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Preliminary Study on Diverse Carbon Utilization by Transformant *Aspergillus niger*

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***Abstract*— *Aspergillus niger* have been widely recognized as producer of metabolites and reported as good expression hosts for homologous and heterologous proteins. For recombinant expression systems, nature of metabolite production would change when the expression host system is modified via plasmid development. In order to study the diversity of carbon utilization of transformant *A.niger* and their relation to specific carbon sources that could trigger mannanase production, a new screening system was introduced using Biolog technique to evaluate the growth of the transformant performed on 95 carbon sources. As a result, the transformed *A.niger* were found able to utilize dextrin and other 27 carbohydrates with majority preferred carbohydrates were identified as monosaccharide, oligosaccharides and some sugar alcohols as the best chosen carbon sources for growth. The relative simplicity and global carbon sources underlying in the biolog system for screening of carbon source make it as a useful tool for the preliminary screening and identification of carbon sources in order to select the best carbon source for medium development.**

***Keywords*— *A.niger*; FF microplate; joining cluster analysis.**

I. INTRODUCTION

Filamentous fungi are known to have unique biochemical pathways to assimilate a vast array of simple and complex nutrients available to them and produce a variety of metabolites [6]. An important characteristic of filamentous fungi as *Aspergillus niger* has the ability to metabolize a diverse range of organic compounds as carbon sources.

A.niger is an organism that is important for metabolite production. For the selection of the best carbon source aimed for metabolite production, there are some methods and tools that can be applied. Biolog Filamentous Fungi (FF) Microplate was recently useful for the investigation of substrate utilization, growth, secondary metabolite and antimicrobial profiles [6]. This method is a useful tool to select media components in media optimization for secondary metabolite production by various cultures [5]-[6]. Biolog FF Microplate can also be used to determine the availability of biological control agent serves as limiting carbon source by comparing the profiles of carbon utilization used for testing nutritional requirements of antagonist interactions [3]. The nutritional requirements over carbon source is of fundamental importance for fungal growth for synthesis of the cell wall, proteins, nucleic acids and reserved as food materials and sources of energy for

oxidation [4]. The study of carbon utilization profiles is also useful and become the fundamental to understand fungi physiology during growth.

Up till now, there is no information being reported on the global utilization of different sources of carbon by transformant *A.niger*. Therefore, this study is aimed to determine the carbon diversity relative to amount of growth profile of *A.niger* transformant. A 95 wells FF Microplate with each wells containing different carbon source will be used in which the *A.niger* were grown. We apply joining cluster analysis as tools to analyze sole-carbon source utilization profiles. The information derived from this study would be lead to a better understanding of the carbon source utilization by *A.niger* and become important reference for selection the best carbon sources in medium development.

II. EXPERIMENTAL PROCEDURE

The strain of recombinant *Aspergillus niger* PY 11was obtained from the School of Bioscience and Biotechnology, Faculty of Science and Technology, National University of Malaysia. The characteristic of strain is given in Table 1. Carbon utilization profiles were investigated by FF Microplates (Biolog, Inc., Hayward, CA) containing test panel with 95 wells provided with different carbon-compounds and a single well with water as a control. The medium in the microplate was prefilled with dried nutrients and test reagents placed in the 96 wells of the microplate. A 200 µl of spore suspension containing 1 x 10^{11} spores/ml were prepared on 2% (wt/vol) malt extract agar under ambient daylight at 26 ^{0}C for 4 to 5 days until sporulation occurs. To prepare the inoculums, the spores were suspended and mixed gently with 16 ml of sterile phytagel in disposable borosilicate test tubes. The suspension was taken at inoculums turbidity of 75% ± 2% at A_{590} nm measured by using galvanometer. After that, 100 µl of the spore suspension was dispensed into each test microplates well and they were incubated at 26 ^{0}C.

Microlog 3 software performed at dual wavelength 490 nm and 750 nm, were used to measure the mycelia growth based on turbidity and the change of optical density. At the absorbance 750nm, the growth of mycelia was monitored after 24, 48, 72 and 96 h of incubation. The mitochondrial activity that plays important rule in evaluation of metabolic reaction caused the formation of a reddish-orange color was measured at absorbance 490 nm. Each samples were analyzed at least three times. Joining Cluster Analysis was used to group carbon sources utilized by *A.niger* and to discriminate the pattern profiles of individual carbon utilization. The programme is available in software package STATISTICA 8 (StatSoft, Inc., Tulsa, Oklahama) and was applied to identify the different groups of carbon sources from the experimental data set. The joining cluster analysis was designed by means of the Euclidean distance with complete linkage used as amalgation rule which is at each stage, the similarity between clusters is determined by two similar variables which has most distant with each cluster.

TABLE I
CHARACTERISTIC OF A.NIGER STRAINS USED IN THIS STUDY

Strain	**Nature**	**Origin and/or Properties**
Aspergillus niger Man 65	Recombinant of *A.niger* PY 11 that inserted with vector that can enhance the production of mannanase	Expression vector modification for mannanase production

III. RESULT AND DISCUSSION

A. Growth Profiles of A.niger Man 65

General carbon-source utilization profiles for Man 65 are represented by three distinct clusters given in Fig. 1. From the analysis, growth on 95 carbon sources and water as a control has shown variation of its growth pattern from one carbon to another.

Cluster I shown in Fig. 2a contain carbon source in the category that lead to very slow growth of biomass formation and some of them have been observed to cause inhibition to the growth. The incubation was done until 96 h and the normalized OD found were less than 0.6. For the case of the control experiment containing water, it was grouped within this type of carbon source. Most carbon being categorized under this group are gluconeogenic carbon source. The most dominant groups of this kind are amino acid (L-serine, L-aspartic acid, L-threonine, L-phenylalanine, L-ornithine, L-glutamic acid, N-acetyl-L-glutamic acid, L-asparagine, adenosine and glucuroamide) and TCA-cycle intermediates (α-keto-glutaric acid, D-malic acid, L-malic acid, Glycyl-L-Glutamic acid, D-saccharic acid, L-lactic acid and sebaric acid). Other types of carbon source fall under this cluster are esters, alcohols, phosporylated and aromatics groups. Of fifty one carbohydrates used, only ten belongs to this cluster.

Cluster II (Fig. 2b) can be described as a group of slow growth profiles which could be due to most carbon source supplied are polymers (Tween 80, glycogen, α-cyclodextrin and β-cyclodextrin) and also the TCA-cycle intermediates which consists quinic acid, D-gluconic acid, γ-Amino-butyric acid, succininc acid, D-glucuronic acid, 2-keto-D-gluconic acid, D-galacturonic acid, bromosuccinic acid and succinamic acid. Amino acids and some carbohydrates are also identified to give slow formation of biomass in this cluster.

Cluster III (Fig. 2c) performed the best utilizable carbon sources for this strain, which led to the fastest growth. This group of carbon comprises mainly carbohydrates which are monosaccharide, disaccharides, oligosaccharides, polysaccharides and polyols. This findings are consistent with the result published by reference [1] and [5] in the case of *Hypocrea atrovidis* (fungi) that do also utilize carbohydrates as the best carbon source and led to the fastest growth. Of the other two carbons found under this category are alcohol (glycerol) and polymer (dextrin) group. In most cases of the profiles in each clusters, it was observed that the cultivation were slowing down after 48 h and maximum growth achieved was at 72 h of incubation.

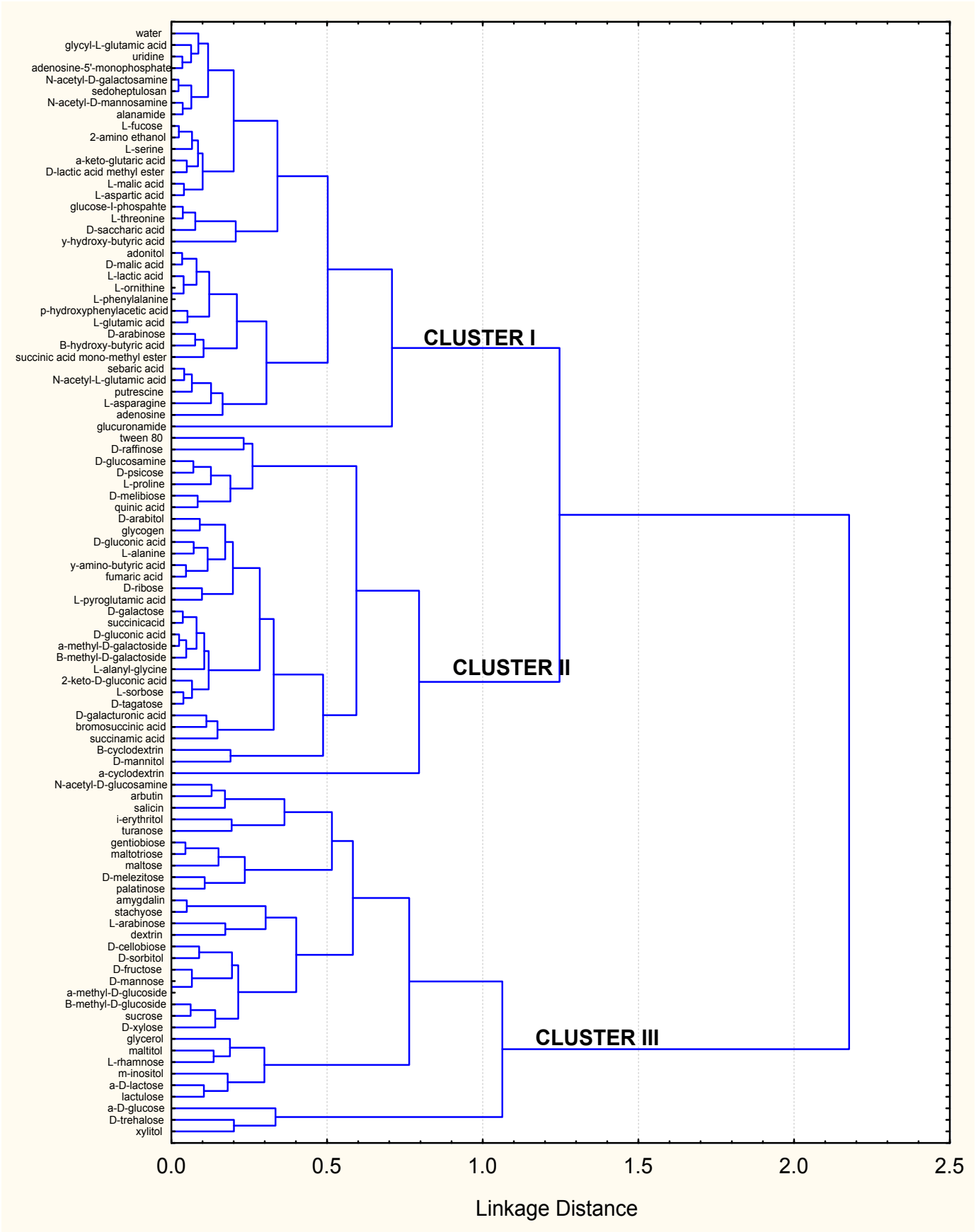

Fig. 1 Joining cluster analysis of *A.niger* Man 65

B. Diversity of Carbon Sources Utilization in A.niger

Generally, *A.niger* was classified as a member of eukaryotic organisms that able to utilise a wide variety of substrate to grow compared to yeast and bacteria as it is shown in this study. Previous studies by reference [2] have reported that for assimilation of various substrates, *A.niger* has the capability to switch between several different biochemical pathways. The diversity of carbon utilization may be due to structural variation and configuration of molecules from various carbon source compounds. From our results, cluster I experienced to very slow growth as a results of carbon source being the group of amino acids, carboxylic acids, phosphorylated, aromatic, ester and alcohol group, which in contrast to cluster II and III that consist mostly carbohydrate groups. The compounds in Cluster I have complex structural of molecules, compared to carbohydrate group which resulted the fastest growth and can be considered as the best carbon source utilized by *A.niger*.

Results also proved that *A.niger* can be classified as versatile organisms, with respect to the ability to utilize wide variety of carbon sources such as monosaccharide, disaccharides, oligosaccharide, polysaccharides and some alcohols and organic acids. This study shows that *A.niger* posses the ability to synthesize a diverse range of carbon sources by having broad substrate specificities. The reactions that are essential for growth on the different carbon sources mainly involved in major catabolic pathways, namely tricarboxylic acid cycle, pentose phosphate pathway, glycolisis/gluconeogenesis and glyoxylate shunt and oxidative phosphorylation.

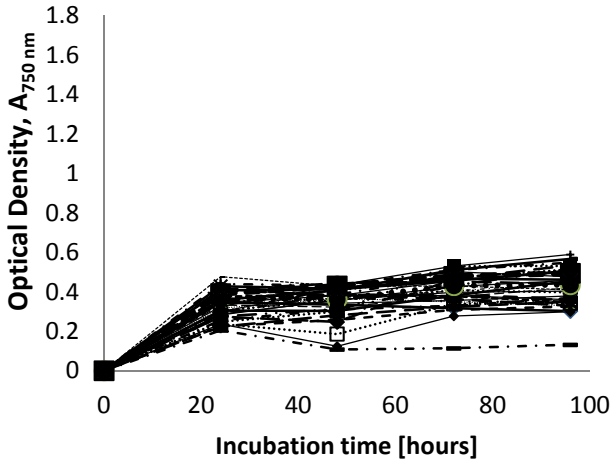

Fig. 2a Growth profile of *A.niger* Man 65 for cluster I

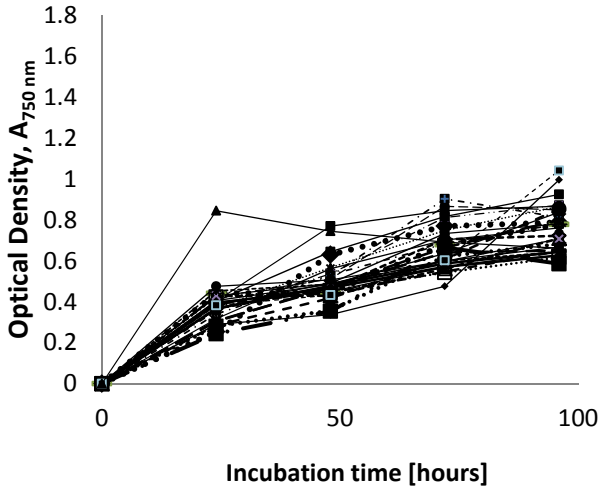

Fig. 2b Growth profile of *A.niger* Man 65 for cluster II

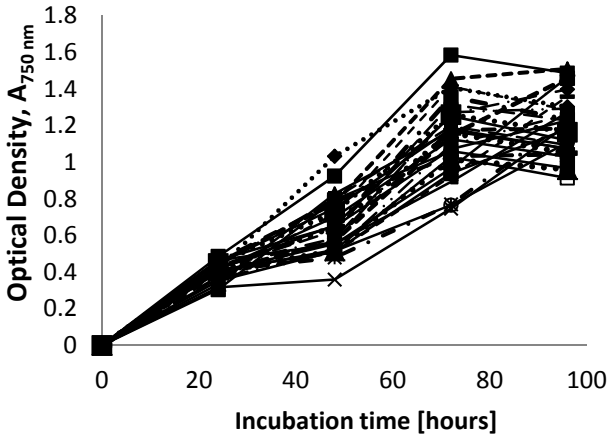

Fig. 2c Growth profile of *A.niger* Man 65 for cluster III

IV. Conclusions

In conclusion, this study indicates that transformed *A.niger* Man 65 as versatile organisms that can utilize diverse range of carbon source from carbohydrate group and others group (amino acids, carboxylic acids, polymers, aromatics, esters, phosphorylated and sugar alcohols). The application of cluster analysis as tool of carbon utilization studies being practical for quick review data especially data generated by biolog system.

Acknowledgment

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REFERENCES

[1] I.S. Druzhinina, M. Schmoll, B. Seiboth and C.P. Kubicek, "Global carbon utilization profiles of wild-type, mutant and transformant strains of *Hypocrea jecorina*," *Applied and Environmental Microbiology*, vol.72, pp. 2126-2133, Mar. 2006.

[2] W.E. Hintz, I. Kalsner, E. Plawinski, Z. Guo and P.A. Lagosky, "Improved gene expression in *Aspergillus nidulans*," *Canadanian Journal of Botany*, vol. 73, pp. 876-884, 1995.

[3] D.P.S. Lakhesar, D. Backhouse and P. Kristiansen, "Nutritional constraints on displacement of *Fusarium pseudograminearum* from cereal straw by antagonists," *Biological Control*, vol. 55, pp. 241-247, Dec. 2010.

[4] M. Madan and K.S. Thind, *Physiology of the Fungi*, SB Nangia and APH Publishing, Ansari Road, Darya Ganj, New Delhi, 1998.

[5] V. Seidl, I.S. Druzhinina and C.P. Kubicek, "A screening system for carbon sources enhancing β-N-acetylglucosaminide formation in *Hypocrea atrovidis* (*Trichoderma atroviride*)," *Microbiology*, vol. 152, Mar. 2006.

[6] M.P. Singh, "Application of Biolog FF Microplate for substrate utilization and metabolite profiling of closely related fungi," *Journal of Microbiological Methods*, vol. 77, pp. 102-108, Jan. 2009.