CHAPTER 32

MICROBIOLOGY

Doctoral Theses

01. KASHYAP (Amuliya)
Biochemical and Molecular Characterization of Lipase Lip 11 from Yarrowia Lipolytica MSR80.
Supervisors: Prof. Rani Gupta
Th 26190

Abstract

Lipase Lip11 of oleaginous yeast Yarrowia lipolytica was expressed in Pichia pastoris and its native secretion-signal was effectively recognised by the host machinery with 92% expression compared to a-secretion factor. Ser228, Asp295, and His357 were confirmed to form the catalytic triad in Lip11 by mutagenesis. Further, disruption of N-terminal putative Nglycosylation site (Asn17) in Lip11 improved its catalytic efficiency by 2.7-fold resulting in a variant N1, while disruption of the C-terminal site (Asn389) ruinously affected its catalytic activity. Additionally, N1 possessed higher thermal and acid stability than its glycosylated counterpart concomitant with distinct secondary and tertiary structural modifications such as an alternate ratio of alpha-helices and beta-sheets and higher surface hydrophobicity. Further, Lip11 showed an extended N-terminus upon alignment with Lip2 and N-truncation of 58 residues generated a 3.3-fold catalytically efficient variant T3, and further truncation emerged detrimental. Importantly, N-truncation abolished substrate inhibition in Lip11 and characterization of T3 revealed: higher thermal and acid stability while substrate specificity remained unaltered. Following N-truncation, b-sheet content increased from 8% to 16%, which perhaps is a structural basis for improved catalytic activity. Ahead, the lid-loop of Lip11 was determined to be hydrophilic in contrast to hydrophobic lid loop of Lip2, and imparting hydrophobicity to the lid-loop of T3 improved its catalytic efficiency by 7-fold. This correlated with the outcome of in-silico analysis where a comparatively flexible lid and a wider catalytic triad-volume were ascertained. Substrate specificity remained unaltered following lid mutations while sn-1, 3 regiospecificity and interfacial-activation subsided. In the end, a novel Pichia pastoris expression platform was developed via genomic integration of lipase gene for sustained release of methanol from methyloleate. Methyloleate successfully instigated PAOX-1 induction of two reporter proteins: amylase from Bacillus licheniformis, and g-CGTase from Evansella caseinilytica. The platform emerged versatile, and functioned sustainably even at higher cell densities.

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02. SAINI (Kuldeep)
Expression, Characterization and Application of γ-Cyclodextrin Glycosyltransferase from Evansella Caseinilytica.
Supervisors: Prof. Rani Gupta
Th 26191

Abstract

Cyclodextrin glycosyltransferase (CGTase; EC 2.4.1.19) belongs to the glycosyl hydrolase superfamily. CGTase is a biotechnologically important enzyme that hydrolyzes α-glycosidic bond between two sugar molecules leading to synthesis of cyclodextrins and other transglycosylation products. The present work focuses on expression and characterization of γ-CGTase from Evansella caseinilytica and its application in starch to γ-CD conversion. E. caseinilytica secreted a γ-CGTase and resulted in 5.5-fold enhancement with 240.5 ± 5.46 U/L γ-CGTase production titres after media optimization. Purified wCGTase was a monomer (~65 kDa) with optima at 60°C, pH 11 along with high thermostability and specific γ-CD production was identified on HPLC. Peptide fingerprinting revealed maximum identity with 'IPT-TIG domain containing protein' in the proteome of E. caseinilytica that shared high sequence homology with γ-CGTase from E. clarkii. CGTase was expressed in E. coli majorly as inclusion bodies and the soluble fraction protein was 3.5 fold enhanced using a combination of temperature and salt. The rCGTase was relatively thermolabile to wCGTase. Structures generated after MD simulations in low and high salt conditions indicated more compact catalytic pocket and salt bridges with better structural rigidity in high salt conditions conferring higher thermostability. It showed that same protein expressed under saline conditions in halophilic E. caseinilytica (wCGTase) had different conformation to the rCGTase produced under non-saline conditions in E. coli. Functionally active CGTase expression was achieved in Pichia pastoris which exhibited optima at pH 9.0 and 50°C. The present study also demonstrated cell surface expression of γ-CGTase on E. coli along with immobilization of free enzyme via covalent crosslinking on chitosan microspheres and electrostatic interactions on Purolite ECR8310F Amino C2 acrylate resin. Surface expressed enzyme was used as a whole cell biocatalyst in the conversion reaction and all the immobilized enzymes efficiently converted starch to γ-CD along with high recyclability and storage stability.

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03. SINGH (Ashutosh)
Multigene Phylogeny and MALDI-TOF MS Characterization of Melanized fungi and Determination of their Antifungal Susceptibility Profiles.
Supervisors: Prof. Anuradha Chowdhary
Th 26192

Abstract

Melanized fungi are a heterogeneous group of fungi causing cutaneous to systemic diseases with high mortality and are highly relevant because of their potential to infect otherwise healthy individuals. Melanized fungi are generally under estimated as an etiologic agent of diseases, due to difficulties in classical identification owing to poor growth and sporulation. In this study, we examined, using molecular methods and matrix-assisted laser desorption ionization–time of flight mass spectrometry

(MALDI-TOF MS), the diversity of melanized fungi isolated from patients in 19 medical centers in India. A total of 215 melanized fungal isolates collected in the repository of Medical Mycology Unit, Department of Microbiology, VPCI during 2012-2019 were used for characterization, by sequencing the internal transcribed spacer (ITS) region of the ribosomal deoxyribonucleic acid (rDNA), the D1/D2 domain of the large subunit rDNA (28S) and β- tubulin gene and by proteomic approach using MALDI- TOF MS. Sequencing of MF isolates showed that these isolates represented 21 genera comprising 29 species, the majority of them belonging to the orders Pleosporales (61%) and Chaetothyriales (16%). Of 215 MF species characterized Alternaria alternata (37.5%) was found to be most species, followed by Cladosporium cladosporioides (16%) and Curvularia lunata (13%). Among the 29 melanized fungal species identified in this study, only 6 (20%) species were identified by the MALDI-TOF MS due to the limited commercial database. However, a 100% identification rate of 20 unidentified species was obtained by constructing an in-house database. Further, the CLSI broth microdilution method revealed low MICs for posaconazole (≤1 µg/ml) and voriconazole (≤2 µg/ml) in 99% and 95.5% of isolates, respectively. Skin/subcutaneous phaeohyphomycosis due to MF were diagnosed in 21% (n =46) of cases. Also, 5% of patients had central nervous system involvement, and 2 cases of fungal osteomyelitis due to Cladophialophora bantiana were observed.

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1. Review of melanized fungi: their characterization and antifungal susceptible profile 2. Phenotypic characterization of melanized fungi 3. Molecular characterization of melanized fungal isolates by using ITS, D1/D2 and β-tubulin gene sequencing 4. Characterization of melanized fungal isolates by using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) and creation of the in-house database 5. In-vitro antifungal susceptibility of melanized fungi against conventional clinical antifungal agents and a novel antifungal agent olorofim. Summary and conclusion. Appendix and list of publications.