The expression of csdA, encoding an RNA helicase, was induced at 3°C in *Yersinia pseudotuberculosis*. The role of CsdA in *Y. pseudotuberculosis* under cold conditions was confirmed by impaired growth of insertional csdA mutants at 3°C. The results suggest that CsdA is crucial for *Y. pseudotuberculosis* survival in the chilled food chain.

*Yersinia pseudotuberculosis* is an enteropathogen able to grow at low temperatures (25). Recently, infections have been linked to contaminated fresh produce stored at low temperatures (14, 23, 30). Chilled storage gives *Y. pseudotuberculosis* a competitive advantage over many bacteria (8), but only a few studies on cold tolerance mechanisms of *Y. pseudotuberculosis* have been published (24, 25).

The deaD encoding a member of the highly conserved DEAD-box (asp-glu-ala-asp) protein family (18) was previously demonstrated to complement a mutation in rpsB encoding ribosomal protein S2 in *Escherichia coli* (31). Moreover, DeaD stabilizes mRNAs without ribosomes in this bacterium (12). DeaD was later renamed CsdA (cold shock dead-box protein), because it was found to be induced in *E. coli* under cold conditions (15). At low temperatures, CsdA facilitates translation initiation (15, 19) and contributes to the degradation of mRNAs in *E. coli* (17, 28, 33). It is also required for the biogenesis of 50S ribosomal subunits at low temperatures (7, 27) or at 37°C (27) during the early exponential growth phase. Furthermore, at low temperatures CsdA is needed in *E. coli* for the synthesis of stationary-phase sigma factor RpoS (29). The role of CsdA in enteropathogenic *Yersinia* is unknown.

Expression of csdA at 3°C relative to its expression at 28°C in *Y. pseudotuberculosis* IP32953 (gratefully received from Elisabeth Carniel, Institut Pasteur, Paris, France) was investigated using quantitative real-time reverse transcription-PCR as described previously (24). The relative expression level of csdA in the early logarithmic growth phase at 3°C was 9.4-fold higher (*P < 0.001; Student’s t test*) than the expression level at 28°C, suggesting that elevated csdA transcript levels are needed for *Y. pseudotuberculosis* growth at low temperature. To further investigate the role of CsdA at low temperatures, we constructed three insertional mutations as reported earlier (24). A search for domains of csdA (YPTB0486) was performed with the InterProScan tool (6, 11). The Pfam domains were PF00270 (DEAD/DEAH-box helicase; residues 32 to 196), PF00271 (helicase-conserved C-terminal domain; residues 266 to 342), PF03880 (DbpA RNA binding domain; residues 503 to 573), and PF12343 (cold shock protein DEAD-box A; residues 591 to 664). The TargeTron (Sigma-Aldrich Co., St. Louis, MO)–based mutations were targeted to the DEAD/DEAH-box helicase domain (PF00270) between bases 483 and 484 (csdA483–484::Ltr Kan’ mutant; referred to as csdA483 here) or to the DbpA RNA binding domain (PF03880) between bases 1548 and 1549 (csdA1548–1549::Ltr Kan’ mutant; referred to as csdA1548 here) or 1578 and 1579 (csdA1578–1579::Ltr Kan’ mutant; referred to as csdA1578 here). The primers are listed in Table 1. Single-intron insertion in the mutant genomes was confirmed by Southern blotting (24) (Fig. 1). Growth experiments were performed with the wild-type strain and the mutants at 3°C and 28°C as described previously (24). The values of optical density at 600 nm (OD600) for the wild-type and mutant strains correlated with the numbers of viable bacteria.

The completely abolished growth of csdA483 and the severely impaired growth of csdA1548 and csdA1578 at 3°C (Fig. 2A) indicate that CsdA, particularly its DEAD-box helicase domain, is indispensable for the growth of *Y. pseudotuberculosis* IP32953 at low temperatures. At the optimal growth temperature of 28°C, mutations had no effect on growth of *Y. pseudotuberculosis* (Fig. 2B), which is in line with findings for *E. coli* in which CsdA was found to be dispensable at 37°C (1, 7, 15, 32). The insertion of the group II intron into the *Y. pseudotuberculosis* csdA in both sense (csdA1548) and antisense (csdA483 and csdA1578) orientations hindered growth; thus, the observed growth defect was considered to be due to lack of functional CsdA and not to polar effects resulting from expression of the strong kanamycin promoter in the intron. CsdA was previously reported to be essential for optimal growth at 25°C or below in mesophilic *E. coli*, indicating an important function of CsdA at low temperatures (1, 7, 15, 32). In *Bacillus cereus*, transposon insertion into the 5′ untranslated region of cshA (BC0259) encoding a CsdA homolog resulted in impaired growth at 10°C (5), while deletion of the entire BC0259 abolished growth at 10°C (26).

During construction of the mutant, the virulence plasmid (pYV) was lost from csdA483, and despite several attempts, pYV could not be retained in csdA483. However, the other mutants, csdA1548 and csdA1578, kept their pYV. Frequent loss of the pYV in *Y. enterocolitica* has been previously reported (4). However, the loss of this plasmid is not expected to have distorted the results, since pYV-negative strains generally grow faster than pYV-positive strains and the difference is pronounced at low temperatures (9).

An important role of CsdA at low temperatures was further confirmed by successful complementation of the csdA mutation.
TABLE 1 Primers used in the study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence (5′→3′)</th>
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<tr>
<td>cdA-RT-qPCR-left</td>
<td>GTGATGTGAGTCGACGGAGAG</td>
</tr>
<tr>
<td>cdA-RT-qPCR-right</td>
<td>AGCATGCAAGTGGGACAGAA</td>
</tr>
<tr>
<td>16S-RT-qPCR-left</td>
<td>GCCTGGTCAGGAAATCTGGG</td>
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<tr>
<td>16S-RT-qPCR-right</td>
<td>TATGTGGTCGAGCCTGCTTC</td>
</tr>
<tr>
<td>cdA483-484-IBS</td>
<td>AAAAAAACCTTAATTATCTTGTGAGCTGCCGCGCCCGAGATAGGGTG</td>
</tr>
<tr>
<td>cdA483-484-IBS1d</td>
<td>CAGATTGTAGAAATGTTGCTGAAACATACCTTGTTTCTTTGTTG</td>
</tr>
<tr>
<td>cdA483-484-EBS2</td>
<td>TGAAGGCAAGTTTCATATTCTTGTGAGGCTGCTGAGTAg</td>
</tr>
<tr>
<td>EBS universal</td>
<td>TAAATCGACTGACTATAGG</td>
</tr>
<tr>
<td>T7</td>
<td>TTGTTTGGTGATACCCGGCTT</td>
</tr>
<tr>
<td>cdA483-484-flank-left</td>
<td>AGAGAAGACCGCGGTCTGCTT</td>
</tr>
<tr>
<td>cdA483-484-flank-right</td>
<td>AAAAAAACCTTAATTATCTTGTGAGCTGCCGCGCCCGAGATAGGGTG</td>
</tr>
<tr>
<td>cdA1548-1549-IBS</td>
<td>CAGATTGTAGAAATGTTGCTGAAACATACCTTGTTTCTTTGTTG</td>
</tr>
<tr>
<td>cdA1548-1549-EBSS1d</td>
<td>TGAAGGCAAGTTTCATATTCTTGTGAGGCTGCTGAGTAg</td>
</tr>
<tr>
<td>cdA1578-1579-IBS</td>
<td>TGAAGGCAAGTTTCATATTCTTGTGAGGCTGCTGAGTAg</td>
</tr>
<tr>
<td>cdA-flank-left</td>
<td>TCTCTGGTGATACCCGGCTT</td>
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<td>cdA-flank-right</td>
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<tr>
<td>cdA5</td>
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</tr>
<tr>
<td>RP</td>
<td>TTTCACAGGAAACAGCCTATAGC</td>
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</table>

CDsA and its putative native promoter, as predicted by BPROM (softberry, Inc., Mount Kisco, NY), were amplified by PCR (Table 1) and ligated to the high-copy-number plasmid pBluescript II KS+ (here called pBluescript) (Stratagene, La Jolla, CA), resulting in pBluescript-cdsA. The complemented mutants showed enhanced growth at 3°C compared to the vector-only controls, although the phenotype of the wild-type strain was not fully restored (Fig. 2C to E). However, as the complemented strains continued to grow throughout the experiment, loss of the complementation plasmid was very unlikely. Introduction of pBluescript-cdsA into the wild-type strain did not influence growth (Fig. 2F).

To investigate the possible role of cdsA in freeze-thaw tolerance, duplicate aliquots of overnight cultures of the IP32953 wild-type strain and the mutants were frozen to −20°C. Cryovials were thawed and refrozen 15 times at 24-h intervals by incubation for 10 min in a 25°C water bath and freezing again to −20°C. The numbers of CFU per milliliter were determined before and upon freezing at 72-h intervals. The average numbers of viable cells of the wild-type, cdsA1548, and cdsA1578 strains decreased from 108 to 102 and those of the cdsA483 decreased from 108 to 101 (P < 0.05; Student’s t test). Thus, mutations in cdsA did not affect the freeze-thaw tolerance of Y. pseudotuberculosis. This is in line with a previous study showing that a mutation in an RNA helicase gene prevented growth of Listeria monocytogenes at low temperatures but had no effect on freeze-thaw tolerance (2).

The maximum and minimum growth temperatures of the IP32953 wild-type and mutant strains were determined using a Gradipate W10 temperature gradient incubator (BCDE Group, Helsinki, Finland) under aerobic conditions as described previously (10). Strains IP32953, cdsA1548, and cdsA1578 grew throughout the temperature gradient (1.7 to 6.3°C), indicating that the minimum growth temperatures of these strains are lower than 1.7°C. The minimum growth temperature of cdsA483 was 5.6°C and was thus significantly higher (P < 0.05, Student’s t test) than that of the wild-type strain. The maximum growth temperatures of IP32953, cdsA483, cdsA1548, and cdsA1578 were 43.0°C, 43.7°C, 43.2°C, and 43.2°C, respectively, indicating no significant differences between strains.

Apparently, the main function of cdsA in Y. pseudotuberculosis occurs at a low temperature. In E. coli, the main role of CsdA at a

![FIG 1 Southern blots to demonstrate single insertions. Single bands of approximately 11.5 kb in size indicate single-intron insertion in mutants cdA483, cdsA1548, and cdsA1578. Linear vector pACD4K-C served as a positive control. Lanes: M, molecular weight marker; 1, pACD4K-C; 2, wild type; 3, cdA483; 4, cdA1548; 5, cdsA1578.](image)
FIG 2 (A and B) Growth curves and growth rates (GR; expressed in OD units per hour [3]) of the Yersinia pseudotuberculosis IP32953 wild-type strain and csdA mutants at 3°C (A) and at 28°C (B). (C to F) Data represent growth of the mutants, complemented mutants, vector-only controls, and the wild-type strain at 3°C. In the graphs in panels A and C to F, measured values are shown at 20-h intervals; in panel B, measured values are shown at 1-h intervals. Error bars indicate minimum and maximum values.
low temperature was proposed to relate to its helicase function (1, 13, 32) and mRNA decay (1, 33). Also, cold-shocked Y. enterocolitica cells refused to resume growth until cold shock protein mRNAs were degraded (21, 22). Whether CsdA is responsible for cold shock protein mRNA degradation at low temperatures in Y. pseudotuberculosis warrants further investigation.

ACKNOWLEDGMENTS

This work was supported by the Finnish Center of Excellence in Microbial Food Safety Research, Academy of Finland (grants 118602 and 141140), and the Medical Fund of the University of Helsinki. The Doctoral Program of the Faculty of Veterinary Medicine of the University of Helsinki, the Finnish Veterinary Foundation, the Walter Ehrström Foundation, and the Ehrström Foundation, and the Medical Fund of the University of Helsinki.

We thank Erika Pitkänen, Kirsi Ristkari, and Heimo Tasanen for technical assistance.

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