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Screening of microorganisms for hydrolyases with commercial potential

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Abstract

Advancement in green chemistry has increased the use of microbial hydrolyases in various industries and chemical processes because of high catalytic efficiency, specificity, cost-effectiveness and eco-friendly nature. Bioconversion of tannins such as tannic acid is achieved by tannin acyl hydrolase, also known as tannase. It converts tannic acid into glucose and gallic acid by catalyzing the hydrolysis of ester and depside linkages in tannic acid. Tyrosinase is monophenol and *O*-diphenol oxidase a copper containing enzyme catalyzes the oxidation of tyrosine and generates different types of pigment such as melanin. Xylanases hydrolyze xylan into its constituent sugar with the help of several debranching enzymes. Microbial strains isolated from various sources were screened for these hydrolyases: Bhavnagar marine salterns (*Bacillus megaterium* BVUC_01 and *Bacillus licheniformis* BVUCh_02); Okhamadhi marine salterns *Aspergillus versicolor*; Spoiled/infected pomegranate (*Xenoacremonium falcatum,* two strains PGF1 and PGF4, *Bacillus velezensis*PGF2 and *Candida freyschussii*PGF3. The other laboratory maintained bacterial cultures namely, *Bacillus subtilis, Pseudomonas aeruginosa*, *Staphylococcus aureus, Salmonella typhi* were also used in this study. *Asp. versicolor* and *Xen. falcatum* (PGF1) produced all the three enzymes (tannase, tyrosinase and xylanase). *B. licheniformis, B. megaterium, B. subtilis, B. velezensis* produced tyrosinase and xylanase. *Xen. falcatum* (PGF4) and PGF2 produced tannase and xylanase. PGF3 produced tannase and tyrosinase. While, *Bacillus megaterium* and *Salmonella typhi* showed only tyrosinase activity. *Candida freyschussii* showed tannase activity. *Staphylococcus aureus* did not produce any of these enzymes.

Keywords: *Bacillus velezensis; Xenoacremonium falcatum; Candida freyschussii; Tannase; Tyrosinase; Xylanase*

1. Introduction

Enzymes are biocatalyst which catalyzes reaction with high catalytic efficiency and specificity. With advancement in green chemistry, the use of enzymes in various industries and chemical processes enormously increased because of its cost-effectiveness and eco-friendly nature [1, 2]. Bioconversion of tannins such as tannic acid is achieved by tannin acyl hydrolase (TAH; EC 3.1.1.20), also known as tannase. It converts tannic acid into glucose and gallic acid by catalyzing the hydrolysis of ester and depside linkages in tannic acid [3, 4]. Tannin is a water soluble, phenolic compound with capability to precipitate proteins from solution [5]. It is widely present in plants which are used for human consumption [5, 6]. Microbial tannase is more stable than plant and animal tannase, so microorganisms become most favored source of tannase [7]. Tannase have wide range of applications in various industries such as, food [8], cosmetics [9, 10], leather [11, 12], pharmaceutical [13, 14], animal feed and in treatment of waste waters [15, 16]. Despite of its applications, there are very few report on its sources, throughout the world [17, 18]. Tannins have toxic effect on microbes and inhibit their growth [6, 18]. Tyrosinase is a monophenol and *O*-diphenol oxidase, copper containing enzyme which catalyzes oxidation of tyrosine and generates different types of pigment such as melanin. It is widely distributed among prokaryotes as well as eukaryotes [19]. The mode of action of tyrosinase is that, hydroxylation of its substrate (monophenol) to *O*-diphenol followed by oxidation of diphenol to *O*-quinones. In whole sequential process it requires

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molecular oxygen [20, 21, 22]. If substrate is tyrosine then, upon hydroxylation it will be converted into 3,4-dihydroxyphenylalanine or DOPA, and upon oxidation of DOPA it is converted into dopaquinone followed by series of enzymatic and non-enzymatic reactions which ultimately results in production of melanin pigment [23]. In fungi, tyrosinase plays an important role in browning, pigmentation [24, 25], defense and virulence mechanism [26, 27], stability of spores [28] and also contributes in resistance to some radiation and extreme temperatures [29, 30]. Plant contains a variety of phenolic compounds which are oxidized by tyrosinase browning as a primary immune response during injury of tissues [31, 32, 33]. Tyrosinase is used for bioremediation of industrial waste waters and soil containing phenolic compounds as a common pollutant, which is toxic in nature and hazardous for human health [33, 34, 35, 36]. Apart from this tyrosinase is widely used in pharmaceutical, food processing and cosmetic industries [19, 37, 38].Main components of lignocellulosic material are hemicellulose and cellulose [39, 40]. Hemicellulose consists of xylan, arabinan, mannan and galactan as major heteropolymers [41, 43]. In higher plants about one third dry weight of hard wood hemicellulose contains xylan [40, 42, 43]. Xylanases hydrolyze xylan into its constituent sugar with the help of several debranching enzymes [42, 43, 44]. The biotechnological and industrial applications of xylanases have increased enormously in the recent years [45, 46, 47]. With the development in the use of these enzymes in biotechnology and other industries has led to increased demand for new sources of these enzymes. Therefore, the objective of this study was to screen microorganisms from various sources for the production of hydrolyases tannase, tyrosinase and xylanase.

2. Methodology

Microbial strain used in this study: *Bacillus megaterium*, *Bacillus licheniformis,* BVUC_01, BVUCh_02 isolated from Bhavnagar marine salterns [48]; *Aspergillus versicolor* isolated from Okhamadhi marine salterns [48];*Xenoacremonium falcatum*(two strains PGF1 and PGF4)*, Bacillus velezensis*, *Candida freyschussii,* PGF2, PGF3, isolated from spoiled pomegranate [49]. Apart from this four bacterial strains - *Bacillus subtilis, Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus were* also used in this study.

2.1. Screening for production of extracellular tannase, tyrosinase and xylanase enzymes

Primary screening of the isolates for production of tannase, tyrosinase and xylanase was done by using plate assay method. For fungi pH of the medium was adjusted to 6.5-7.0, for bacteria pH of the medium was adjusted to 7.2-7.4.

2.1.1. Screening for tannase production

Screening of microbial cultures for production of tannase was done by using Tannic acid agar medium (TAA) containing (g/L): tannic acid, 10.0; $NaNo_3$, 3.0; $KH2PO_4$, 1.0; $MgSO_4$. $7H_20$, 0.5; KCI 0.5; $FeSO_4$. $7H_2O$, 0.01; Agar-agar powder, 30.0 [52].

2.1.2. Screening for tyrosinase production

Tyrosinase activity of cultures was screened by using medium containing (g/L): Peptone, 5.0; Yeast Extract, 3.0; L-tyrosine, 5.0; Agar-agar powder, 20.0 [53].

2.1.3. Screening for xylanase production

Screening of cultures for production of xylanase was done by using media containing (g/L): (A) for bacteria: Xylan, 5.0; $MgSO_4 \cdot 7H_2O$, 0.05; NaCl, 0.05;$CaCl_2$, 0.01; yeast extract, 0.2;peptone, 0.5; Agar-agar powder 20.0 [50]; (B) for fungi: Xylan, 5.0; $MgSO_4 \cdot 7H_2O$, 0.05; $CaCl_2$, 0.05; $NaNO_3$, 0.005; $FeSO_4 \cdot 7H_2O$, 0.009; $ZnSO_4$, 0.002; $MnSO_4$, 0.012; KCl, 0.23; KH_2PO_4, 0.23; peptone, 2.0; Agar-agar powder 19.0g [40, 43].

The solid media were spot inoculated with the actively growing cultures and incubated as follows: bacterial cultures were incubated at 37°C for 24-48 hrs; while fungal cultures were incubated at room temperature (20-25°C) for 48-72 hrs.

After incubation tannase and tyrosinase activity was detected by color change. Formation of brown halos around microbial colony indicated production of respective enzyme [19]. Xylanase activity was detected by flooding the plate with 0.4% Congo red dye and after 10 minutes washed with 1.0M NaCl. Formation of clear zone around growth indicated the production of xylanase [43].

3. Results and discussion

Microorganisms from marine environments as well as from spoiled foods, fruits, vegetables, etc. have been reported for production of extracellular hydrolyases. Some of these hydrolyases such as amylase, lipase, pectinase, protease, etc. have been commercially exploited. To the best of our knowledge this is the first report which describes screening of microbes isolated from Bhavnagar and Okhamadhi marine salterns for tyrosinase and tannase enzyme production.

3.1. Screening of various hydrolytic extracellular enzymes

In the present study *Aspergillus versicolor, Xenoacremonium falcatum* (PGF1) produced all the three enzymes namely, tannase, tyrosinase and xylanase. *Bacillus licheniformis, Bacillus velezensis, Bacillus subtilis,* BVUC_01 and BVUCh_02 produced tyrosinase and xylanase. *Xenoacremonium falcatum*(PGF4) and PGF2 produced tannase and xylanase. PGF3 produced tannase and tyrosinase. *Bacillus megaterium* and *Salmonella typhi* showed only tyrosinase activity. Only tannase activity was detected in *Candida freyschussii* tannase, while, *Staphylococcus aureus* did not produce any of these enzymes (Figure 1, 2 and 3; Table 1).

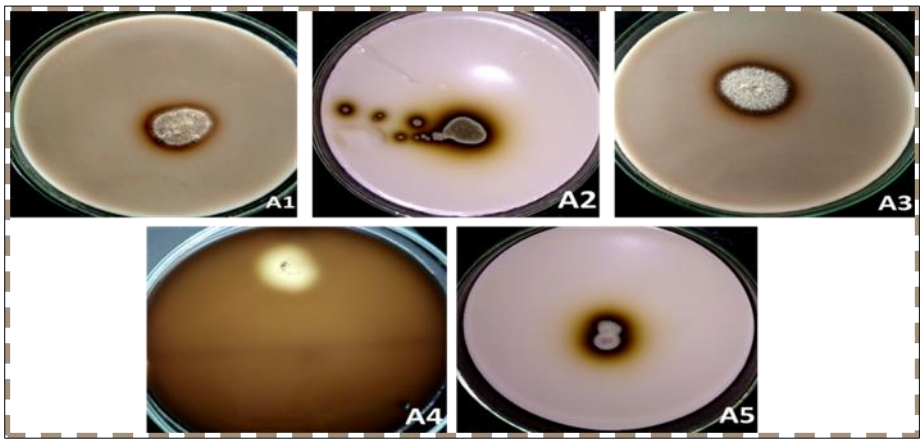

Figure 1 Results of screening of microorganisms for tannase production: A1, *Xenoacremonium falcatum* (PGF1); A2, PGF2; A3, *Xenoacremonium falcatum* (PGF4); A4, *Candida freyschussii*; A5, *Aspergillus versicolor*.

Table 1 Screening of cultures for tannase, tyrosinase and xylanase enzyme production

Isolates	Tannase	Tyrosinase	Xylanase
BVUC_01	-	+	+
Bacillus megaterium	-	+	-
Bacillus licheniformis	-	+	+
BVUCh_02	-	+	+
Aspergillus versicolor	+	+	+
Bacillus velezensis	-	+	+
Xenoacremoniumfalcatum(PGF1)	+	+	+
PGF2	+	-	+
PGF3	+	+	-
Xenoacremoniumfalcatum(PGF4)	+	-	+
Candida freyschussii	+	-	-
Bacillus subtilis	-	+	+
Pseudomonas aeruginosa	-	+	+
Staphylococcus aureus	-	-	-
Salmonella typhi	-	+	-

Note: "+" positive result and "-" negative result after incubation

To the best of our knowledge this is the first report which shows production of tannase, tyrosinase, xylanase by *Xenoacremonium falcatum* and *Candida freyschussii*; tannase and tyrosinase by *Aspergillus versicolor*; and tyrosinase by *Salmonella typhi*.

These studied organisms also previously screened for production of various hydrolytic as well as therapeutic enzymes such as amylase, cellulase, chitinase, lipase, pectinase, protease, arginase, asparaginase, glutaminase [48, 49, 54]. These studies revealed that *Aspergillus versicolor* produced all the twelve (12) enzymes, while *Bacillus velezensis* showed all except, tannase activity. *Bacillus velezensis,* BVUC_01 and BVUCh_02 did not show pectinase and tannase activity [48, 54].

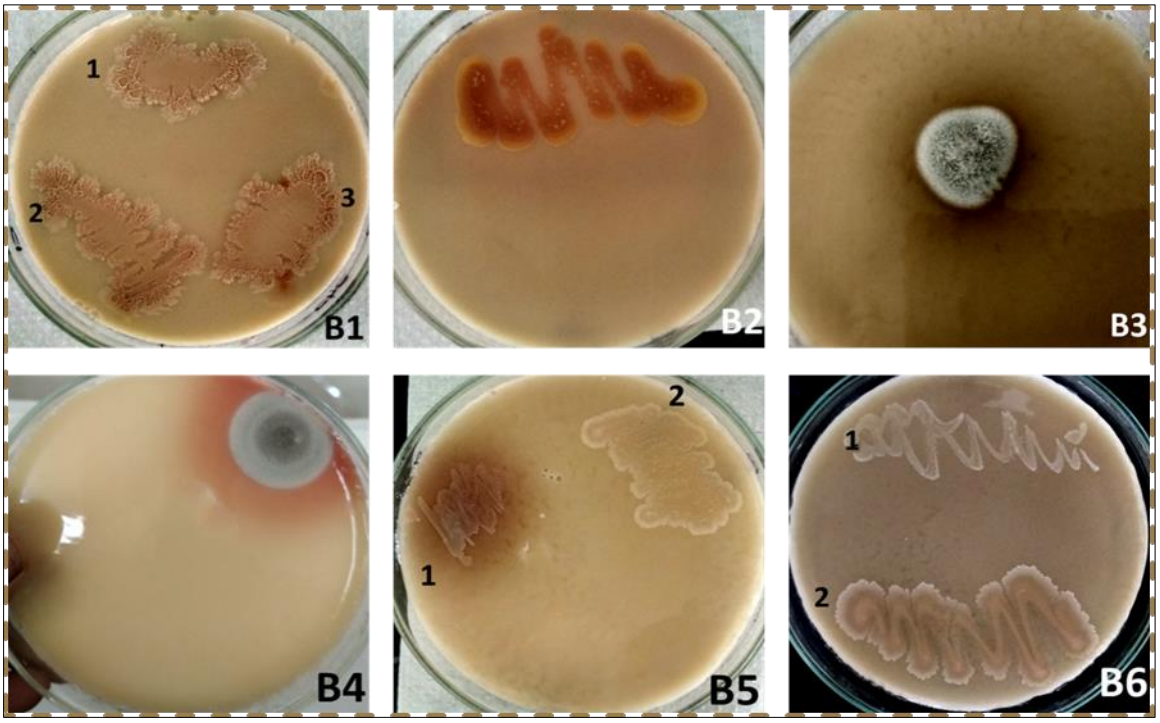

Figure 2 Results of screening for tyrosinase production; B1, (1-BVUCh_02, 2-*Bacillus licheniformis,* 3-BVUC_01); B2, (1-*Bacillus megaterium,* 2-*Bacillus subtilis*); B3, *Aspergillus versicolor*; B4, *Xenoacremonium falcatum*(PGF1); B5, *Salmonella typhi*; B6, (1-*Candida freyschussii,* 2-*Bacillus velezensis*)

Lima et al. [55] used various species of *Aspergillus* and *Penicillium* for production of tannase from agro-industrial waste and leaves of Barbados cherry and mangaba fruit. They reported that *P. montanense* URM 6486 was the best producer of tannase using Barbados cherry residue as substrate. Many authors revealed that use of agro-industrial waste oil palm drastically increases tannase production [55, 56]. Mukesh et al. [57] isolated *K. pneumoniae* from soil and screened it for tannase production and also optimized various parameters for its production. Production of tannase from bacteria provides additional advantages such as rapid growth, ease of handling and short incubation period. The common genera reported for tannase production are *Bacillus* [58, 59, 60]; *Lactobacillus* [60], *Pseudomonas* [61], *Staphylococcus* [62], *Klebsiella* [58, 63], etc. Among microorganism fungi are widely used for research, industrial production as well as applications of tannase. The most commonly reported fungal genera for tannase production are *Aspergillus*[64, 65], *Penicillium* [64, 66] and *Trichoderma* [67]. Tannase production has also been reported in the yeast *Candida* sp..Saxena et al. [68] reported tannase production from *Candida guilermondii* and *Candida tropicales.* Yu et al. [69] and Albertse [70] studied recombinant yeast *Pichia pastoris* and *Saccharomyces cerevisiae,* respectively for tannase production. Farag et al. [71] isolated *Aspergillus nomius* GWA 5 from marine sediment of Western Harbour, Alexandria, Egypt and found that it had great ability to produce tannase that that can be active in wide range of pH and temperature suitable for industrial and pharmaceutical purpose.

Roy et al. [72] isolated marine actinobacteria from Marina beach (13.05°N, 80.28°E), Chennai, Tamil Nadu, India and identified it as *Streptomyces espinosus* strain LK4. It produced tyrosinase which was stable at high temperature and pH, and greater stability as well as yield than mushroom tyrosinase. They also found that the purified tyrosinase had great potential for the removal of phenol from aqueous solution. Immobilization of tyrosinase improved its thermal stability, protected it from proteolysis, increased efficiency of reusability, stability and viability [73-76]. Some fungi reported for

tyrosinase production include *Agaricus bisporus* [77], *Neurospora crassa* [78, 79], *Amanita muscaria* [80], *Aspergillus oryzae* [81], Portabella mushrooms, *Pycnoporus sanguineus* [82], and *Lentinula boryana*[83, 84]. Bacterial tyrosinase is generally exploited for melanin production. The most common bacterial genera reported for tyrosinase production are *Rhizobium, Symbiobacterium, Pseudomonas, Marinomonas, Thermomicrobium, Bacillus*, *Streptomyces* [84 - 88]. Among these tyrosinases from *Streptomyces* spp. Are widely studied and characterized [84].During production of tyrosinase incubation in dark condition showed high enzyme activity, but the preferable condition for incubation is in light because it inhibits laccase production which is largely produced in dark condition [89, 90]. Zaidi et al. [91] attempted extraction, purification and characterization of mushroom tyrosinase and found that it remained active at 30-65°C temperature and at neutral to slight acidic pH. Mushroom tyrosinase showed high similarities with human tyrosinase, therefore can be a potential source of this enzyme for therapeutic use in melanogenesis.

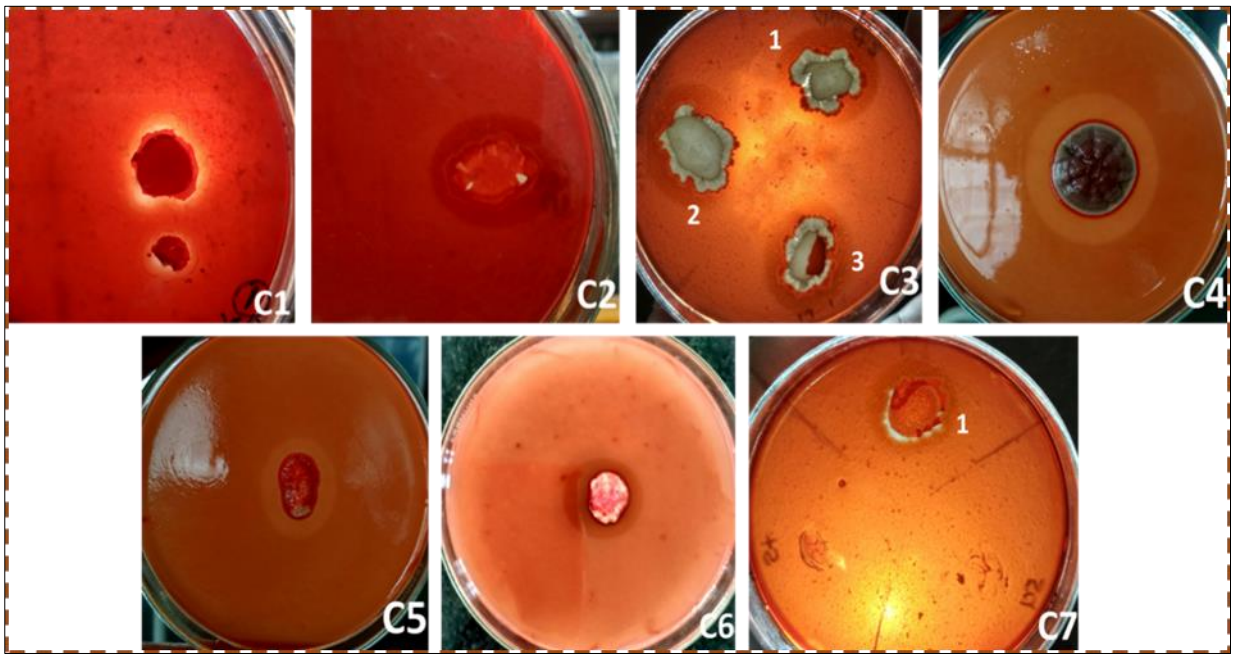

Figure 3 Results of screening of isolates for xylanase production; C1, *Bacillus velezensis*; C2, *Bacillus subtilis*; C3, (1-BVUCh_02, 2-BVUC_01, 3-*Bacillus licheniformis*); C4, PGF2; C5, *Xenoacremonium falcatum* (PGF4); C6, *Aspergillus versicolor*; C7, *Pseudomonas aeruginosa*

Tannase has applications in various industries such as foods, animal feeds, cosmetic, pharmaceutical, chemical, leather industries, etc. [11, 53, 92, 93]. Tannase is used in food and pharmaceutical application which includes, in the manufacture of instant tea, stabilization of malt polyphenols, clarification of beer and fruit juices [94]. Tyrosinase is used in amalgam-biosensors for the detection of inconsequential level of volatile phenolics [95]. Xylanase plays a crucial role in pulp and paper industry. It has various roles in bioprocessing of fabrics, bioleaching of pulp, waste water recycling and bioconversion of various valuable products, food and feed [42, 45, 96].

To the best of our knowledge this is the first report which describes screening of microorganisms isolated from Bhavnagar and Okhamadhi marine salterns for tyrosinase and tannase enzyme production. Further, research work on optimization for production and applications is envisaged.

4. Conclusion

A variety of hydrolyases is produced by the microorganisms isolated from marine salterns (Bhavnagar and Okhamadhi) as well as spoilt pomegranate. On the basis of the results, it can be concluded that tannase is produced by *Aspergillus versicolor, Xenoacremonium falcatum* (PGF1 and PGF4), PGF2, PGF3 and *Candida freyschussii.* All the organisms screened except PGF2, *Xenoacremonium falcatum* (PGF4), *Candida freyschussii* and *Staphylococcus aureus* produced tyrosinase. Xylanase activity was detected in most organisms, except *Bacillus megaterium,* PGF3, *Staphylococcus aureus* and *Salmonella typhi*. Further studies can be helpful for commercial exploitation of these isolates.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Authors not have any conflict of interest.

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