A novel strain of *Aureobasidium* sp. TeO12 for theophylline production from caffeine

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Abstract A total of 40 fungal cultures were isolated for their ability to grow on caffeine as a sole source carbon and nitrogen, and further screened for theophylline-producing activities under the growing cell system. Based on thin-layer chromatography and high-performance liquid chromatography analyses, the potent strain *Aureobasidium* sp. TeO12 was chosen for its capability to generate theophylline via biotransformation of caffeine. It was identified based on phenotypic characteristics and its ITS1–5.8S–ITS2 rDNA sequencing data (GenBank accession number no. KT439072). To improve theophylline yield, the effects of various factors, such as resting cell density, Fe(II) concentration, and course of the transformation of caffeine, were studied in a biotransformation reaction containing 0.1 M sodium phosphate buffer (pH 7), *Aureobasidium* sp. TeO12 resting cells as the whole-cell catalyst and caffeine (2.5 g/L) as the substrate, and the reaction was incubated at 30 °C on an orbital shaker (200 rpm). The results indicated that optimal combination included resting cell density 6 g/L, Fe(II) concentration 75 mg/L, and the biotransformation time 72 h. Under these optimal reaction conditions, the highest theophylline concentration of 1.55 g/L (molar yield of 67%) with an average degradation yield of the substrate of about 83% was obtained in the biotransformation process. This is the first report on the biotransformation of caffeine into theophylline by a novel strain of the genus *Aureobasidium*.

Keywords *Aureobasidium* sp. · TeO12 · Biotransformation · Caffeine · Resting cell · Theophylline

Introduction

Theophylline (1,3-dimethylxanthine), a naturally occurring alkaloid, is found in small amounts in tea, coffee, and cocoa plants and is produced commercially for use in cosmetic products as a tonic and as an anticellulite product (Council of Europe Publishing 2008). Theophylline is also used in the treatment of bronchial asthma, idiopathic apnea of prematurity, and diuresis, which is due to its desirable pharmacological properties such as diuretic, cardiac stimulation, and smooth muscle relaxation (Barnes 2010). Furthermore, several feasibility studies are developed to evaluate the therapeutic potential of theophylline with its application as antitumor and/or antiviral agents due to its structural similarity to adenine (Lentini et al. 2010; Zheng et al. 2011). Most of the theophylline sold commercially is prepared from dimethylurea and ethyl cyanoacetate by traditional chemical transformations (Winek et al. 1980). However, the chemical transformation of theophylline has several drawbacks such as high cost, low efficiency, and use of catalysts that are not environment friendly. Hence, the microbial transformation of caffeine offers a cleaner, more economical alternative for the natural production of theophylline and its application in pharmaceutical formulations (Patra 2007). Caffeine (1,3,7-trimethylxanthine) is one of the most popular and commercially important plant-derived purine alkaloids. It is found in over 100 plant species among which *Coffea arabica* (coffee), *Camellia sinensis* (tea), *Cola nitida* (Cola) and *Theobroma cacao* (cacao) genera are prominent (Ashihara et al. 2008). Additionally, caffeine as a by-product of decaffeination

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and from the coffee and tea processing plants is a renewable and economic substrate for the production of theophylline through biotransformation process. Despite this, only few reports have been published on microbial theophylline production from caffeine. Kurtzman and Schwimmer (1971) reported the first biotransformation of caffeine to theophylline using a strain of *P. roqueforti* isolated on caffeine-containing agar. Nevertheless, the resultant theophylline yield was very low, due to further degradation of theophylline to other dimethyl and monomethyl xanthines. Until now, several pure fungal isolates belonging to the genus *Penicillium*, *Aspergillus*, *Stemphylium*, *Rhizopus*, and *Fusarium* capable of degrading caffeine have been identified. However, only *Penicillium citrinum* and *Fusarium solani* produced substantial amounts of theophylline from caffeine biotransformation, as these fungi can produce theophylline in concentrations of 0.78 g/L (molar yield 85%) and 0.33 g/L (molar yield 35.5%), respectively (Patra 2007; Nanjundaiah et al. 2016). In view of this background, the study focuses on the isolation and identification of caffeine-degrading fungi to explore their ability in the biotransformation of caffeine to theophylline. The effects of initial cell density, Fe(II) concentration, and incubation period on the biotransformation of caffeine to theophylline by a newly isolated strain, TeO12, identified as *Aureobasidium* sp., were studied under resting cell system.

Materials and methods

Chemicals and media

The standards of caffeine (1, 3, 7-trimethylxanthine, 99%) and theophylline (1, 3-dimethylxanthine, 99%) were purchased from Sigma-Aldrich (UK) and were used for biotransformation experiments. HPLC-grade acetonitrile was obtained from Merck (Darmstadt, Germany). Acetic acid (99%) and n-butanol were acquired from Thermo Fischer Scientific (Breda, The Netherlands) and employed for TLC experiments. Potato dextrose agar and broth media were purchased from HiMedia laboratories (India). All other materials used were of the analytical grade and of highest purity.

Enrichment of caffeine-degrading cultures

For enrichment of fungal isolates capable of degrading caffeine, a modified minimal salt medium designated as M9-caffeine agar (Sambrook et al. 1989) including (g/L): Na_2HPO_4 : 6; KH_2PO_4 : 3; NaCl : 0.5; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$: 0.05; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.5; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$: 0.01; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$: 0.01; $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$: 0.01; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$: 0.01; agar: 20;

and caffeine: 2.5 g/L was used. Chloramphenicol was included at 100 mg/L to suppress bacterial growth. The pH of the medium, post-sterilization, was adjusted to 5.8 ± 0.1 . A total of 50 soil samples were collected randomly from tea plantation soils in the northern regions of Iran, serially diluted (10^{-1} to 10^{-6}), and plated on the enrichment media, as mentioned above, and then incubated for 5 days at 25 °C. The pure cultures of fungal isolates were picked and maintained on PDA slants at 4 °C.

Isolation of caffeine-transforming culture

Caffeine-degrading isolates were screened for their ability to transform caffeine into theophylline under growing cell experiments. The biotransformation experiments were performed using a two-step procedure (Aguirre-Pranzoni et al. 2011). Spore suspensions (5×10^6 CFU/mL) of 72-h-old slants of different fungal isolates were harvested by homogenization with deionized water (0.85% NaCl, 1% Tween 80) and inoculated into 50 mL of liquid PDB media in 250-mL Erlenmeyer flasks and incubated at 25 °C, on a rotary shaker at 200 rpm for 48 h and then caffeine (2.5 g/L) was added to the cultures. Two days after adding substrate, samples were collected and centrifugated at $10,000 \times g$ for 10 min. The supernatant was then subjected to TLC and HPLC analyses to check for theophylline production from caffeine. Control experiments without substrate and without fungal isolates were conducted under same culture conditions.

Identification of the isolate TeO12

Preliminary identification of the fungal isolate TeO12 was performed using macroscopic and microscopic examination of the culture followed by physiological and biochemical tests (Kurtzman et al. 2011; Peterson et al. 2013). Light microscopy of the fungal isolate was performed with Olympus, model BX40 (Japan). Molecular characterization of the isolate based on sequencing data of the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) has been used for phylogenetic analysis. To isolate genomic DNA, fungal pellets were grown to mid-log phase (18 h growth) in liquid PDB medium, harvested by centrifugation ($10,000 \times g$ for 10 min), and washed with 0.1 M sodium phosphate buffer (pH 7) at 30 °C and 200 rpm. DNA was efficiently extracted from the fungal mycelia by freezing cells in liquid nitrogen followed by grinding with glass bead beating and then using phenol–chloroform extraction (Amberg et al. 2005). Amplification of the PCR amplicons corresponding to the ITS1–5.8S–ITS2 rDNA regions was performed using universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGA

TATGC-3') (White et al. 1990). PCRs included an initial denaturation at 94 °C for 5 min followed by 30 cycles of 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 45 s. The final extension was for 10 min at 72 °C. The purified product was sent for sequencing to Macrogen Company in South Korea. Data from the ITS sequencing were searched for the similarity in the GenBank database using the BLASTN algorithm. ClustalX program was used for multiple ITS sequence alignments. The phylogenetic tree was constructed by maximum likelihood analysis of ITS1–5.8S–ITS2 region sequences based on Kimura two-parameter model with 1000 bootstrap replicates in the MEGA software (version 6.0) (Tamura et al. 2013).

Resting cell biotransformation

To prepare resting cells of isolate TeO12 which is used as a whole-cell catalyst in biotransformation reactions, fungal pellets were cultured for 36 h to the end of the exponential growth phase in 250-mL Erlenmeyer flasks containing 50 mL of a modified CZAPEK/DOX broth (g/L): glucose 30; NaNO₃ 3; yeast extract 5; MgSO₄·7H₂O 0.5; KCl 0.5; FeSO₄·7H₂O 0.01; KH₂PO₄ 1; (pH 7 ± 0.1) in a rotary shaker at 200 rpm at 30 °C. Fungal mycelium was harvested from the supernatant by filtration through a Whatman No. 1 filter paper, and the resulting pellets were washed three times with sodium phosphate buffer (100 mM, pH 7) before being resuspended in the same buffer. The biotransformation of caffeine to theophylline was evaluated with different initial resting cell concentrations (g dry weight/L) ranging from 2 to 10. The effect of various Fe(II) concentrations (25, 50, 75, 100, and 125 mg/L) on theophylline production was also investigated in the reaction mixture under optimum cell mass concentration. The biotransformation experiments were performed in 250-mL flasks containing 50 mL of 0.1 M sodium phosphate buffer (pH 7) supplemented with a sterile solution of caffeine (2.5 g/L). The experiments were performed in triplicate at 30 °C and 200 rpm for 48 h. To determine the influence of incubation time on theophylline production under resting cell experiments, the reaction mixture [buffer (pH 7) and 2.5 g/L caffeine] was incubated for 24, 48, 72, 96, and 120 h at 30 °C and 200 rpm at the optimal concentrations of cell mass and Fe(II). Time course samples were taken and subjected to TLC and HPLC analyses to check for biotransformation of caffeine to theophylline. The molar yield (percent) of theophylline formed during biotransformation experiments is calculated as follows:

Molar yield [%]

$$= 100 \times \frac{\text{g theophylline accumulated} \times \text{mol. wt. of caffeine}}{\text{g caffeine supplemented} \times \text{mol. wt. of theophylline}}$$

Analytical methods

Growth was determined by calculation of optical density of spore suspensions at 530 nm (OD₅₃₀) in a UV–Vis Analytik Jena's spectrophotometer (SPECORD 210, Carl Zeiss Technology, Germany) or by the measurement of dry weight of the fungal pellets after drying at 105 °C until a constant weight was achieved (Saraswathy and Hallberg 2005). Qualitative TLC analysis of caffeine and its transformation to theophylline was done on silica gel 60F254 plates (20 × 20 cm, Merck, Germany) developed in solvent system n-butanol/acetic acid/water (4:1:1 v/v) as described previously (Patra 2007). After development, the plates were air dried and observed in a UV chamber (UV transilluminator TS-36, USA). Quantitative analysis of substrate and transformed product was performed using HPLC with a reversed-phase C18 column (5 µm, 4.6 × 250 mm, Phenomenex) and PU4225 UV detector (Philips, Cambridge, UK). A mixture of acetonitrile/0.5% glacial acetic acid (1:4 v/v) was used as the isocratic mobile phase at a flow rate of 1 mL/min and UV detection at 278 nm. Triplicate, uninoculated controls with the above same conditions were also included. The crude reaction product (theophylline) was further purified by preparative TLC and subjected to FTIR and mass spectroscopy analyses with the view to determine its chemical structure. The FTIR spectra of the biotransformed theophylline were recorded using a Bruker Vector 22 spectrophotometer in KBr pellets in the 400–4000 cm^{−1} region. Mass spectra for the theophylline formed from caffeine biotransformation were acquired with electron impact (EI) ionization at 70 eV on an Agilent 5975C Mass Spectrometer.

Results and discussion

Due to environmental considerations and economic aspects, there is an increasing popularity on microbial transformation processes for the synthesis of bio-based products. Theophylline has been identified as the major intermediate of the caffeine-degrading pathways by fungi that could potentially be exploited for pharmacology and therapeutic applications. Thus, the present study was directed towards isolation of caffeine-degrading fungal cultures from plantation soil and evaluation of their ability for the synthesis of theophylline from caffeine using the whole-cell catalyzed biotransformation. An enrichment culture was used to screen for fungal cultures capable of degrading caffeine on a minimal salt medium containing caffeine as described in “Materials and methods”. A total of 40 fungal cultures were isolated from tea plantation soils in the north of Iran upon their ability to grow on caffeine as

the sole carbon and nitrogen source. Based on TLC analysis, only 12 isolates were able to produce theophylline from caffeine under growing culture experiments. From the results, the isolate TeO12 has been shown to produce much higher levels of theophylline compared to the other isolates tested (data not shown). The isolates were further checked in shake flask experiments for theophylline production from caffeine by HPLC analysis of the culture supernatant. Figure 1 represents % biotransformation of caffeine into theophylline by the isolated fungal strains after 48-h reaction under growing cell experiments. From the results obtained, isolate TeO12 was the most effective among the isolates and exhibited the highest transformation yield ($22.30\% \pm 1.29$). Based on TLC and HPLC analyses, isolate TeO12 was selected for further studies. The macroscopic and microscopic studies were first used for preliminary characterization of the strain TeO12. The colonies of the isolate on potato dextrose agar or broth media were fast growing, smooth, and covered with slimy masses of spores. The surface was initially white and gradually turned to black due to chlamydospore production. The blastoconidia, arthroconidia, and the presence of numerous hyphal elements producing spores of various shape and size could be seen under light microscopic examination (Fig. 2). The isolate hydrolyzed gelatin and urea but not starch. Nitrate and nitrite are not reduced by the isolate. Growth in the presence of 10% NaCl/5% glucose is positive. Growth occurs at 20–35 °C and pH 4–10 (optimal growth at 30 °C and pH 7.0). To confirm its relationship among members of the genus *Aureobasidium*, genomic DNA derived from the fungus was amplified based on ITS (ITS1 and ITS2) regions and 5.8S rDNA using universal primers ITS1 and ITS4. Total length of ITS1–5.8S–ITS2 sequence region (620 bp) of the isolate has been deposited in GenBank under accession number KT439072. The multiple alignments showed more than

97% similarity of this sequence with members of genus *Aureobasidium* in the GenBank databases of the NCBI. The phylogenetic tree was obtained using neighbor-joining algorithm showing the position of strain TeO12 among species of the genus *Aureobasidium* (Fig. 3). Sequencing data from the ITS1–5.8S–ITS2 region coupled with morphological and physiological characteristics suggested that strain TeO12 belongs to the genus *Aureobasidium*. It is known that strains of the genus *Aureobasidium* spp. are recognized as excellent producers for the production of organic compatible solutes, synthesis of extracellular enzymes, production of fungal exopolysaccharides, synthesis of dicarboxylic acids as well as the production of biosurfactants (Saha and Racine 2011; Peterson et al. 2013; Kim et al. 2015) which have possible industrial applications in food, medical, pharmaceutical, agricultural, and chemical industries. However, this is the first report to our knowledge on the production of theophylline from a newly isolated strain *Aureobasidium* sp. TeO12. The resting cell strategy was also employed for improving yield of theophylline production. It is significantly advantageous than the original use of growing cell biotransformation since biomass duplication can be effectively inhibited, initial cell density can be artificially controlled with the substrate, the biotransformation process can be run under non-sterile condition, downstream processing is more easy and, therefore, can be scaled up for industrial purposes (Ashengroph et al. 2012). The effects of various factors such as resting cell initial concentration, Fe(II) concentration, and the biotransformation time on the formation of theophylline was investigated. To understand the relationship between the amount of biomass (dry weight) and the biotransformation rate, the influence of various biomass concentrations on the yield of theophylline was studied (Fig. 4a). Increasing the biomass concentration to 6 g/L resulted in an increase in theophylline concentration at

Fig. 1 Biotransformation of caffeine to theophylline by the isolated fungal strains under growing cell experiments. Caffeine was fed at 48 h with an initial concentration of 2.5 g/L. The reactions were carried out at 30 °C for a 48-h incubation with shaking at 200 rpm and 50 mL of liquid PDB in 250-mL Erlenmeyer flasks. *Y-error bars* indicate standard deviation ($\pm$ sd) among the three parallel replicates

Fig. 2 Light microscopy of *Aureobasidium* sp. TeO12 after growth for 48 h at 30 °C. **a** Vegetative cells grown in PDB broth medium, **b** arthroconidia and hyaline blastoconidia produced on the PDA agar medium. Bar, 10 μ

Fig. 3 The phylogenetic tree of the strain TeO12 and the closest relatives based on a maximum likelihood analysis of ITS1–5.8S–ITS2 region sequences. Bootstrap support values from 1000 replicates (cutoff value of 50%) are shown at the nodes. The tree was rooted to

Rhodotorula araucariae. GenBank accession numbers for ITS sequences are shown in parentheses. Bar, 0.01 substitutions per nucleotide position

which biomass concentration the maximal yield of theophylline ($41\% \pm 1.38$) was obtained. Therefore, 6 g/L was selected as the optimum biomass concentration for the biotransformation process. At higher biomass concentrations, lesser products were observed to accumulate which attributes to the rapid depletion of caffeine at high-density concentration. The influence of Fe(II) concentrations on the biotransformation of caffeine to theophylline has also been determined under the optimal resting cell concentration (Fig. 4b). In the absence of Fe(II) in the reaction mixture, maximum transformation of $41\% \pm 1.13$ was obtained after 48 h of incubation. There was a gradual increase in theophylline production with 25–75 mg/L Fe(II). The highest theophylline yield ($58\% \pm 0.98$) was achieved when a 75 mg/L Fe(II) was added. Hence, 75 mg/L was selected as the optimized concentration for further biotransformation study. These results are in agreement with

previous reports (Ohe and Watanabe 1979; Patra 2007) for other fungal species, which show that the addition of iron to the reaction mixture facilitates the oxidative demethylation of caffeine to theophylline by involvement of putative iron-containing enzymes such as cytochrome P450. The increase in initial Fe(II) concentration beyond 75 mg/L results in further degradation of theophylline due to increased activity of the theophylline-transforming enzymes. Further experimental work that relies on enzymology is urgently needed to justify this conclusion. Figure 5 shows the time course of substrate utilization and product formation measured by HPLC analysis when the resting cells of *Aureobasidium* sp. TeO12 (at final concentration of 6 g/L) as whole-cell catalyst were incubated with 75 mg/L of Fe(II) and 2.5 g/L of caffeine as substrate. After a 24-h biotransformation period, about 0.88 g/L of theophylline with a molar yield of 38% was obtained.

Fig. 4 Effects of initial biomass concentration (dry weight) (a) and Fe(II) concentration (b) on the biotransformation of caffeine to theophylline in the reaction mixture under resting cell system. The experiments were conducted in 250-mL Erlenmeyer flasks containing

50 mL of sodium phosphate buffer (0.1 M $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$, pH 7) supplemented with 2.5 g/L caffeine. The flasks were incubated at 30 °C and 200 rpm for 48 h. Y-error bars indicate standard deviation ($\pm$ sd) among the three parallel replicates

Fig. 5 Time course of biotransformation of caffeine by *Aureobasidium* sp. TeO12 resting cell system. Reaction was performed at 200 rpm for 120-h incubation at 30 °C in 250-mL flasks containing 50 mL of sodium phosphate buffer (0.1 M, pH 7) supplemented with 2.5 g/L of caffeine, 6 g/L biomass, and 75 mg/L Fe(II). Y-error bars indicate standard deviation ($\pm$ sd) among the three parallel replicates

Caffeine was degraded during the first 24 h at a rate of 0.043 g/L/h. The highest theophylline molar yield (about 67%) was achieved within 72 h at which time over 83% of the available caffeine substrate had been degraded. Further increase in biotransformation time greatly reduced the theophylline accumulation which is due to the further degradation of theophylline to other caffeine xanthine derivatives. The study has also shown that resting cells of *Aureobasidium* sp. TeO12 can degrade caffeine in 96 h, with a maximum value of about 98%, pointing to the potential use of this strain in biodecaffeination processes. TLC and HPLC profiles (Fig. 6) of the reaction mixture

after incubation for 48 h under resting cell system show a decrease in the concentration of substrate and an increase of the major metabolite of its biotransformation. Control experiments indicated that the theophylline was produced enzymatically with the resting cells of *Aureobasidium* sp. TeO12. Structural elucidation of theophylline obtained from the biotransformation of caffeine by *Aureobasidium* sp. TeO12 resting cell system was confirmed by analyzing the FTIR and mass spectra (Fig. 7). The corresponding FTIR spectrum (Fig. 7a) shows absorption peaks at 1570 and 1666 cm^{-1} , which are assigned to be due to C=O symmetric and C=O asymmetric stretching vibrations, respectively. The frequencies observed at 980 and 1313 cm^{-1} are assigned to be due to N-CH₃ and C-N stretching vibrations, respectively. The bands observed around 3001 and 3124 cm^{-1} are assigned to be due to C-H and N-H stretching vibrations, respectively. The appearance of sharp vibrational band present at 1708 cm^{-1} could be assigned to C=N stretching vibration. These results are in agreement with those previously reported for FTIR spectra of the authentic theophylline (Gunasekaran et al. 2005). The mass spectrum of biotransformed theophylline showed principal peaks at 180, 95, 68, 53, 123, 151, and also showed a distinct pseudo-molecular ion $[\text{M}+\text{H}]^+$ at $m/z = 181$, which refers to the molecular weight of theophylline (Fig. 7b). Numerous bacterial and fungal strains belonging to *Acinetobacter*, *Alcaligenes*, *Aspergillus*, *Bacillus*, *Brevibacterium*, *Klebsiella*, *Leifsonia*, *Penicillium*, *Pseudomonas*, *Rhodococcus*, *Salinivibrio*, *Serratia*, *Stemphyllum*, *Thermomonospora*, and *Trichosporon* have been reported in the literature on caffeine degradation, which may be useful in the development of an environmental friendly and cost-effective biodecaffeination process (Ibrahim et al. 2016; Ashengroph 2017). However,

Fig. 6 TLC (I) and HPLC (II) chromatograms for biotransformation of caffeine to theophylline using *Aureobasidium* sp. TeO12 resting cell system. Reaction mixture contained 0.1 M sodium phosphate buffer (pH 7), 2.5 g/L caffeine, 6 g/L biomass, and 75 mg/L Fe(II),

and reaction was performed (200 rpm) at 30 °C for 48-h incubation. A standard caffeine, B standard theophylline, C control (uninoculated) and D biotransformed reaction. a control (uninoculated) and b biotransformed reaction

Fig. 7 FTIR (a) and mass spectroscopy (b) spectra of theophylline produced during the biotransformation of caffeine with *Aureobasidium* sp. TeO12 resting cell system

very few studies have been conducted on the biotransformation of caffeine to theophylline and other methylxanthines for commercial application. *P. citrinum* strain MTCC 5215 is isolated from the soil of a tea plantation for its ability of transforming caffeine and reasonable accumulation of theophylline under optimal reaction conditions. Development of a combined approach for optimization of theophylline production using single factor and response surface methodology resulted in 85% yield with a substrate concentration of 1 g/L after 42-h incubation at 28 °C in shake flask (Patra 2007). *F. solani* capable of degrading caffeine was isolated from caffeine-contaminated soil. Response surface optimization for decaffeination and theophylline production by the fungus showed that a pH of 5.8, temperature of 24 °C, and inoculum size of 4.8×10^5

spores/mL have resulted in a complete biodecaffeination of caffeine as well as the production of theophylline with a yield of 33% (Nanjundaiah et al. 2016). Compared with the previous reports, this work shows that resting cells of *Aureobasidium* sp. TeO12 expose a great potential to be used as biocatalyst to transform caffeine to theophylline with a molar yield of 67% within 72 h of incubation, without further optimization.

Conclusion

The current study was focused on the isolation and characterization of caffeine-degrading fungi to prepare the useful biocatalyst for the biotransformation of caffeine to

theophylline. *Aureobasidium* sp. TeO12 has been successfully isolated for its ability to use caffeine as the sole carbon and nitrogen source which was further used for the production of theophylline from caffeine using whole-cell biocatalyst. This is the first study on the biotransformation of caffeine to theophylline using *Aureobasidium* genus. The result suggested that whole cells of *Aureobasidium* sp. TeO12 can be used as potential catalysts for biodecaffeination process along with the production of biotransformed theophylline.

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