

## PCR Detection of Virulence Genes in *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* and Investigation of Virulence Gene Distribution

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PCR-based assays were developed for the detection of plasmid- and chromosome-borne virulence genes in *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*, to investigate the distribution of these genes in isolates from various sources. The results of PCR genotyping, based on 5 virulence-associated genes of 140 strains of *Y. enterocolitica*, were compared to phenotypic tests, such as biotyping and serotyping, and to virulence plasmid-associated properties such as calcium-dependent growth at 37°C and Congo red uptake. The specificity of the PCR results was validated by hybridization. Genotyping data correlated well with biotype data, and most biotypes resulted in (nearly) homogeneous genotypes for the chromosomal virulence genes (*ystA*, *ystB*, and *ail*); however, plasmid-borne genes (*yadA* and *virF*) were detected with variable efficiency, due to heterogeneity within the bacterial population for the presence of the virulence plasmid. Of the virulence genes, only *ystB* was present in biotype 1A; however, within this biotype, pathogenic and apathogenic isolates could not be distinguished based on the detection of virulence genes. Forty *Y. pseudotuberculosis* isolates were tested by PCR for the presence of *inv*, *yadA*, and *lcrF*. All isolates were *inv* positive, and 88% of the isolates contained the virulence plasmid genes *yadA* and *lcrF*. In conclusion, this study shows that genotyping of *Yersinia* spp., based on both chromosome- and plasmid-borne virulence genes, is feasible and informative and can provide a rapid and reliable genotypic characterization of field isolates.

*Yersinia enterocolitica* and *Y. pseudotuberculosis*, both members of the family *Enterobacteriaceae*, are comprised of strains with different degrees of pathogenicity. Both pathogenic and nonpathogenic strains are frequently isolated from various animals (birds, mammals, and reptiles) as well as from the environment (water and soil). Rodents (mice and rats), hares, rabbits, and birds serve as reservoirs for *Y. pseudotuberculosis* (1). Pathogenic strains of *Y. enterocolitica* and *Y. pseudotuberculosis* are frequently present in pigs without normally causing disease in these animals. Other food-producing animals, such as cattle, harbor mostly nonpathogenic strains of *Y. enterocolitica*.

In humans, *Y. enterocolitica* and *Y. pseudotuberculosis* are well-known food-borne pathogens and are mainly transmitted through ingestion of contaminated pork, milk, or water. Yersiniosis frequently occurs in young children as enterocolitis with fever, diarrhea, and abdominal cramps. Although the disease is usually self-limiting, complications (e.g., septicemia) are not uncommon in immunocompromised hosts. Furthermore, sequelae, such as reactive arthritis, have been reported (21).

The identification and further typing of subspecies, aiming at recognition of pathogenic strains of *Yersinia* spp., are traditionally based on phenotypic tests. *Y. enterocolitica* can be classified into biotype 1A, generally regarded as nonpathogenic (9), and the pathogenic biotypes 1B, 2, 3, 4, and 5. Both species can also

be divided into serotypes with predictive values for pathogenicity. Serological and biochemical classification, however, are time consuming and are not generally available in routine laboratories. Alternative phenotypical tests, such as calcium-dependent growth at 37°C, Congo red binding (26), pyrazinamidase testing (16), autoagglutination testing, and serum resistance testing (2, 4, 5, 6, 13, 28) all have limited predictive value for the pathogenicity of *Y. enterocolitica* and *Y. pseudotuberculosis*. The tests are frequently ambiguous to read, and their outcome may be unreliable, since they depend on the presence and expression of (plasmid-borne) virulence genes and the virulence plasmid pYV can easily be lost depending on the culture conditions. Therefore, differentiation of pathogenic strains should not rely solely on the expression or detection of the virulence plasmid but also on the detection of chromosomal virulence factors.

The aim of this study was to develop PCR assays for the detection of plasmid- and chromosome-borne virulence genes of *Y. pseudotuberculosis* and *Y. enterocolitica*. The obtained results were compared to classical phenotypic subtyping methods. The presence or absence of various virulence genes was compared in strains isolated from human patients, food, (food-producing) animals, and the environment. The following chromosomal virulence genes were included in the analysis: *ail*, the *Y. enterocolitica* attachment invasion locus gene, reported to be present in pathogenic strains only (22, 23); *ystA*, which is responsible for the production of a heat-stable enterotoxin in *Y. enterocolitica* (12); *ystB*, which has been observed to encode an enterotoxin present mainly in biotype 1A strains of *Y. enterocolitica* (27, 29, 33); and *inv*, which is present in pathogenic *Y.*

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