ISOLATION OF ENDOPHYTIC HUPERZINE A-PRODUCING FUNGI FROM *Huperzia cuernavacensis*

Olga L Cruz Miranda[1], Rosa Cerros Tlatilpa[2], Aniceto Mendoza Ruiz[2], María Luisa Villarreal[1], Alexandre Cardoso Taketa[1]

[1]Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos, México
[3]Laboratorio de Sistemática y Morfología, Universidad Autónoma del Estado de Morelos, México
[4]Laboratorio de Biología de Pteridofitas, Universidad Autónoma Metropolitana, México
olgacumi@hotmail.com, ataketa@uaem.mx

Key words: endophytic fungi, huperzine A

Introduction. Alzheimer's disease (AD), an irreversible neurodegenerative disorder primarily targeting elderly populations, affects approximately 36 million people worldwide according to the 2010 estimations (1). No known cure for AD currently exists; although the use of cholinesterase inhibitors (AChEI) is one of the most accepted strategies in AD treatment (2). Huperzine A (Hup A), fig. 1, an alkaloid isolated from *Huperzia serrata,* has been shown to be a powerful and selective AChEI, with unique pharmacological activities and low toxicity (3). However, *H. serrata* actually produces very low Hup A content, and has very limited distribution, and extremely slow growth. Due to its high demand it is important to find new sources of Hup A (4). Previous studies reported that several endophytic fungi associated with Huperziaceae plants could produce Hup A, suggesting that fermentation processes using Hup A-producing microorganisms may be a promising approach to its production (5).

Methods. Healthy wild-plants of *H. cuernavacensis* were collected from the natural populations from Cuernavaca Morelos. The samples were thoroughly washed in running tap water, and then sterilized by washing in 75% ethanol (v/v) for 1 min, 3.4% NaClO for 10 min and 75% ethanol for 30 s, respectively. Afterwards, plants were rinsed 4–5 times in sterile distilled water and then the roots and stems of the samples were cut into 0.5-cm lengths. Leaves were cut into 0.5-cm^2 sections, and transferred to Petri dishes in four different media: potato dextrose agar (PDA), corn meal agar (AHM), malt extract agar (AEM) and water agar (AA) (amended with 60 ug/mL streptomycin and 100 ug/mL ampicillin). The Petri dishes were incubated at 28 °C in darkness and monitored every day to check the growth of endophytic fungi. Each fungal culture was checked for purity and transferred to another PDA plate by the hyphal tip method and incubated at 28 °C. The Hup A produced by endophytic fungi will be screened through thin layer chromatography (TLC) using silica gel plates developed in a solvent system (chloroform: acetone: isopropanol at 4:4:2 v/v/v). The spots will be visualized by ultraviolet light and Wagner reagent. Dry crude extracts will be dissolved in 1 mL methanol and subjected to TLC with authentic Hup A as control.

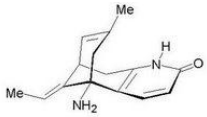

Fig.1 Huperzine A structure

Results. We obtained a total of 22 fungal isolates, as shown in Table 1. The greatest number of isolates was obtained from PDA, mainly from roots. From AA, AEM and AHM media were obtained the same number of isolates, however in the last two media were not isolated any fungi of stems. According to morphological and microscopic observations the strains could belong to the genera *Penicillium, Fusarium, Alternaria, Acremoniun Cladosporium*, among others (Fig. 2). These genera have been reported to produce Hup A. E.g. *Cladosporium cladosporioides* was reported by Zhang et al. (2011), and produced Hup A (56.8 ug/L), which was identified and purified by thin layer chromatography and HPLC.

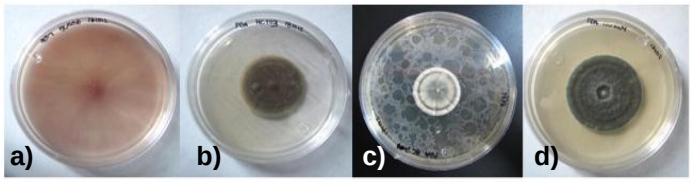

Fig.2 Representative isolates genera *Fusarium* (a), *Cladosporium* (b), *Penicillium* (c) y (d).

Table 1. Number of isolates of vegetable samples and culture medium.

	Parts Plant			
Media	Leaves	Stems	Roots	Total
PDA	2	3	8	13
AEM	1	0	2	3
AHM	3	0	0	3
AA	1	1	1	3

Conclusions. In this work, 22 fungal isolates of *H. cuernavacensis* were obtained, which could give promising results in the production of Hup A.

Acknowledgements. Thanks to CONACYT for the financial support to this work. Project N° 156276.

References.

1. Prince M, Bryce R, Ferri C. 2011. London, UK: Alzheimer's Disease International (ADI); 2012.
2. Darvesh S, Walsh R, Kumar R, Caines A, Roberts S, Magee D,Rockwood K, Martin E (2003). Alzheimer Dis Assoc Disord 17:117–126.
3. Wang R, Yan H, Tang XC 2006. Acta Pharmacol Sin 27(1):1–26
4. Ma XQ, Gang DR (2004). Nat Prod Rep 21:752–772
5. Wang, Y.; Zeng, Q. G.; Zhang, Z. B.; Yan, R. M.; Wang, L. Y.; Zhu, D. 2010. Journal of Industrial Microbiology and Biotechnology DOI 10.1007/s10295-010-0905-4
6. Zhang, Z. B; Zeng, Q. G; Yan, R. M; Wang, Y; Zou, Z. R; Zhu, D. 2011. World J Microbiol Biotechnol (2011) 27:479–486