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Editorial

Blue Native Page in Combination with Mass Spectrometry as an Effective Tool to Study Multi-Enzyme Complex's in Secretome of Aerobic Fungus

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Introduction

The past decade has seen a large global interest in the development of renewable energy sources, and bio-products based on plant biomass. This is owing to the abundance of lignocellulosic material on the planet and due to their high sugar content. However plant biomass is a complex matrix of hemicellulose and aromatic lignin interacting with core cellulose making it remarkably resistant towards enzymatic saccharification [1,2]. In nature many microorganisms decompose plant biomass by secreting battery of carbohydrases capable of degrading cellulose and hemicellulose. The carbohydrate active enzyme system of bacteria and fungus can be largely considered as complexed or non-complexed enzymes. Several anaerobic cellulolytic bacteria are known to produce cell associated cellulosome. Cellulosome is a large extracellular multicomponent complex containing several cellulolytic and xylanolytic enzymes tightly bound to scaffolding protein. Such an organisation of hydrolases in close proximity to bacterial cell enhances synergy between constituent enzymes and allows efficient uptake of the hydrolysis products by minimizing the distance over which these oligomers needs to diffuse in order to reach host cell. On the other hand, several aerobic fungi and bacteria secrete free form of various carbohydrases that act synergistically on lignocellulose. However, few recent reports documented the formation of multienzyme complex in secretome of aerobic fungi and suggested enzyme complex formation as an effective strategy for efficient hydrolysis in aerobic fungi [3-5]. Protein-protein interaction among these carbohydrate active enzymes has also been proposed to potentially improve their stability [6,7].

Blue Native PAGE as a Tool to Study Multienzyme Complex in Fungal Secretome and its Advantages

Blue Native PAGE (BN-PAGE) has been implemented to study multi-enzymes interactions in secretome of *Penicillium purpurogenum* [5] and *Trichoderma harzianum* [3]. Blue Native PAGE is an electrophoresis-based separation method that fractionates protein complexes based on external charge induced by coomassie dye (CBB) and according to their molecular mass [8]. Coomassie binds to hydrophobic domains on protein complex surface providing negative charge that not only converts the hydrophobic protein into a water soluble protein but also conserve native configuration as well provides mobility to protein towards the anode. Major advantage of BN-PAGE offer over other protein- protein interaction methods (like co-immunopurification, yeast two hybrid screens, chromatography etc.,) is that it provides useful information about composition of secretome and relative abundance of multienzyme complexes within secretome in their native conformation. As BN-PAGE fractionates enzyme complexes in their native state, this technique is compatible with in-gel activity assays. Zymogramcoupled to BN-PAGE efficiently identifies cellulolytic and xylanolytic activity in the multienzyme complex in secretome. Moreover electrostatic repulsion due to the negative charge on complex surface reduces the protein aggregation; which makes BN-PAGE perfectly suitable tool for studying protein complexes in fungal secretome. Furthermore the combination of first dimension BN-PAGE with second dimension SDS-PAGE can disassociate multi-enzyme complex into its constituent individual enzymes. Finally Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) of individual SDS-PAGE band provides identification of enzyme to deliver the structural and functional information.

Drawbacks of BN-PAGE

Although BN-PAGE can efficiently fractionate enzyme complex's, based on to their molecular size and external charge induced by coomassie dye, but accurate determination of complex size by BN-PAGE is biggest challenge. Molecular weight estimation by BN-PAGE is generally found to be significantly smaller than the theoretical molecular mass. This may be partially being due to differences in CBB binding to the hydrophobic surface of proteins. Likewise, major challenge faced

while using BN-PAGE is to investigate the biological significance of the protein-protein interaction and to understand the nature as well as reason of interaction. Formation of multienzyme complex in secretome of few filamentous fungi has been reported, but the mechanism by which this interaction take place is still un-known. Though cohesion-dockerin type of interactions has been suggested based on the strong association between components of multienzyme complex, but no scaffoldin or cohesion-dockerin was found in any of the studied complex so far. Another experimental challenge with BN-PAGE is that the protein complexes with identical molecular mass tend to migrate together and subunit from two different protein complexes may be falsely identified as being part of the same protein complex. Nonetheless, this problem can be resolved by adding for those particular protein complexes a pre-purification Colorless Native PAGE (CN-PAGE) step prior to BN-PAGE.

Despite of above mentioned challenges, BN-PAGE has been successfully applied to study protein-protein interaction in different types of samples and with many different aims which range from oligomeric state of protein complexes to the analysis of complex mixtures of protein complexes. With the future scope for advancement in molecular and bioinformatic tools it is conceivable that BN-PAGE will provide better understanding of the protein-protein interaction mechanism.

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References

1. Badhan AK, Chadha BS, Kaur J, Sonia KG, Saini HS, and Bhat MK. Role of trans glycosylation products in the expression of multiple xylanases in *Myceliophthora* sp. IMI 387099. Current Microbiology. 2007; 54: 405-409.

2. Ribeiro GO, Gruninger RJ, Badhan A, and McAllister TA. Mining the rumen for fibrolytic feed enzymes.Animal Frontiers. 2016; 6: 20-26.

3. Silva AJd, Gómez-Mendoza DP, Junqueira M, Domont GB, Filho EXF, et al. Blue native-PAGE analysis of *Trichoderma harzianum* secretome reveals cellulases and hemicellulases working as multi-enzymatic complexes. Proteomics. 2012; 12: 2729-2738.

4. Ohtsuki T, SuyantoYazaki S, Ui S, Mimura A, et al. Production of large multienzyme complex by aerobic thermophilic fungus Chaetomium sp. nov. MS-017 grown on palm oil mill fibre. Letters in Applied Microbiology. 2005; 40: 111-116.

5. Gonzalez-Vogel A, Eyzaguirre J, Oleas G, Callegari E, and Navarrete M. Proteomic analysis in non-denaturing condition of the secretome reveals the presence of multienzyme complexes in *Penicillium purpurogenum*. Applied Microbiology Biotechnology. 2011; 89: 145-155.

6. Chang BS, and Mahoney RR. Enzyme thermostabilization by bovine serum albumin and other proteins: evidence for hydrophobic interactions. Biotechnology and Applied Biochemistry. 1995; 22: 203-214.

7. Mukhopadhyay A, Hazra PP, Sengupta T, Saha R, Nandi R and Sengupta S. Protein-Protein Interaction Conferring Stability to an Extracellular Acetyl (Xylan) Esterase Produced by *Termitomycesclypeatus*. Biotechnology Progress. 2003; 19: 720-726.

8. Darie CC. Investigation of Protein-Protein Interactions by Blue Native-PAGE & Mass Spectrometry. Modern Chemistry application. 2013; 1: e116.