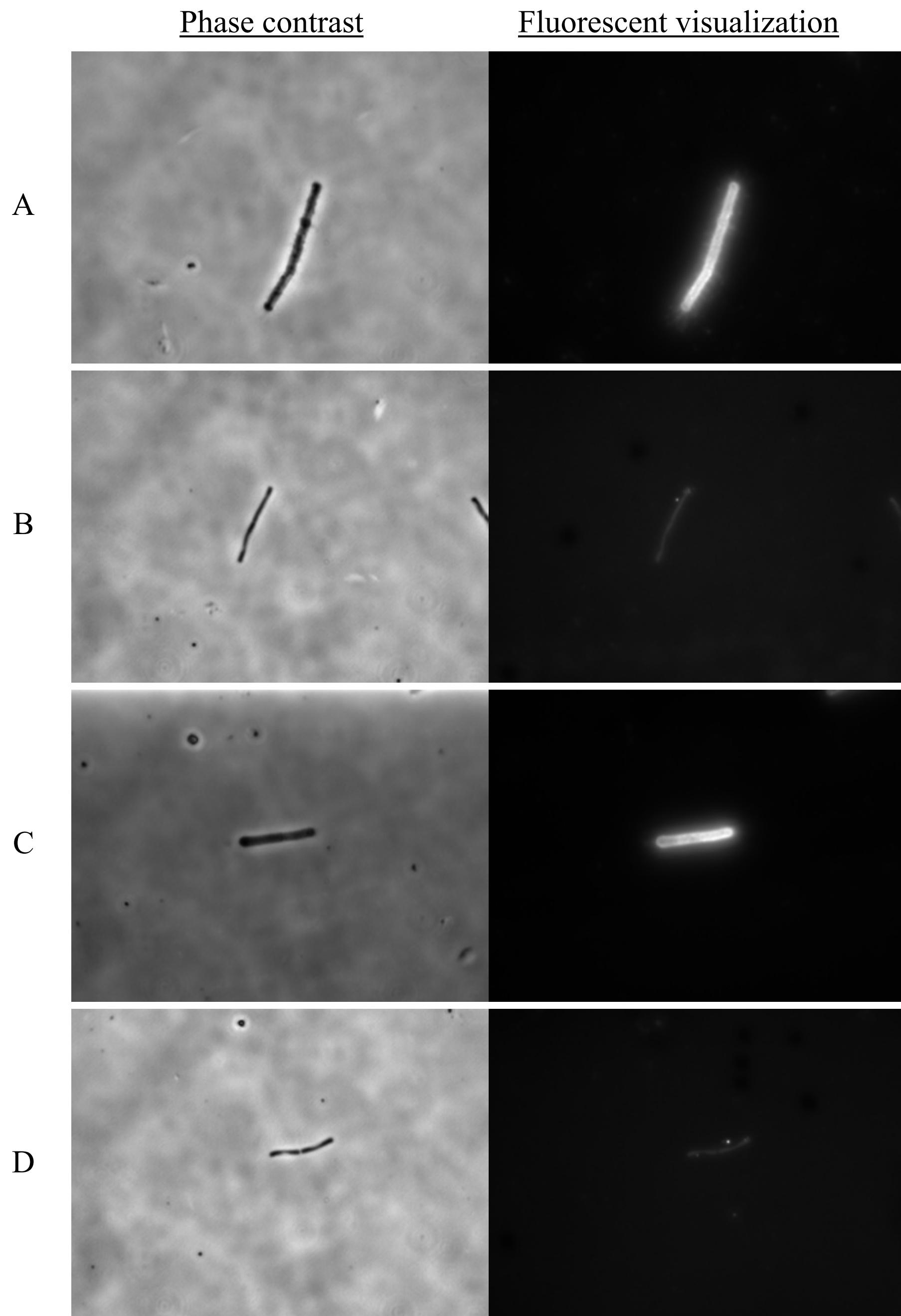

Complementation of the capBCAD-null mutant phenotype

To clone the *capBCAD* operon, we amplified *B. anthracis* DNA sequences extending from 906 nts upstream of the translational start codon of *capB* to 49 nts downstream of the translational stop codon of *capD* using the PCR with the following primers: 5'-ATTGCGGCAGCTAAAAAGAA-3' and 5'-ATCGGCATAATCCATCCAAA-3'. The

Bacillus replication origin from pHT304 (Arantes et al., 1991) was amplified using primers 5-CTGCAGTCGACCCGGGAGAGTGAGTTTTATGTCGC-3' and 5'-ATCGTGGTGTTCAAAAACGC-3'. The amplified DNA fragments and the kanamycin-resistance cassette from pUTE618 (Chen et al., 2004) were cloned into pGEM-T (Promega) to generate pUTE684. The replication origin and kanamycin-resistance cassette without the *capBCAD* sequence were cloned into pGEM-T for the vector control. The plasmids were electroporated into *B. anthracis* as described previously (Koehler et al., 1994)

The figure shows the capsule phenotypes of (A) parent strain UM23C1-1td10, (B) the *capBCAD*-null mutant, (C) the mutant complemented with *capBCAD* cloned *in trans* on a plasmid vector, and (D) the mutant containing the cloning vector without *capBCAD* (negative control). Cultures were grown on nutrient broth yeast agar (Green et al., 1985) supplemented with 0.8% (wt/vol) sodium bicarbonate at 37 °C under 20% CO_2 atmosphere. Immunofluorescence staining of *B. anthracis* cells was carried out as described previously (Xu et al., 2004) using a mouse monoclonal antibody against *B. anthracis* capsule(Kozel et al., 2004). Cells of the parent and complemented strains appeared larger and were more highly fluorescent than those of the *capBCAD*-null mutant and the *capBCAD*-null mutant containing the vector only. These data indicate that deletion of *capBCAD* abolishes the ability of *B. anthracis* to produce capsule material and a plasmid borne *capBCAD* restores the ability of the *cap*-null mutant to synthesize capsule.