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NOTE

Serotyping of *Cryptococcus neoformans* Strains Isolated in Korea

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Twenty strains of *Cryptococcus neoformans* isolated from environmental and clinical sources in Korea were examined for their serotypes. Two environmental isolates from pigeon excreta belonged to *C. neoformans* var. *neoformans* serotype A. Of the 18 isolates from clinical specimens, 17 belonged to *C. neoformans* var. *neoformans* (serotype A : 16, serotype D : 1) and one belonged to *C. neoformans* var. *gattii* serotype B, which was culturally unusual, producing mucous colonies. This is the first report of the identification of *C. neoformans* var. *gattii* serotype B from a patient in Korea.

Key words: serotype, *Cryptococcus neoformans var. neoformans, Cryptococcus neoformans var. gattii*

Cryptococcus neoformans is an encapsulated yeast-like fungus that causes life-threatening infections, particularly in patients with compromised immune systems (Lewis *et al.*, 1972; Casadevall *et al.*, 1992; Baro *et al.*, 1999). The incidence of cryptococcal infection has increased in recent years as a result of a large increase in AIDS cases and the expanded use of immunosuppressive drugs (Dismukes and W.E. 1988; Ito-Kuwa *et al.*, 1994; Sullivan *et al.*, 1996). *C. neoformans* has been divided into two varieties, *C. neoformans* var. *neoformans* and *C. neoformans* var. *gattii* (Ikeda *et al.*, 1982; Bennett *et al.*, 1997). These two varieties of *C. neoformans* are easily differentiated by their biochemical properties, and have distinctive serotypes based on the antigenic composition of those capsular polysaccharides, which play an important role in pathogenicity (Bennet *et al.*, 1978; Baro *et al.*, 1998). *C. neoformans* var. *neoformans* corresponds to serotypes A, D, and AD, whereas *C. neoformans* var. *gattii* corresponds to serotypes B and C (Bennet *et al.*, 1977; Kwon-Chung *et al.*, 1982). Differences between the two varieties with regard to pathogenicity and geographical distribution have been documented (Kabasawa *et al.*, 1991; Baro *et al.*, 1998).

C. neoformans var. *neoformans* has a worldwide distribution and has been associated with a variety of environmental sources, in particular, bird excreta and decaying wood (Walter *et al.*, 1968; Ruiz *et al.*, 1981). The most common isolate responsible for human infection is *C. neoformans* var. *neoformans* serotype A (Ellis and D.H., 1987). Cryptococcus reported to be isolateed from AIDS patients has almost exclusively been of serotype A, even in areas where serotypes B or C are isolated more frequently in the general population (Rinaldi *et al.*, 1986). *C. neoformans* var. *gattii* has a more restricted global distribution occurring in tropical and subtropical areas (Kwon-Chung *et al.*, 1984), and plant debris associated with a number of *Eucalyptus species* (Ellis *et al.*, 1990; Pfeiffer *et al.*, 1992; Sorrell *et al.*, 1996). These trees, native to Australia, are its natural habitat (Halliday *et al.*, 1999). These trees have also been exported to other countries in the world and *C. neoformans* var. *gattii* infections are also found in these regions where the trees landed (Padhye *et al.*, 1993; Chakrabarti *et al.*, 1997). Despite the increasing number of studies on the epidemiology of *C. neoformans*, the role of these trees and the nature of infectious propagule are not well understood. However, identification of the two varieties of *C. neoformans* isolated from clinical and environmental sources provides useful information for epidmiological and ecological investigations.

In the present study, we examined serotypes of *C. neoformans* isolated from environmental and clinical sources. A total of 20 strains obtained from environmental and clinical sources are listed in Table 1. Two environmental isolates of *C. neoformans* were obtained from weathered pigeon excreta from the parks in Pusan, South Korea. To recover *C. neoformans* from pigeon excreta, approximately 2.0 g of pigeon excreta were added to 10 ml of sterilized saline. The samples were allowed to stand for

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20 min with frequent vortexing and centrifuged at 1500×g for 5 min. Aliquots of 100 μl of the supernatant were inoculated onto esculin based media (EBM) for detection of the typical brown pigmentation of *C. neoformans* (Edberg *et al.*, 1980). All plates were incubated at 30°C for 2 to 10 days. A brown colony was picked and streaked onto a Sabouraud dextrose agar (SDA) plate for identification. Eighteen clinical isolates originating from two cities, Seoul and Pusan, were identified as *C. neoformans* by hospital microbiology laboratories using standard criteria.

All isolates were examined and reconfirmed on the basis of their biochemical characteristics, such as the assimilation of sugars, using API 20C AUX system (bioMerieux, Marcy-l'Etoile, France), positive urease activity on Christensen's urea broth, inability to reduce nitrate, and the ability to grow at 37°C. Phenol oxidase activity was also determined by using DL-3,4-dihydroxylphenylalanine (DL-DOPA) as a substrate (Kabasawa *et al.*, 1991). For separation of the two varieties of *C. neoformans*, canavanine-glycine-bromthymol blue (CGB) agar and disks of D-proline were prepared and tested as described by Kwon-Chung *et al.* (1982) and Nishikawa *et al.*, (1996), respectively. A color change from light yellow-green to cobalt blue was considered a positive result for the CGB test, indicating *C. neoformans* var. *gattii* (serotype B/C). No change from light yellow-green to cobalt blue was considered negative, indicating the var. *neoformans* (serotype A/D). A strong growth around the disk was considered a positive reaction, which meant the utilization of D-proline as the sole source of nitrogen. Serotyping was performed via the Cryptocheck agglutination test (Iatron Laboratories Inc., Tokyo, Japan). The serotyped strains were evaluated with the CGB test and D-proline assimilation.

All isolates were identified as *C. neoformans* based on the results of analysis using the API 20C AUX system, their ability to grow at 37°C, and a positive reaction for phenol oxidase in both EBM and DL-DOPA media. Two environmental isolates and 17 of the 18 clinical isolates belonged to *C. neoformans* var. *neoformans* (serotype A: 16, serotype D: 1) on the basis of no color change on CGB agar and the inability to assimilate D-proline. The one exception was culturally unusual with mucous colonies on SDA plates (Fig. 1), and a positive reaction on CGB medium (Fig. 2) and D-proline test. This isolate belonged to *C. neoformans* var. *gattii* serotype B (Table

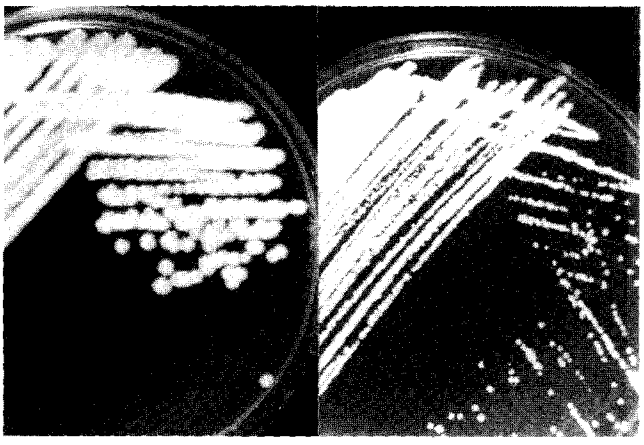

Fig. 1. *Cryptococcus neoformans* colonies grown for 5days on SDA plates. (A) CH3 strain (B) ATCC 66031

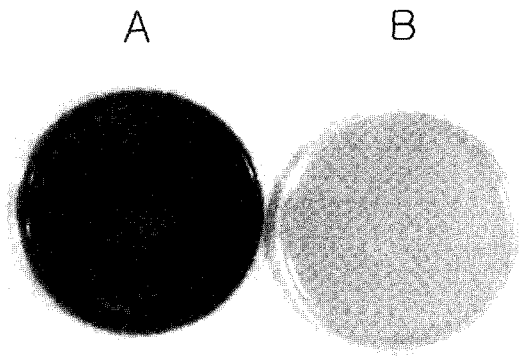

Fig. 2. Color change of *Cryptococcus neoformans* strains on CGB agar plate.Positive reaction (cobalt blue color) of *C. neoformans* var. *gattii* CH3 Strain, Negative control (light yellow-green color) of *C.neoformans* var. *neoformans* ATCC 66031

Table 1. Sources and serotypes of *C. neoformans* isolated in South Korea

Strain	Source	Year of isolation	Place of isolation	Serotype
CH 1	CSF[a]	1993	Seoul	A
CH 2	CSF	1993	Seoul	A
CH 3	CSF	1993	Pusan	B
CH 4	CSF	1993	Pusan	D
CH 5	CSF	1993	Pusan	A
CH 6	Pigeon excreta	1993	Pusan	A
CH 7	Pigeon excreta	1993	Pusan	A
CH 8	CSF	1996	Pusan	A
CH 9	CSF	1996	Pusan	A
CH10	CSF	1996	Pusan	A
CH11	CSF	1997	Pusan	A
CH12	CSF	1997	Pusan	A
CH13	CSF	1997	Seoul	A
CH14	Blood	1997	Seoul	A
CH15	CSF	1997	Seoul	A
CH16	CSF	1999	Seoul	A
CH17	CSF	1999	Seoul	A
CH18	CSF	2000	Seoul	A
CH19	CSF	2000	Seoul	A
CH20	CSF	2000	Pusan	A

[a]CSF, Cerebrospinal fluid

1). I found a complete agreement between the biochemical characteristics and serotypes of the isolates. This is the first report of *C. neoformans* var. *gattii* isolated from a Korean patient with chronic meningitis, although the origin of infection and route of transmission in this patient has yet to be determined.

Until now, there are few Korean epidemiological surveys of the serotypes of *C. neoformans*. In 1986, Kim *et.al.* (1986) studied 10 clinical isolates of *C. neoformans* from Korea and reported that all isolates were *C. neoformans* var. *neoformans* (serotype A : 7, serotype D : 3). Japanese clinical isolates were also found to be predominantly of *C. neoformans* var. *neoformans* serotype A (Kwon-Chung *et al.*, 1984; Yamamoto *et al.*, 1995). Ikeda *et al.* (1982) based on the study with 62 clinical isolates of *C. neoformans* from Japa: 58 of them were serotype A, three were serotype A-D, and one was serotype D. The study by Bennett *et. al.* (1978) revealed that isolates of *C. neoformans* var. *gattii* were infrequent causes of infection, except in southern California, while the isolates of *C. neoformans* var. *neoformans* serotype A predominant cause of infection in the United States. A high prevalence of serotype B was recognized in Thailand and Vietnam, Australia, and Brazil and Central Africa (Kwon-Chung *et al.*, 1984). Also, three of the clinical isolates from India were identified as serotype B (Padhye *et al.*, 1993). In Europe, serotype D and AD of *C. neoformans* var. *neoformans* were more prevalent than in any other regions studied (Mishra *et al.*, 1981).

Our results, although limited by the number of isolates tested, showed that *C. neoformans* var. *neoformans* serotype A was the predominant isolate from environmental and clinical sources in Korea, and the identification of *C. neoformans* var. *gattii* from a patient with chronic meningitis might have some importance in explaining the epidemiological significance of serotypes of *C. neoformans*.

Further studies will establish the molecular typing of *C. neoformans* to establish a correlation between the biological properties of the isolates and their environmental sources in Korea.

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