５　学術雑誌掲載論文要旨

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An interlaboratory study on the detection method for *Escherichia albertii* in food using real time PCR assay and selective agars

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Escherichia albertii is an emerging enteropathogen. Although *E. albertii*-specific detection and isolation methods have been developed, their efficiency on food samples have not yet been systematically studied. To establish a series of effective methods for detecting *E. albertii* in food, an interlaboratory study was conducted in 11 laboratories using enrichment with modified *E. coli* broth supplemented with cefixime and tellurite (CT-mEC), realtime PCR assay, and plating on four kinds of selective agars. This study focused on the detection efficiency of an *E. albertii*-specific real-time PCR assay (EA-rtPCR) and plating on deoxycholate hydrogen sulfide lactose agar (DHL), MacConkey agar (MAC), DHL supplemented with rhamnose and xylose (RX-DHL), and MAC supplemented with rhamnose and xylose (RX-MAC). Chicken and bean sprout samples were inoculated with *E. albertii* either at 17.7 CFU/25 g (low inoculation level) or 88.5 CFU/25 g (high inoculation level), and uninoculated samples were used as controls. The sensitivity of EA-rtPCR was 1.000 for chicken and bean sprout samples inoculated with *E. albertii* at low and high inoculation levels. The Ct values of bean sprout samples were higher than those of the chicken samples. Analysis of microbial distribution by 16S rRNA gene amplicon sequencing in enriched cultures of bean sprout samples showed that approximately >96 % of the population comprised unidentified genus of family *Enterobacteriaceae* and genus *Acinetobacter* in samples which *E. albertii* was not isolated. The sensitivity of the plating methods for chicken and bean sprout samples inoculated with a high inoculation level of *E. albertii* was 1.000 and 0.848–0.970, respectively. The sensitivity of the plating methods for chicken and bean sprout samples inoculated with a low inoculation level of *E. albertii* was 0.939–1.000 and 0.515–0.727, respectively. The *E. albertii*-positive rate in all colonies isolated in this study was 89–90 % in RX-DHL and RXMAC, and 64 and 44 % in DHL and MAC, respectively. Therefore, the sensitivity of RX-supplemented agar was higher than that of the agars without these sugars. Using a combination of enrichment in CT-mEC and *E. albertii* isolation on selective agars supplemented with RX, *E. albertii* at an inoculation level of over 17.5 CFU/25 g of food was detected with a sensitivity of 1.000 and 0.667–0.727 in chicken and bean sprouts, respectively. Therefore, screening for *E. albertii*-specific genes using EA-rtPCR followed by isolation with RX-DHL or RX-MAC is an efficient method for *E. albertii* detection in food.

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食品および環境水からの *Escherichia albertii* 分離法の検討および分離株の解析

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Escherichia albertii is an emerging enteropathogen and its distribution in various foods and environmental samples has been reported in many regions around the world. In this study, we aimed to identify effective isolation and detection methods for *E. albertii* in various foods and environmental water samples. *E. albertii*-specific polymerase chain reaction (PCR) was positive in chicken, oyster, river water, and wastewater samples, and *E. albertii* was isolated from these PCR-positive samples except the wastewater sample. *E. albertii* was not isolated from any of the samples without screening PCR; therefore, PCR is useful for the detection and isolation of *E. albertii* in foods and environmental water samples. The effect of two-step enrichment with four kinds of selective enrichment broth was compared with cycle threshold (Ct) values of the *E. albertii*-specific real-time PCR assay and the isolation results. The Ct values in three out of five samples were lower in the second enriched culture than those of the first enriched culture, and *E. albertii* was isolated from enriched cultures showed Ct values <25. These results suggest that the population of *E. albertii* in these three samples increased in the second enriched culture compared with the first enriched culture, and isolating *E. albertii* from an enriched culture showing Ct values <25 is an efficient method. Genetic analysis was performed to *E. albertii* isolates from food, environmental water, and human fecal samples, and all the isolates possessed *eae*, and isolates from chicken, pork, and river water samples showed the same EAOg type as *E. albertii* isolated from human fecal samples. Therefore, it was suggested that a continuous attention should be paid to *E. albertii* in food and environment.