中文題目：南台灣環境中分離之伺機感染性黴菌：隱球菌和阿氏絲孢酵母

英文題目：Isolation of *Cryptococcus* Species and *Tricosporon asahii* from Environment in Southern Taiwan

作　　者：李至恭 [1,2]　張學文 [2] 林清江 [3]

服務單位：輔英科技大學附設醫院感染科 [1] 國立中山大學生物科學系 [2]
輔英科技大學附設醫院醫學檢驗科 [3]

Background： The increasing infection of *Cryptococcus* species and *Tricosporon asahii* emerged in clinical patients who were immunocompromised. These opportunistic fungal pathogens usually invaded lung, skin, brain and other organs and caused systemic diseases in human. If no effective treatment, morbidity and mortality in immunocompromised patients will be higher than normal healthy people. Isolation of *Cryptococcus neoformans* from birds' droppings was noted by previous reports. *Cryptococcus neoformans var. grubii* (serotype A) infections were reported in clinical cases predominantly and were found in birds' droppings in large amount. Relatively, *Cryptococcus neoformans var. gattii* (serotype B,C) had a natural life in plants. Eucalypticus trees were reported to be isolated frequently. Isolation from other trees was also reported increasingly in the tropical and subtropical areas. Comparing to *Cryptococcus* species, *Tricosporon asahii* is a saprophytic fungus of soil. *Tricosporon asahii* is seldom isolated from other creatures in tropical area. Try to realize the relationship between these opportunistic fungal pathogens and human diseases, we attempted to search the distributive area of these opportunistic fugal pathogens in southern Taiwan. We collected samples of racing birds' droppings and eucalyticus trees for serial analyses. Furthermore, the relationship between human and these opportunistic pathogens will be understood and provide prompt information for diseases control and prevention.

Materials and Methods：The environmental investigation about *Cryptococcus* species and *Tricosporon asahii* in southern Taiwan was performed from Sep. 2009 to Sep. 2010. We collected 120 specimens of racing pigeons' droppings from 7 breeding farms in Donggang Township, Xinyuan Township and Xinpi Township in Pingtung County. We also collected 114 specimens of eucalypticus trees in Chengcing Lake and Nauzi Dist in Kaohsiung City. All samples were mixed with phosphate buffer saline which included powders of sodium penicillin G and streptomycin sulfate. Shaking completely then standing for 15-30 minutes and 0.1ml solution was drawn. The ten time of dilutions were smeared over sabouraud dextrose agars. After 3 to 7 days of incubation in 35^0C incubator, all the suspected creamy white colonies were analyzed. The Gram's stains, India ink stains and germ tube tests were used to initially identify these suspected colonies for opportunistic fungal pathogens. All the most likely

colonies were smeared over sabouraud dextrose agars again for pure colony cultures. The identification of these pure colonies were confirmed by VITEK 2 system （bioMérieux, Inc. Durham, USA）finally. For antibiotic susceptibility test, only *Cryptococcus neoformans* was available in VITEK 2 system. All isolates of *Cryptococcus neoformans* were operated for the susceptibility of Amphotericin B and Fluconazole and Flucytosine. For serotyping of these fungal colonies, *Cryptococcus neoformans* and *Tricosporon asahii* were prepared for molecular biology analyses. First, these fungal DNAs were extracted individually by Genomic DNA purification Kit. Second, serotypes of *Cryptococcus neoformans* were identified by polymerase chain reaction – fingerprinting (PCR-fingerprinting). The minisatellite-specific core sequence of the wild-type phage M13 (5'GAG GGT GGC GGT TCT 3') was used as single oligonucleotide primer for PCR. Three, molecular biology analysis of *Tricosporon asahii* was performed by PCR. Ribosomal DNA intergenic space 1 regions were amplified. 26SF (5'ATC CTT TGC AGA CGA CTT GA 3') and 5SR (5'AGC TTG ACT TCG CAG ATC GG 3') were used for oligonucleotide primers. These specific patterns of electrophoretic analysis chart were compared to previous reports of serial references. The serotypes of *Cryptococcus neoformans* and molecular identification of *Tricosporon asahii* were obtained the confirmation at last.

Results： Among 120 samples of racing pigeons' droppings from 7 breeding farms in southern Taiwan, 30 colonies of *Cryptococcus neoformans* were isolated from racing pigeons' droppings (25%). Besides, 4 colonies of *Cryptococcus laurentii* (3.3%) and 2 colonies of *Cryptococcus albidus* (1.7%) were isolated also. In addition, 25 colonies of *Tricosporon asahii* (20.8%) were isolated from racing pigeons' droppings. The total isolation rate of these opportunistic fugal pathogens was 46.7%. But, among 114 samples of eucalypticus trees, none of opportunistic fungal pathogens was isolated (0%). Serotyping of *Cryptococcus neoformans* was finished by core phage M13 PCR-fingerprinting. Comparing the patterns of electrophoretic analysis chart, all 30 colonies of *Cryptococcus neoformans* isolated from racing pigeons' droppings were *Cryptococcus neoformans var. grubii* (serotype A, VN Ⅰ). Under the detection of VITEK 2 system, antibiotic susceptibility tests of 30 colonies of *Cryptococcus neoformans* identically showed sensitive to Amphotericin B (minimal inhibitory concentration ≦0.25μg/ml) and Fluconazole (minimal inhibitory concentration 2μg/ml) and Flucytosine (minimal inhibitory concentration ≦1μg/ml). All 25 colonies of *Tricosporon asahii* isolated from racing pigeons' droppings showed the identical patterns of electrophoretic analysis chart by PCR amplification for ribosomal DNA intergenic space 1 regions.

Conclusion：To sum up, *Cryptococcus* species and *Tricosporon asahii* were isolated from racing pigeons' droppings in our study, especially *Tricosporon asahii* in large

amount. Racing pigeons for gambles was a fad in Taiwan, especially in southern Taiwan. The immunocompromised patients increased yearly in Taiwan and their surrounding contact environment played an important role in opportunistic infection. Opportunistic infection caused by these fungal pathogens should be given more attention to racing pigeons which had intimate contact with human. Intensive investigation and surveillance will be carried out in the future to provide a valid information for diseases control and prevention.