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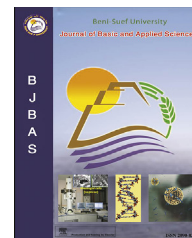

Short Communication

Culture conditions for the production of thermostable lipase by *Thermomyces lanuginosus*

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ARTICLE INFO

Article history:
Received 21 August 2016
Received in revised form 22 November 2016
Accepted 22 November 2016
Available online

Keywords:
Thermomyces lanuginosus
Oils
Culture medium
pH
Temperature
Lipase production

ABSTRACT

In the present investigation lipase production by three strains of thermophilic *Thermomyces lanuginosus* (GSLMBKU-10, GSLMBKU-13 and GSLMBKU-14) was carried out in submerged fermentation process. Olive oil and triacetin (0.1%) were added to the basal medium, which stimulated the lipase production. The maximum lipase was produced by GSLMBKU-10 and GSLMBKU-13 in yeast extract starch medium supplemented with triacetin (0.1%). The optimum pH was recorded at 6.0, 6.5 and 7.0 by GSLMBKU-10, GSLMBKU-13 and GSLMBKU-14 respectively. *T. lanuginosus* GSLMBKU-10 strains failed to produce lipase at pH 8.0. The optimum temperature for lipase production was observed at 45 °C by GSLMBKU-14 and GSLMBKU-10, while that for GSLMBKU-13 was at 50 °C. The marginal temperature ranged from 45 °C to 50 °C for both lipase production and vegetative growth by the three strains of *T. lanuginosus* under study. In conclusion, the GSLMBKU-13 strain was comparatively superior in the production of lipase than the other two strains under investigation.

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1. Introduction

Lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) are a biotechnologically important group of enzymes which act on the carboxyl ester bonds present in triacylglycerols and liberate fatty acids and glycerol (Abrunhosa et al., 2013). They are also involved in a wide range of conversion reactions such as esterification, interesterification, transesterification, alcoholysis, acidolysis and aminolysis in non-aqueous media (Charles and James, 2011). Lipases are used extensively in food and dairy industry for the hydrolysis of milk fat, cheese ripening, flavour enhancement and lipolysis of butterfat and cream (Ray, 2012). These are also used as additives in washing powder, for removal of oil/fat stains, and to increase fabric absorbency in the textile industry (Shivika and Shamsher, 2014). In addition, these enzymes are used as a catalyst for the production of different products used in the cosmetic industry, such as pulp and

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http://dx.doi.org/10.1016/j.bjbas.2016.11.010
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Please cite this article in press as: B. Sreelatha, V. Koteswara Rao, R. Ranjith Kumar, S. Girisham, S.M. Reddy, Culture conditions for the production of thermostable lipase by *Thermomyces lanuginosus*, Beni-Suef University Journal of Basic and Applied Sciences (2016), doi: 10.1016/j.bjbas.2016.11.010

paper, synthesis of biodiesel, degreasing of leather and resolution of racemic mixtures in the pharmaceutical industry (Ferreira-Dias et al., 2013). Lipases are also employed in wastewater treatment for degreasing of lipid clogged drains and in the medical industry as a diagnostic tool in blood triglyceride assay (Verma and Prakash, 2014).

Lipases are reported to be produced by many species of animals, plants, bacteria, yeasts and fungi (Sztajer et al., 1998). Among these, microbial lipases have gained wide industrial applications in view of high yields, rapid growth of microorganisms and ease of genetic manipulation (Andualema and Gessesse, 2012). Extracellular lipase production has been observed in several species of *Mucor*, *Rhizopus*, *Geotrichum*, *Pencillium*, *Acremonium*, *Candida*, *Humicola*, *Cunninghamella* and *Aspergillus* (Abrunhosa et al., 2013; Cihangir and Sarikaya, 2004; Gopinath et al., 2000, 2002, 2003; Prabhakar et al., 2012). With the advancement of molecular biology, fungal classification and phylogenetic studies have shifted to DNA sequence based methods (Shenoy et al., 2007). These methods play an important role in fungal identification and in understanding of genetic diversity of fungi (Rajesh et al., 2013). The internal transcribed spacer (ITS) regions are more variable and used to analyse interspecies and sometimes intra-species relations (Divakara et al., 2015). The 5.8 S r-DNA and the flanking ITS regions are conserved regions frequently used in phylogenetic studies. A homology search by BLAST has shown that the ITS region and 5.8 S rDNA sequences were highly conserved in thermophilic fungi, which suggests a possible recent taxonomic divergence in the fungal community.

Thermophilic fungi are widespread and its great importance in many natural processes has been reported (Ranjith Kumar et al., 2010). *Thermomyces lanuginosus* is a thermophilic fungus formerly known as *Humicola lanuginosa*, frequently isolated from self-heating organic debris and a variety of decaying plant materials (Anand et al., 1990; Gomes et al., 1993; Haarhoff et al., 1999; Hoq et al., 1994; Ranjith Kumar et al., 2010; Sreelatha et al., 2013). Most of the mesophilic fungi grow at temperatures between 10 °C and 40 °C and have an optimum growth at around 25–30 °C, while thermophilic fungi grow optimally at 40 °C–50 °C and sometimes exhibit growth even at an elevated temperature of 60 °C, but fail to grow below 20 °C (Cooney and Emerson, 1964). A further characteristic feature of thermophiles is that their enzymes are more heat stable than those of mesophiles when extracted and tested in cell-free systems. In view of the potential biotechnological applications of lipases in the oleochemical industry lead to the manufacturing of useful products such as cocoa butter substitute. However, several comprehensive reviews and information are available on lipases produced by mesophilic fungi (Almeida et al., 2013; de Almeida et al., 2016; Edwinoliver et al., 2010; Fernanda et al., 2015; Gutarra et al., 2009; Hosseinpour et al., 2012; Maldonado et al., 2016; Mukherjeea and Gupta, 2016; Mukhtar et al., 2016; Nwuche et al., 2013; Punitha et al., 2016; Rajan and Nair, 2011; Ramos-Sánchez et al., 2015; Santhosh Kumar and Ray, 2014; Sarkar and Laha, 2013; Yogesh and Prabhune, 2011). To date only limited information is available on lipases produced by *T. lanuginosus* (Ávila-Cisneros et al., 2014). Therefore, in the present investigation, production of thermostable lipase by three strains of *T. lanuginosus* was studied.

2. Materials and methods

2.1. Reagents and standards

Whatman filter paper No. 42, sodium chloride, citrate buffer (pH 8.0), triacetin, toluene, absolute alcohol, olive oil, rhodamine B, phenolphthalein, yeast extract, starch, $MgSO_4{\cdot}7H_2O$, $KH_2PO_4{\cdot}7H_2O$, agar and all the chemicals employed were from Merck Chemicals (Mumbai), India.

2.2. Basal medium

The yeast starch medium (basal medium) used in the present study consists of yeast extract 5.0 g, starch 15.0 g, $MgSO_4{\cdot}7H_2O$ 0.5 g, $KH_2PO_4{\cdot}7H_2O$ 1.0 g, pH 6.5 and Milli-Q ultrapure water (Millipore).

2.3. Sampling

Thermophilic fungi were isolated from dung of different animals (cow, sheep, turkey, pigeon, poultry, bear, duck, rabbit, monkey), zoo waste, banana peel and bird nest material collected from Zoo Park, Warangal, Telangana, India.

2.4. Cultures used in the present study

Aspergillus fumigatus, *A. flavus*, *Chaetomium thermophilum*, *Chrysosporium* species, *Humicola grisea*, *H. insolens*, *H. stellata*, *Malbranchea pulchella*, *Mucor miehei*, *M. pusillus*, *Myriococcum albomyces*, *Penicillium duponti*, *Rhizopus arrhizus*, *Torula thermophila* and *T. lanuginosus* (formerly known as *H. lanuginosus*) were used in the present study.

2.5. Morphological identification

The collected samples were analysed for the mycological examination of the fungi using dilution plate technique (Apinis, 1963a) and paired petri plate technique (Waksman et al., 1939). *T. lanuginosus* isolates were morphologically identified by standard manuals and protocols (Apinis, 1963b; Barnett and Hunter, 1972; Cooney and Emerson, 1964).

2.6. DNA extraction and PCR assay

Isolates of different strains of *T. lanuginosus* were cultured in 100 mL yeast-extract broth in 250 mL Erlenmeyer flasks 2–4 d at 120 rpm on a rotary shaker. Mycelium was harvested over a Büchner funnel and freeze dried overnight, and total genomic DNA was extracted from approximately 50–100 mg pulverized mycelium with a cetyl trimethyl-ammonium bromide (CTAB) method as suggested (Ramana et al., 2012). Thus, obtained genomic DNA was dissolved in 50 µL of 2 mmol/L Tris–EDTA and the concentration was estimated using a NanoDrop 2000c spectrophotometer (Thermo Scientific, Waltham, MA, USA). The DNA samples and stored at –20 °C for further analysis.

2.7. Molecular identification

The morphological identification of *T. lanuginosus* strains was further confirmed by molecular methods by polymerase chain

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