which can be easily extracted and do not require expensive extraction methods (Couri et al., 1998). Studies on the production of tannase using solid, liquid and submersed fermentation have been reported (Lekha and Lonsane, 1994; Pinto, 2003). Solid-state fermentation have many advantages over conventional enzyme production spacially submerged fermentation(Sum).Several studies on tannase production by Aspergillus Sp. indicate the significant higher production of tannase in SSF than in SmF. This could be due to the absence of proteolytic activity in SSF as reported by Aguilar et al. (2002) as well as the complete secretion of enzyme extracellularly in SSf than SmF where partial enzyme secretion is intracellular (Lekha & Lonsane, 2004)

There are several potential industrial applications for tannase; however, due to the high production costs and limited knowledge of its catalytic action, there are currently only a few applications.

2.Fungal Sources

In nature a variety of Tannase producers have been identified which includes bacteria, fungi, yeasts & plant sources. Among them many researches proved that fungi are the most prominent producers of tannase, in which Aspergillus &

Penicillium genus was found to be most promising .A brief list of fungal sources is given in Table 1.(A Pandey et al. 2006)

3.Screening and Selection of Tannase Producers

Many of the literature suggested that the tannase producing fungus can be screened by initially in tannic acid (1%) & agar (3%) containing media in which tannic acid act as sole carbon source. Bradoo et al.(1996)had screened almost 50 isolates for tannase producing activity on tannic acid agar plates (TAA) containing 1% tannic acid and 3% agar.Batra & Saxena (2005) examined Tannase-producing ability of 35 Aspergilli and 25 Penicillii on tannic acid agar plates and quantitatively in broth & found among Aspergilli, *Aspergillus fumigatus* (8.3 IU/ml), *Aspergillus versicolor* (7.0 IU/ml), *Aspergillus flavus* (4.95 IU/ml) and *Aspergillus caespitosum* (4.47 IU/ml) and Amongst Penicillii, Penicillium Charlesi (4.82 IU/ml), *Penicillium variable* (4.70 IU/ml), *Penicillium crustosum* (4.7 IU/ml) and *Penicillium restrictum* (4.47 IU/ml), the potent producer of tannase. The crude tannase from these fungi showed pH optima of 5.0, except in *A. caespitosum*, *P. crustosum* and *P. variable*, which had pH optima of 6.0. Optimum tannase activity was at 60 ◦C in most of the potent producers, except in *A. caespitosum*, *P. charlesii*,*P. crustosum* and *P. restrictum*, which showed temperature optimum of 40 ◦C. amongst the selected Aspergilli and Penicillii, tannase from *A. versicolor* and *P. restrictum* was stable in a broad pH range of 3.0–8.0 for 24 h. The tannase from *A. versicolor* is heat stable as it retained 67% activity at 70 ◦C after 1 h. .S.Chand et al. (2000) reported the culture of isolated *A. awamori* on Honey–Peptone–Barley medium. (50 g of Pearl barley, 9 ml of solution containing honey (10%w: v) and peptone (1%) at 37°C for 6–7 days. Saxena et al. (2008) cultured the *Penicillium variable* on Potato dextrose agar (PDA) slant supplemented with 0.1% tannic acid at 30°C for 6 days.

4.Solid State Fermentation

Solid state fermentation is found to be more significant than other conventional fermentation techniques in terms of higher extracellular enzyme production & ease of purification.Subhash Chand et al. (2000) used *Aspergillus awamori* for tannase enzyme production in the medium containing tannic acid (varying concentrations),. Tannic acid concentration, agitation speed and pH during the fermentation were identified as important process parameters effecting cell growth and enzyme synthesis by *Aspergillus awamori*. These parameters were optimized in a laboratory bioreactor by response surface methodology & found that 35.0 g/ l of substrate conc. pH -5, 37oC & 350 rpm gives the maximum enzyme activity A.Pandey et al. (2005) used Palm kernel cake (PKC), and tamarind seed powder (TSP) for the production of tannase under solid-state fermentation *Aspergillus niger* was grown on the substrates without any pretreatment. In PKC medium, a maximum enzyme yield was obtained when SSF was carried out at 30 °C, 53.5% initial substrate moisture, 33 · 109 spores/5 g substrate inoculum size and 5% tannic acid as additional carbon source after 96 h of fermentation. In TSP medium, maximum tannase yield was obtained at 30 °C, 65.75% initial substrate moisture, 11 · 109 spores/5 g substrate inoculum, 1% glycerol as additional carbon source and 1% potassium nitrate as additional nitrogen source after 120 h of fermentation. Jitendra Sharma et al.(2007) used different fungal strains for tannase production using agro waste as substrate. *Aspergillus ruber* gave maximum enzyme yield under solid state fermentation using different tannin rich substrates like ber leaves (Zyzyphus Mauritiana), jamun leaves (Syzygium cumini), amla leaves (Phyllanthus emblica) And jawar leaves (Sorghum vulgarism). Jamun leaves were found to be the best Substrate for enzyme production under solid-state fermentation (SSF). At 30 °C after 96 h of incubation. Tap water was found to be the best moistening agent, with pH 5.5. Gustavo Viniegra-Gonza´lez et al. (2001) checked .Induction and repression patterns of tannase production by *Aspergillus niger* Aa-20 in solid-state (SSC) and submerged culture (SmC) & found that in SSC an increase in tannic acid enhances the expression of tannase activity than that of glucose as carbon source. Rintu Banerjee (2006) worked on strain improvement by UV, Heat & mutagen treatment. Spores from the co-culture of *Aspergillus foetidus* and *Rhizopus oryzae* were subjected to UV, heat and NTG (3-nitro, 5-methylguanidine) Mutagenesis. The best mutant isolated from the heat treatment (60 °C for 60 min) .

5.Enzyme Purification

Beverini & Metche (1990) reported acetone precipitation as initial step for the purification of tannase. K.Mahapatra(2005) further purified the acetone precipitated fraction by using G-100 sephadex column.S.Sharma et.al.(2008)purified the tannase using ultrafilteration using membrane cartridges of different molecular weight cutoffs. Followed by gel filteration chromatography using G-200 sephadex column. first step resulted 97% yield & 5 .0 fold purification while the second

step of purification gave 91% yield & 135 fold purification. Costa et al. (2012)purified the extracellular tannase by using two chromatography techniques, filteration chromatography using G-150 sephadex column followed by ion exchange chromatography in a DEAE Sephadex column. which allowed the separation of two isoforms of tannase designated as TAH I and TAH II among that TAHI is responsible for more than 70% of total tannase activity.

6. Enzyme characterization

6.1 Temperature tolerance:

The optimum temperature and the temperature range for tannase activity of the selected tannase producers were determined by carrying out the reaction at different temperatures ranging from 30 to 80 ◦C at their respective optimum pH by different scientists.Batra & Saxena(2005) found that the functional temperature range of the tannase is 30–70 ◦C with optima at 60 ◦C for *A. flavus, A.fumigatus, A. versicolor and P. variable, whereas A. caespitosum, P. charlesii, P. crustosum and P. restrictum* had an optimum activity at 40 ◦C. These results are also S.Sharma et al. (2008) experimentally bring it to notice that the three different strains of *Penicillium variable* showed optimum tannase activity at 50°C.K Mahapatra et al.(2005) reported that the optimum temp. For tannase activity was 35°C for *A. oryzae & P.chrysogenum*.

6.2 pH tolerance:

The optimum and the pH range for tannase activity of the selected fungi was found to be 3.0 to 8.0.Barthomeuf C et al, (1994) reported for *A. niger* pH optimum of 5.0–6.0,Batra & Saxena(2005) reported that tannase from *A. fumigatus* and *A. flavus* is stable at pH 4.0. & doesn't show tannase activity at alkaline pH of 8.0.In contrast, *A. versicolor* tannase showed a relatively wider range of pH stability at pH 8.0 and les stability at pH 3.0 with a maximum (100%) at pH 6.0.S.Sharma et al.(2008) reported the optimum activity of tannase by *P.variable* at pH 3.0. so it can be concluded that the fungal tannase is an acidic protein & needed an acidic environment to be active(K. Mahapatra ,2005) .

7.Conclusion:

Tannase has the potential for a wide range of application, but due to higher production cost & lower yield, they currently have limited uses. This review article suggest the type of fermentation method & optimum conditions for the tannase production .Generalization of the conditions cannot be done for the production of tannase as it vary from organism to organism. In view of growing demand for the tannase for industrial application, it is very important to develop high yielding & cost effective process.

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Table 1:

Fungal Source	
Ascochyta biochemical	Lekha & Lonsane (1997)
Ascochyta boltshauseri	Lekha & Lonsane (1997)
Ascochyta Pisi	Lekha & Lonsane (1997)
Ascochyta viciae	Lekha & Lonsane (1997)
Rhizopus Oryzae	Hadi et al.(1994)
A.Aculeatus	Banerjee et al. 2001
A.Aureus	Bajpai & Patil,1996
A.Awamori	Bradoo et al. 1996
A.Carneus	Lekha & Lonsane 1997
A.Fischerii	Bajpai & Patil, 1996
A.Fleviceps	Lekha & Lonsane 1997
A.flavus	Yamada et al. 1968
A. fumigates	Lekha & Lonsane 1997
A.japonicus	Bradoo et al. 1996
A,nidulans	Lekha & Lonsane 1997
A.niger	Lekha & Lonsane 1994
A.oryzae	Bradoo et al 1996
A.parasiticus	Bajpai & Patil 1996
A.phoenicis	Van de lagemaat et al. 2000
A.rugulosus	Bradoo et al.1996
A.tamari	Lekha & Lonsane 1997
A.tereus	Bajpai& Patil 1996
A.ustus	Lekha & lonsane 1997
Chetomium lobosum	Lekha & Lonsane 1997
Cryphonectria parasiticus	Farias et al. 1994
F.oxysporium	Bradoo et al. 1996
F.solani	Bajpai & Patil 1996
Helicostylum sp.	Bradoo et al. 1996
Mucar pranii	Lekha & Lonsane 1997
Neurospora sp.	Lekha & Lonsane 1997
Penicillium acrellanum	Bradoo et al.1996
P.carilophylum	Bradoo et al.1996
P.charlesii	Bradoo et al.1996
P. chrysogenum	Bajpai & Patil 1996
P.citrinium	Bradoo et al.1996
P.commune	Van de lagemaat et al. 2000
P.digitatum	Bradoo et al.1996
P. fellutanum	Lekha & Lonsane 1997
P.glabrum	Van de lagemaat et al. 2000
p.islandium	Lekha & Lonsane 1997
P.notatum	Lekha & Lonsane 1997
P.variable	Lekha & Lonsane 1997
Trichoderma hamantum	Bradoo et al.1996
T. harzianum	Bradoo et al. 1996