Shake flask production of prolyl oligopepetidase inhibitor by *Fusarium* sp. CMI397470

Ng, L.T.*[1, 2]; Kuek, C.[1] and Yeo, Y.T.C.[2]

[1]*Swinburne University of Technology,*
School of Engineering, Computing, and Science,
Jalan Simpang Tiga, 93350,
Kuching, Sarawak, Malaysia
[2]*Sarawak Biodiversity Centre*
KM20, Jalan Borneo Heights, Semengoh,
Locked Bag No. 3032, 93990 Kuching, Sarawak, Malaysia
*Corresponding author email: ltng@swinburne.edu.my

Fusarium sp. CMI 397470, an endophytic fungus isolated in Sarawak exhibits inhibitory activity against prolyl oligopeptidase (POP), an enzyme associated with Alzheimer's Disease. To understand the cultural parameters required to produce the POP inhibitor(s) (POPI) in shake flask culture, various manipulations were studied. A glucose-based chemically defined medium was found to be suitable for producing POPI. From the patterns of growth and POPI titre over time, POPI has the characteristic of a secondary metabolite. POPI production was improved when glucose supply was increased from 5 to 10 g L^{-1} but remained the same when increased from 10 to 20 g L^{-1}. At 20 g L^{-1} the POPI yield coefficient (POPI titre/substrate consumed) was less efficient. Biomass yield coefficient (biomass weight/substrate consumed) decreased as supplied glucose was increased from 10 to 20 g L^{-1}. In the temperature range 25 to 35^{o}C, POPI production was similar at 25 and 30^{o}C but apparently hampered at 35^{o}C.

1.0 Introduction

An endophytic fungus was isolated from a yam plant near Kuching, in Sarawak. Molecular profiling undertaken by CAB International, UK identified the fungus as *Fusarium* sp. and the CABI collection number CMI 397470 was assigned. Butanol extracts from culture filtrates of this fungus was found to have high inhibitory activity against a serine protein, prolyl oligopetidase (POP). POP is involved in the degradation of various neuropeptidases *in vitro*, and elevated levels of POP are found in the patients with Alzheimer's Disease (AD) (Aoyogi *et al.*, 1990). The association between AD and POP underlies the interest of pharmaceutical companies in the development of drugs for combating the disease via inhibition of POP activity. The objectives of this study were to characterise in submerged aerobic culture, the pattern of synthesis of POP inhibitor(s) (POPI) by CMI397470, and the determination of some of the physicochemical parameters affecting production of POPI by the fungus.

2.0 Materials and methods

Inoculum production. Fifty mL of chemically defined (Parker) medium (10 g L^{-1} added glucose) in a 250 mL Erlenmeyer flask capped with a silicon rubber foam closure (Sigma Aldrich C1046) was inoculated with six agar plugs containing fungal mycelium mat from a 7-day old Parker Agar culture (10 g L^{-1} of glucose added). The flasks were incubated at 25^{o}C and 200 rpm in a gyratory incubator for 5 days. At harvest, the required number of flasks were combined and homogenized in a sterile Waring Semi-Micro blender vessel.

Shake flask culture. The required number of flasks (sample points x replicates) was each inoculated with 5 mL of inoculum. The flasks are incubated at 25^{o}C and 200

rpm in a gyratory incubator. Culture from 3 flasks was sampled daily and mycelial dry weight was determined after filtration and drying at 60°C. The pH of the culture filtrate was determined, and assayed enzymatically for residual glucose and POPI titre.

POPI assay. Inhibitor titre was determined using culture filtrate, POP (0.3 U mL^{-1} in 0.1 mM, pH 7.0 phosphate buffer, and 5.0 mM Z-gly-pro-ρ-nitroanilide (substrate). The optical density of the reactant solutions were read at a wavelength of 414 nm after incubation. One Prolyl oligopeptidase Inhibitory Unit (PIU) is that amount of substance that reduces the release of 1 µmol of 4-nitroaniline per minute at 30°C, pH 7.

3.0 Results and discussion

3.1 The pattern of POPI production.

Glucose is a suitable carbon source for CMI397470 as biomass accumulation was a reciprocal of the glucose consumption (Fig. 1). POPI titres only increased after growth had slowed down and glucose was depleted. This relationship is clearly demonstrated when data was transformed into specific growth rate (µ) and titre productivity (POPI titre/t). Maximum POPI titres were only observed when µ approaches zero (Fig. 2). This indicates that POPI is a secondary metabolite as its production occurs after the growth phase *i.e.* it is growth dissociated.

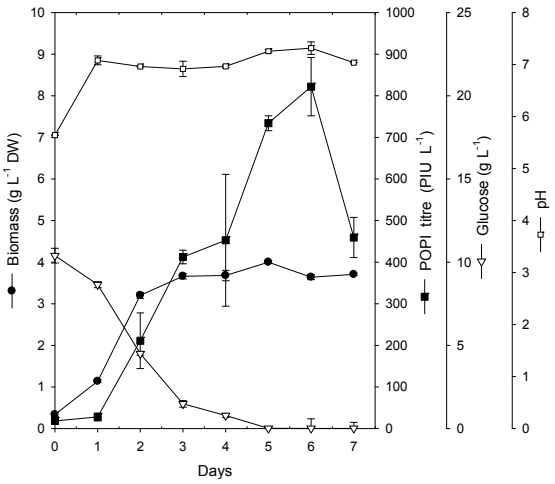

Fig.1: POPI production in shake flask culture of *Fusarium* sp. CMI397470 with Parker medium (10 g L^{-1} added glucose).

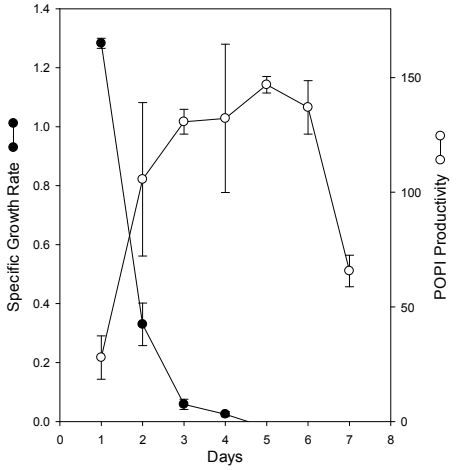

Fig. 2: The growth dissociated relationship between the specific growth rate of *Fusarium* sp. CMI397470 and its POPI productivity.

Studies reporting time course of POPI production by fungi is very limited. A previous study with *Streptomyces eurythermus* (Toda *et al.*, 1992) reported a growth dissociated pattern for the production of POPI similar to that found in our study.

3.2 The effect of glucose quantity supplied in the medium on POPI production

Increasing glucose supply from 5 to 20 g L^{-1} did not appear to result in a definitive trend on biomass accumulation, but a negative effect may be indicated (Fig. 3). However, POPI production responded to an increase in glucose supply from 5 to 10 g L^{-1} in the medium but not thereafter (Fig. 3). The efficiency of conversion ($Y_{p/s}$) of glucose to POPI became poorer when glucose supply was higher than 10 g L^{-1} (Fig.

4). It is clear that the conversion of glucose to biomass ($Y_{x/s}$) was less efficient at the two higher levels of glucose (Fig. 4). Efficient growth of the fungus occurred when glucose was supplied at 5 g L^{-1} but this was associated with poor POPI titre. At 10 g L^{-1} glucose, more glucose was shunted to other metabolic use as indicated by the relatively poorer efficiency of conversion of glucose to biomass but better conversion to POPI. The optimal glucose supply appears to be at middling concentrations indicated by the POPI titre at 10 g L^{-1}. Exceeding this supply can lead to what appears to be repression of POPI production at 20 g L^{-1} glucose.

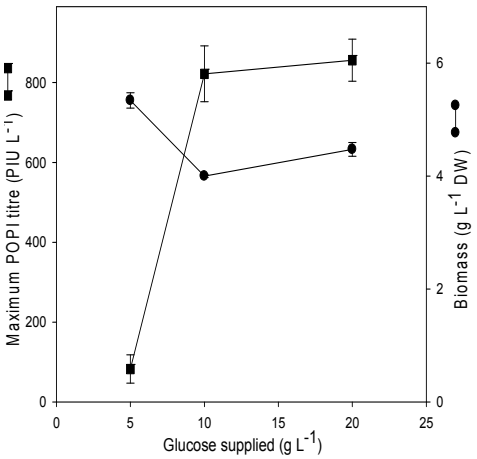

Fig. 3: The maximum POPI titres in shake flask cultures of *Fusarium* sp. CMI397470 when glucose quantity supplied was varied.

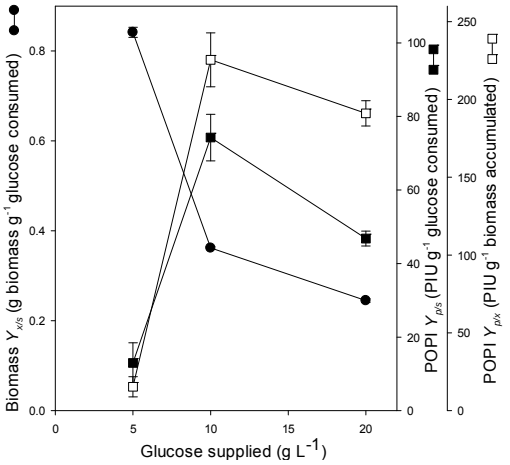

Fig. 4: The efficiencies of the conversion of glucose to biomass and POPI at the point of maximum POPI titre in shake flask culture of *Fusarium* sp. CMI397470

3.2 The effect of incubation temperature on biomass and POPI production.

The fungus grows well at 25 and 30°C (Fig. 5). Growth is clearly inhibited at 35°C. At 30°C, the same quantity of biomass produces more POPI than at 25°C *i.e.* POPI per unit biomass is better (Fig. 6). At 30°C, POPI titre is more than double that at 25°C. The low POPI titres observed at 35°C were a reflection of the lack of biomass due to

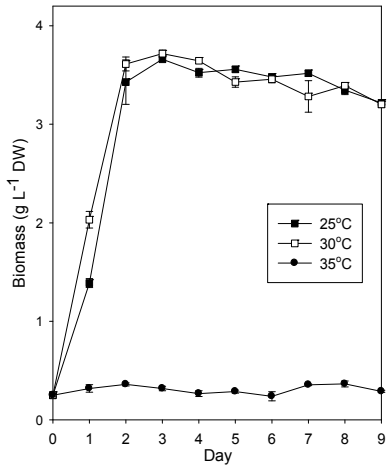

Fig 5: The effect of incubation temperature on biomass accumulation in shake flask culture of *Fusarium* sp. CMI397470.

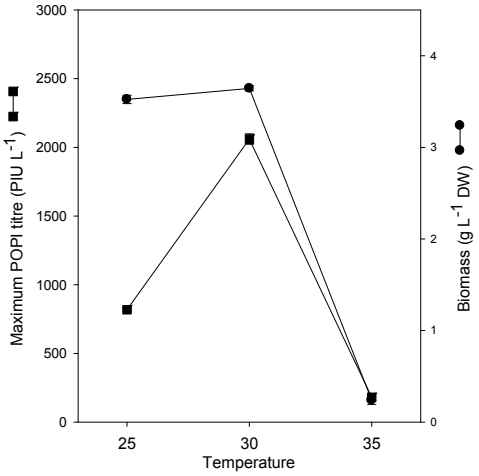

Fig. 6: The effect of incubation temperature on maximum POPI titre and the associated biomass quantity in shake flask culture of *Fusarium* sp. CMI397470.

poor growth. A similar relationship between biomass and metabolite productivity (also maximal at 30^{o}C) was found in the production of Cyclosporin A by *Fusarium roseum* (Ismaiel *et al.,* 2010).

In conclusion, POPI was produced by *Fusarium* sp. CMI397470 in shake flask culture. POPI from this fungus appears to be a secondary metabolite from its production pattern in relation to growth. Of the conditions tested, its production is best at 30^{o}C with glucose supplied at 10 g L^{-1}.

4.0 Acknowledgements

We thank the Sarawak Biodiversity Centre for study leave (NLT), the Sarawak state government for project funding, Swinburne University of Technology Sarawak for a scholarship (NLT), and Nimura Genetic Solutions which funded the fungal endophyte screening program at the SBC.

5.0 References

Aoyagi T, Wada T, Nagai M, Kojima, F., Harada, S., Takeuchi, T., Takahashi, H., Hirokawa, K. and Tsumita, T. (1990) Increased γ-Aminobutyrate Aminotransferase Activity in Brain of Patients with Alzheimer's Disease. *Chemical and Pharmaceutical Bulletin* 38: 1748 – 1749.

Ismaiel, A. A., El-Sayed, E.A. and Mahmoud, A.A. (2010) Some Optimal Culture Conditions For Production of Cyclosporin A by *Fusarium roseum. Brazilian Journal of Microbiology* 41: 1112 - 1123.

Toda, S., Obi, Y., Numata, K., Hamagishi, Y., Tomta, K., Komiyama, N., Kotake, C., Furumai, T. and Oki, T. (1992) Eurystatins A and B, new Prolyl Oligopeptidase inhibitor I. Taxonomy, Production, Isolation and biological activities. *The Journal of Antibiotics* 45: 1573 – 1579.

How to cite this paper:

Ng, L.T.; Kuek, C. and Yeo, Y.T.C. (2011) Shake flask production of prolyl oligopepetidase inhibitor by *Fusarium* sp. CMI397470**.** In: "Proceedings of the International Congress of the Malaysian Society for Microbiology 2011"; 8 - 11 December 2011, Penang, Malaysia; R.A. Rahim; W.Z. Saad; S.C. Chin *et al.* (eds.), Malaysian Society for Microbiology, ISBN 978-983-99873-1-7; pp. 492 - 495.