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タヌキマダニ,アカコッコマダニ,ハシブトマダニからのスピロヘータの分離

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Research Note

Isolation of spirochetes from Japanese ixodid ticks, *Ixodes tanuki, Ixodes turdus,* and *Ixodes columnae**

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A recently discovered spirochete, *Borrelia burgdorferi,* has been shown to be the etiologic agent responsible for Lyme disease and related disorders. This disease is transmitted by the bite of ticks belonging to the Ixodidae. The *Ixodes ricinus* complex including *Ixodes ricinus, Ixodes dammini, Ixodes pacificus,* and *Ixodes persulcatus* is an important vector to humans in the Northern Hemisphere. A tick/spirochete survey in various localities of Japan demonstrated the high prevalence of spirochetal infection in questing adults of *I. persulcatus* and *Ixodes ovatus* (Miyamoto *et al.,* 1992; Nakao *et al.,* 1992b), but no cases of Lyme disease caused by *I. ovatus* have been demonstrated in spite of the abundance of human tick bites by this species. Several specimens of ticks recovered from patients of Lyme disease in Japan were identified as female adults of *I. persulcatus* (Kawabata *et al.,* 1987; Miyamoto *et al.,* 1990). Moreover, the protein profiles of spirochetes isolated from several patients were identical with those of the isolates from *I. persulcatus* (Nakao *et al.,* 1992a). These data indicate that the main vector of Lyme disease in Japan is *I. persulcatus.* However, we also isolated spirochetes from *Ixodes tanuki* Saito, 1964, *Ixodes turdus* Nakatsuji, 1942, and *Ixodes columnae* Takada et Fujita, 1992. We report herein the characteristics of spirochetes isolated from these rare species of ticks.

Table 1 Origins of spirochetes examined.

Name of isolate	Species of tick (stage)	Geographic location
Hk501	*Ixodes tanuki* (adult*)	Hokkaido
Iw501	*Ixodes tanuki* (adult)	Iwate
Ya501	*Ixodes turdus* (adult*)	Yamagata
Ac502	*Ixodes turdus* (adult*)	Aichi
Am501	*Ixodes columnae* (nymph)	Aomori
B31	*Ixodes dammini* (adult)	New York

* Engorged ticks collected from wild animals.

Cultivable spirochetes in BSK medium (Barbour, 1984) were isolated from midgut tissues of ticks by the methods of Miyamoto *et al.* (1992). The names and sources of spirochetal isolate are shown in Table 1. Hk501 was derived from a female of *I. tanuki* fed on a raccoon dog, *Nyctereutes procyonoides albus* captured in Tomamae, Hokkaido. Iw501 was derived from an unfed female of *I. tanuki* collected by flagging vegetation in Morioka, Iwate Prefecture. Ya501 was derived from a female of *I. turdus* fed on a bird, *Hypsipetes amaurotis* captured in Tobishima, Yamagata Prefecture. Ac502 was derived from a female of *I. turdus* fed on a bird, *Hypsipetes amaurotis* captured in Atsumi, Aichi Prefecture. Am501 was derived from an unfed nymph of *I. columnae* collected by flagging vegetation in Hachinohe, Aomori Prefecture. The North American B31 strain of *B. burgdorferi* (ATCC 35210) was used as a control.

According to the methods described previously (Nakao *et al.,* 1992b), whole cell lysates of the spirochetes were subjected to sodium dodecyl sulfate-gel electrophoresis (SDS-PAGE) and western blots. Monoclonal antibody (MAb) H9724 reactive with the periplasmic flagella of genus *Borrelia* (Barbour *et al.,* 1986) and MAb H5332 specific for the outer surface protein A (OspA) of *B. burgdorferi* (Barbour *et al.,* 1983) were used

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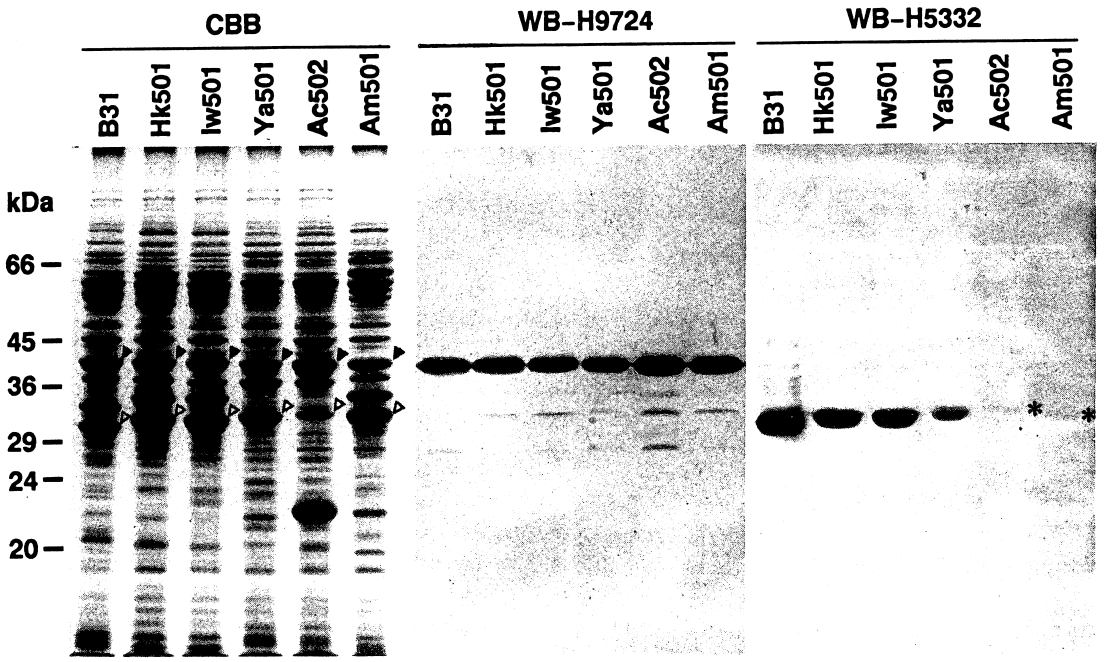

Fig. 1 Coomassie brilliant blue (CBB)-stained proteins of spirochetal isolates in a discontinuous SDS-PAGE gel (12.5%) and western blots (WB) of the isolates reacted with monoclonal antibodies H9724 and H5332.

Closed and open arrowheads indicate the 41 kDa flagellin and OspA proteins respectively. Asterisks indicate the weak reaction of western blots.

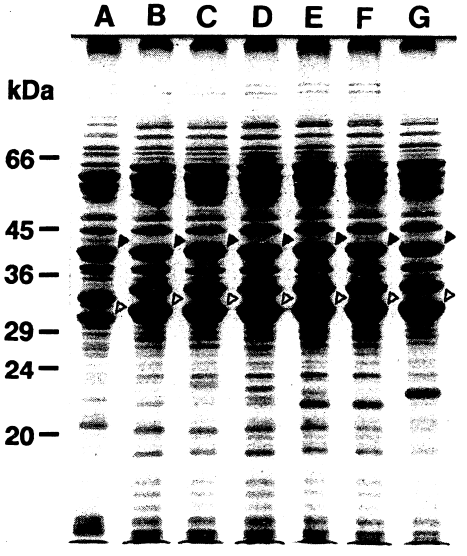

Fig. 2 Protein profile of spirochetes isolated from *Ixodes tanuki* and from rodents.

Lane A, B31 strain (control); lane B, Hk501 from *I. tanuki* in Hokkaido; lane C, Iw501 from *I. tanuki* in Iwate; lanes D–F, 3 isolates from *Clethrionomys rufocanus bedfordiae*; lane G, an isolate from *Apodemus speciosus ainu*. Closed and open arrowheads indicate the 41 kDa flagellin and OspA proteins respectively.

as probes for western blots. The immunocomplexes of MAbs and antigens on nylon membranes (NY13N, Schleicher & Schuell, U.S.A.) were detected by using peroxidase-conjugated protein A (Zymed, U.S.A.). 4-Chloro-1-naphthol was used as chromogen.

The results of SDS-PAGE and western blots are summarized in Fig. 1. None of the 5 isolates from 3 species of Japanese ticks were identical to the North American B31 strain in the comparison of protein profile. The epitope for MAb H9724 were present in the 41 kDa flagellin protein of all isolates examined in this study. Both Hk501 and Iw501 possessed the 32 kDa OspA protein reactive with MAb H5332. MAb H5332 also reacted with the 33 kDa OspA protein of Ya501. The reactivities of MAb H5332 in Ac502 and Am501 were very weak, and its epitope was found on the proteins of 33.5 kDa (Ac502) and 33 kDa (Am501). The reactivities of MAbs confirmed that the isolates examined in this study were strains of *B. burgdorferi*. The 2 isolates from *I. tanuki* (Hk501 and Iw501) were almost identical each other in their protein compositions, although these ticks were collected from different localities. No similarities were observed among the isolates from different species of ticks.

It has been generally assumed that ticks belonging to the *I. ricinus* complex are important vectors of *B. burgdorferi* to maintain the transmission cycles involving wildlife reservoirs. However, in north coastal California, a non-*I. ricinus* complex tick, *Ixodes neotomae* and woodrats, *Neotoma fuscipes* support an enzootic cycle of *B. burgdorferi*, and human-biting ticks, *I. pacificus* become to be infected with *B. burgdorferi* when its immature ticks are feeding on woodrats (Brown and Lane, 1992). In Japan, the transmission dynamics of *B. burgdorferi* in nature is probably different from that seen in California. Our previous study showed that the *I. ricinus* complex tick, *I. persulcatus* harbored the various types of *B. burgdorferi* (Nakao *et al.*, 1992b); however, the types of *B. burgdorferi* from non-*I. ricinus* complex ticks, *I. tanuki, I. turdus* and *I. columnae* examined in this study did not conform to those from *I. persulcatus* (data not shown). Moreover, other non-*I.ricinus* complex tick, *I. ovatus* were infected with a homogeneous type of *B. burgdorferi* which was peculiar to *I. ovatus* (Nakao *et al.*, 1992b). These data suggest that each tick species harbors the characteristic strain(s) of *B. burgdorferi* (possibly including the new species of *Borrelia*), and supports their own transmission cycle of spirochetes together with wildlife reservoirs. Recently, we also isolated the spirochetes from 4 rodents, 3 *Clethrionomys rufocanus bedfordiae* and 1 *Apodemus speciosus ainu* captured in Nanae, Hokkaido, which were quite similar to the isolates (Hk501 and Iw501) from *I. tanuki* (Fig. 2). These rodent isolates were established by culturing earlobe tissues in BSK medium according to the methods of Sinsky and Piesman (1989). This finding suggests that these rodent species are reservoirs susceptible to the spirochetes of *I. tanuki*.

The pathogenicity of spirochetes from *I. tanuki, I. turdus* and *I. columnae* is unknown. These vector potential to humans should be considered epidemiologically, since several cases of human tick bite by these 3 species have been confirmed in Japan (Takada, 1990; Takada and Fujita, 1992; Yoneda *et al.*, 1992).

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摘　要

タヌキマダニ，アカコッコマダニ，ハシブトマダニからのスピロヘータの分離

日本各地で採集されたタヌキマダニ，アカコッコマダニ，ハシブトマダニから5株のスピロヘータを分離培養することに成功した．これらのスピロヘータはモノクローナル抗体 (H9724, H5332) との反応性からライム病病原体 *Borrelia burgdorferi* と同定したが，シュルツェマダニやヤマトマダニが保有するスピロヘータとは菌体表層蛋白の組成が異なっていた．日本ではシュルツェマダニをライム病の主要な媒介者と考えて良い状況にあるが，他のマダニもスピロヘータを保有しているので，媒介者に関する疫学的解釈には慎重になるべきであろう．