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Short Paper

Cross-reactivity of Five Monoclonal Antibodies to Various Isolates of *Alexandrium* as Determined by an Indirect Immunofluorescence Method

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Species within the marine dinoflagellate genus *Alexandrium* Halim (sensu Balech) have been identified on the basis of the morphological features of thecal plates.[1] Some of these features change with environmental conditions or growth stage, and others are difficult to resolve for all but taxonomic experts. It has thus become difficult to distinguish closely related species from each other, and therefore morphological criteria have been controversial.[2]

In previous studies, we used monoclonal antibodies to distinguish among Japanese strains of *A. tamarense* and *A. catenella* and between Japanese and Thai strains of *A. tamarense*.[3,4] Here we investigate the specificity of those antibodies for strains of *Alexandrium* from the United States, Spain, and Thailand by an indirect immunofluorescence method.[3]

A. catenella (BGT1), *A. tamarense* (PWO6), *A. tamarense* (GTLI21), and *A. fundyense* (GTCA29) were isolated from Russian River (California), Prince William Sound (Alaska), Moriches Bay (L. I. New York), and the Gulf of Maine (New Hampshire), respectively, in the United States which were identified by Balech.[5] *A. excavatum* (PEIV) was isolated from Ria de Vigo, Spain, by Dr. I. Bravo and identified by B. Reguera according to the morphological criterion of Balech.[5] *A. tamarense* CU-15 was isolated from the Gulf of Thailand and identified by the criterion described previously.[4] Japanese strains *A. tamarense* OFX151 and *A. catenella* OFX102 were isolated from Ofunato Bay, *A. tamarense* WKS-1 from Kushimoto, and *A. catenella* TNX22 from Tanabe Bay in Japan[3] and identified by the criterion of Fukuyo.[4] All clonal cultures were axenically prepared and maintained in SWIIm medium[3] under a 14:10 h L:D cycle at 100 μEinst. $m^{-2}S^{-1}$ at 20°C, except for OFX151 which was grown at 15°C.[3]

Monoclonal antibody M8751-1 was highly reactive to four strains of *A. tamarense* (PW06, GTLI21, OFX151, and WKS-1), and one strain each of *A. catanella* (BGT1), *A. fundyense* (GTCA29), and *A. excavatum* (PE1V). Antibodies M22-1 and MT15-1 were reactive to all of the strains tested, including *A. tamarense* from Thailand. In contrast, antibody MT15-2 only labeled the Thai strain of *A. tamarense*. MT15-4 acted similarly to MT15-2, although it also showed slight positive reactivity with *A. excavatum* (PE1V) and *A. catenella* (BGT1). These results show that four strains of three species from the United States, one of *A. excavatum* from Spain and two of *A. tamarense* from Japan were immunologically identified as belonging to the same group. This is the same general result that has been obtained several times before following biochemical analyses of strains from this species complex. Hayhome *et al.*[6] found that morphospecies designations were not consistent with biochemical markers such as isozyme banding patterns. Recent molecular studies by Scholin and Anderson[7] using ribosomal RNA sequences shed further light on this issue by demonstrating that members of the *tamarense/catenella/fundyense* species complex cluster together far more logically on the basis of their geographic origin than their morphology.

Numerous studies thus indicate that strains of three different species from North America can be more closely related to each other than two strains (CU-15 and OFX151) of the same species isolated from geographically distant locations. Our immunological results lend further support to the contention that strains from the United States of *A. catenella*, *A. fundyense*, and *A. tamarense* including Japanese strains of *A. tamarense* should not be separate species, but rather varieties of a single species (Scholin and Anderson, personal communication). Furthermore, these data argue that designation of strain or variety names might be better based on biochemical or molecular characteristics than on geographic origin or morphology such as the identification of bacteria. Antibody reactivity may well provide such a distinction in future. Theses antibodies will have high utility as general "tag" or probes for toxic and nontoxic *Alexandrium* species.

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References

1) E. Balech: in "Toxic Dinoflagellates" (ed. by D. M. Anderson, A. W. White, and D. G. Baden), Elsevier, New York, 1985, pp. 33–38.
2) F. J. R. Taylor: in "Toxic Dinoflagellates" (ed. by D. M. Anderson, A. W. White, and D. G. Baden), Elsevier, New York, 1985, pp. 11–26.
3) Y. Sako, M. Adachi, and Y. Ishida: in "Toxic Phytoplankton Blooms in the Sea" (ed. by T. J. Smayda and Y. Shimizu), Elsevier, Amsterdam, 1993, pp. 87–93.
4) M. Adachi, Y. Sako, and Y. Ishida: *Nippon Suisan Gakkaishi*, **59**, 327–332 (1993).
5) E. Balech: Serv. de. Hidr. Naval, Algent. H654, 1–103, pls. 1–12.
6) B. A. Hayhome, D. M. Anderson, D. M. Kulis, and D. J. Whitten: *Mar. Biol.*, **101**, 427–435 (1989).
7) C. Scholin and D. M. Anderson: in "Toxic Phytoplankton Blooms in the Sea" (ed. by T. J. Smayda and Y. Shimizu), Elsevier, Amsterdam, 1993, pp. 95–102.

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