

Geographical Heterogeneity between Far East and Europe in Prevalence of *ypm* Gene Encoding the Novel Superantigen among *Yersinia pseudotuberculosis* Strains

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***Yersinia pseudotuberculosis* is known to produce a novel superantigen designated *Y. pseudotuberculosis*-derived mitogen. In the present study, we demonstrated that there was a distinct geographical heterogeneity between the Far East (higher prevalence) and Europe (lower prevalence) in the prevalence of the *ypm* gene encoding the superantigen among *Y. pseudotuberculosis* strains.**

Yersinia pseudotuberculosis, a gram-negative enteropathogenic bacillus, causes sporadic infection in humans and is sometimes associated with epidemics (5). This organism is capable of inducing not only gastrointestinal symptoms but also a variety of systemic manifestations such as fever, scarlatiniform rash, desquamation, erythema nodosum, and arthritis (9). The clinical pathophysiology of *Y. pseudotuberculosis* infection has many similarities to infections caused by *Staphylococcus aureus* and *Streptococcus pyogenes*, which are each known to produce a superantigen (10). Therefore, it was suggested that a superantigen may also be involved in *Y. pseudotuberculosis* infection. The production by *Y. pseudotuberculosis* of a superantigen which was designated *Y. pseudotuberculosis*-derived mitogen (YPM) was independently demonstrated by two groups (1, 14). YPM was purified to homogeneity from the culture supernatant of a clinical strain of *Y. pseudotuberculosis*, and substantial structural differences between YPM and other bacterial superantigens produced by gram-positive cocci were observed (15). Recently, the gene (*ypm*) encoding YPM was cloned and sequenced (6, 8). In the present study, we examined the distribution of the *ypm* gene among several strains of *Y. pseudotuberculosis* isolated from diverse geographical areas.

A total of 204 strains of *Y. pseudotuberculosis*, which included 142 from Japan, 33 from Far Eastern Russia, 11 from Belgium, 10 from Germany, 6 from Italy, and 2 from The Netherlands, were investigated for the presence of the *ypm* gene by colony hybridization with a PCR-generated probe. The DNA probe for the *ypm* gene was labeled with [α -³²P]dCTP by PCR amplification of a 381-bp DNA fragment (bases 53 to 433) of the *ypm* gene with a primer pair. The nucleotide sequences of the two synthetic primers were 5'-CGTTGGCG ACTGATTATGAT-3' and 5'-TGACAAGGAAGATAGTG AC-3', which were based on the sequences (from 53 to 72 and 414 to 433, respectively) in the reported sequence of the *ypm*

gene (6, 8). Out of the 76 clinical strains investigated, 59 (78%) hybridized with the probe. The culture supernatant fluids of all the *ypm*⁺ strains were positive for proliferative response in human peripheral blood mononuclear cells, while none of the strains lacking *ypm* produced a mitogenic substance. The complete correlation between the presence of the *ypm* gene and the mitogenic activity clearly indicates that strains harboring the *ypm* gene produce YPM.

A geographical pattern in the prevalence of the *ypm* gene among clinical strains of *Y. pseudotuberculosis* was evident in this study. The *ypm* gene was detected in all the clinical strains from Far Eastern Russia and in 95% of the clinical strains from Japan, while only 17% of the European clinical strains harbored the *ypm* gene. A difference in the distribution of serotypes of *Y. pseudotuberculosis* between Japan and Europe has been reported (13). Most of the strains from Europe belong to the serotype O:1a, whereas no strain belonging to this serotype has been isolated in Japan. By contrast, *Y. pseudotuberculosis* strains belonging to nine serotypes (O:1b, 2a, 2b, 2c, 3, 4a, 4b, 5a, and 5b), of which serotypes O:4b and 5b are dominant, have been isolated from patients in Japan. The two serotypes dominant in Japan have never been detected in Europe (13). From Fig. 1, it is clear that the gene is not associated with serotype O:1a, which is dominant in the majority of the strains isolated from Europe. Therefore, it appears that the geographical pattern in the prevalence of the *ypm* gene is directly related to the spatial distribution of serotypes. However, all the European strains belonging to serotypes O:1b, 2a, and 2b were negative, while all the strains of the above-mentioned serotypes from Japan and Far Eastern Russia were positive. This implies that the geographical association in the prevalence of the *ypm* gene is not always dependent on the geographical differences in the serotypes. The low prevalence of the *ypm* gene in Europe appears to be related to the geographical disparity in both serotype and the *ypm* gene itself.

Next, 128 strains of *Y. pseudotuberculosis* isolated from animal and environmental sources were investigated. The geographical and serotype-related distribution of the *ypm* gene among the clinical strains was also reflected among the animal and environmental strains (Fig. 1). The prevalence of the *ypm*

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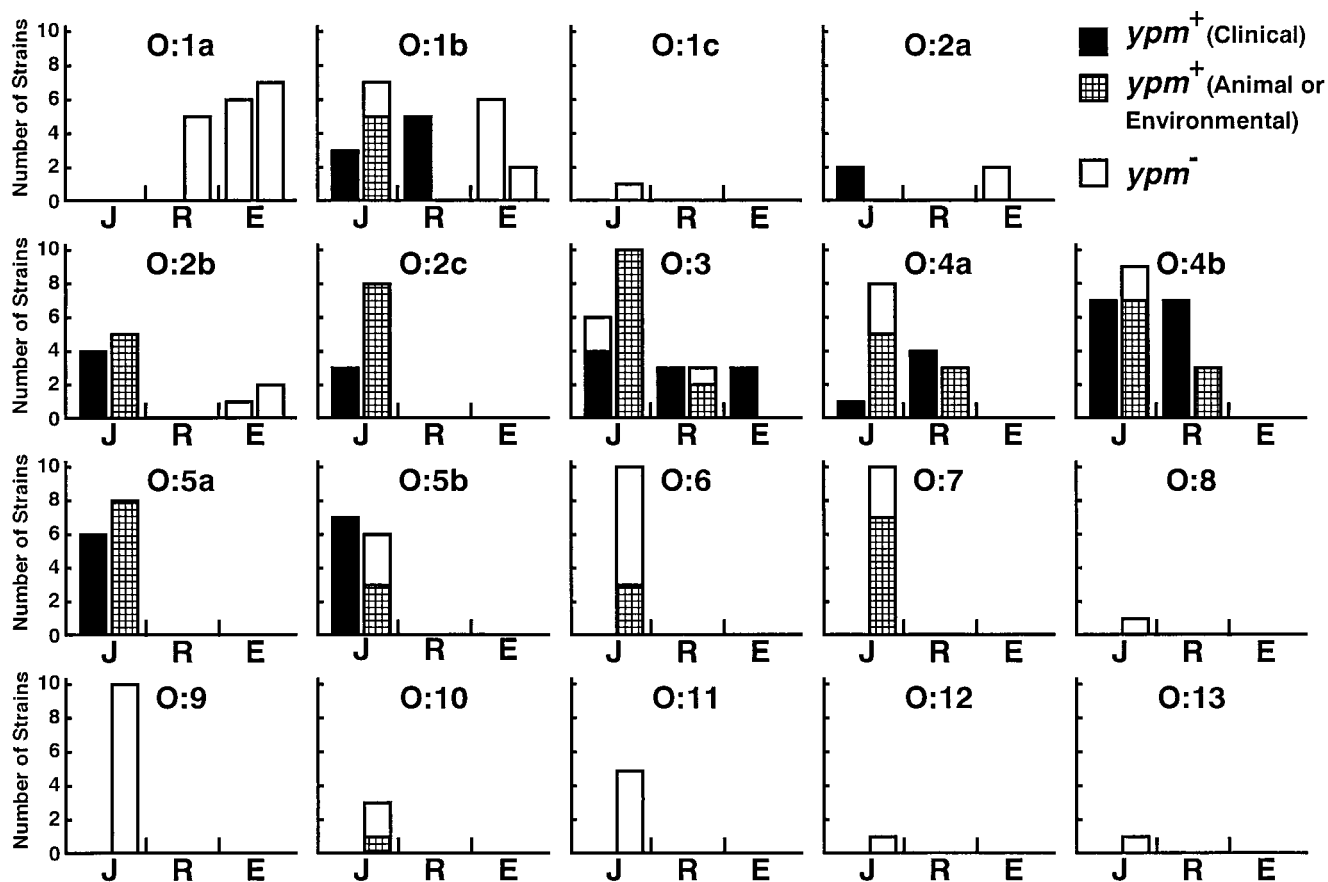


FIG. 1. Prevalence of the *ypm* gene among *Y. pseudotuberculosis* strains in relation to serotype and area of isolation. J, R, and E denote Japan, Far Eastern Russia, and Europe, respectively. The numbers of strains in each area are shown by two columns: *ypm*⁺, strain positive for *ypm* gene.

gene in strains belonging to serotypes O:1c, 6, 7, 8, 9, 10, 11, 12, and 13, which are not associated with disease, was as low as 26%. This further reiterates the role of YPM as a virulence factor of *Y. pseudotuberculosis* infection.

The main clinical manifestations of *Y. pseudotuberculosis* infection seen in Europe are fever and gastroenteric symptoms (3). By contrast, the clinical features of *Y. pseudotuberculosis* infection in Japan (5, 9), Far Eastern Russia (11), and Korea (4, 7) are usually more diverse and severe. The major differences in clinical symptoms between the Far East and Europe are rash and desquamation, which are not seen in European patients but are common in the Far East. Moreover, in Japan approximately 10% of the infected children fulfill diagnostic criteria of Kawasaki disease, including coronary vasculitis (2). Acute renal failure, mostly tubulointerstitial nephritis, is also seen in these patients (4, 12). It appears that the difference in clinical manifestations of *Y. pseudotuberculosis* infection between the Far East and Europe is related to the heterogeneity in the distribution of the *ypm* gene.

A total of 225 strains of *Yersinia enterocolitica*, mostly from Japan, were negative for the *ypm* gene, suggesting that YPM is relevant to the clinical characteristics of *Y. pseudotuberculosis* infection. The virulence factors of *Yersinia* spp., such as invasiveness, serum resistance, and antiphagocytic proteins are equally characterized for *Y. enterocolitica* and *Y. pseudotuberculosis*. However, these mechanisms cannot give an explanation of the severity of the *Y. pseudotuberculosis* infection in Far East.

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