AcmD, a homolog of the major autolysin AcmA of Lactococcus lactis, binds to the cell wall and contributes to cell separation and autolysis

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Published in:
PLoS ONE

DOI:
10.1371/journal.pone.0072167

Publication date:
2013

Supporting Information

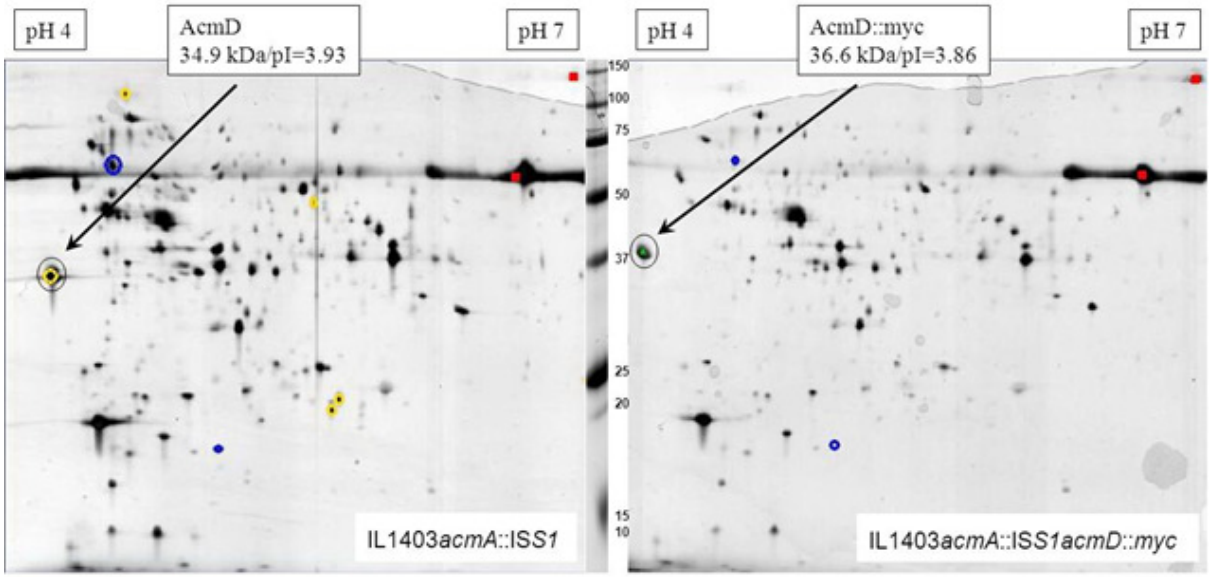

Figure S1.

Comparison of 2D-gel images of supernatant fractions of *L. lactis* IL1403*acmA*::IS*S1* and *L. lactis* IL1403*acmA*::IS*S1acmD*::myc. The amount of protein loaded in both cases was the equivalent of supernatant fraction of 100 ml of a GM17 culture with an optical density at 600 nm of 1.0. The position of the spots of the AcmD and AcmD::myc proteins, identified by Mass-spectroscopic analysis, and their molecular weights and pIs are indicated. Proteins that were more abundant in the supernatant fraction of IL1403*acmA*::IS*S1* (blue) or IL1403*acmA*::IS*S1acmD*::myc (red), and those unique in the supernatant of IL1403*acmA*::IS*S1* (yellow) or IL1403*acmA*::IS*S1acmD*::myc (green) are indicated. Sizes of the pre-stained molecular mass marker (kDa) are indicated in the middle.

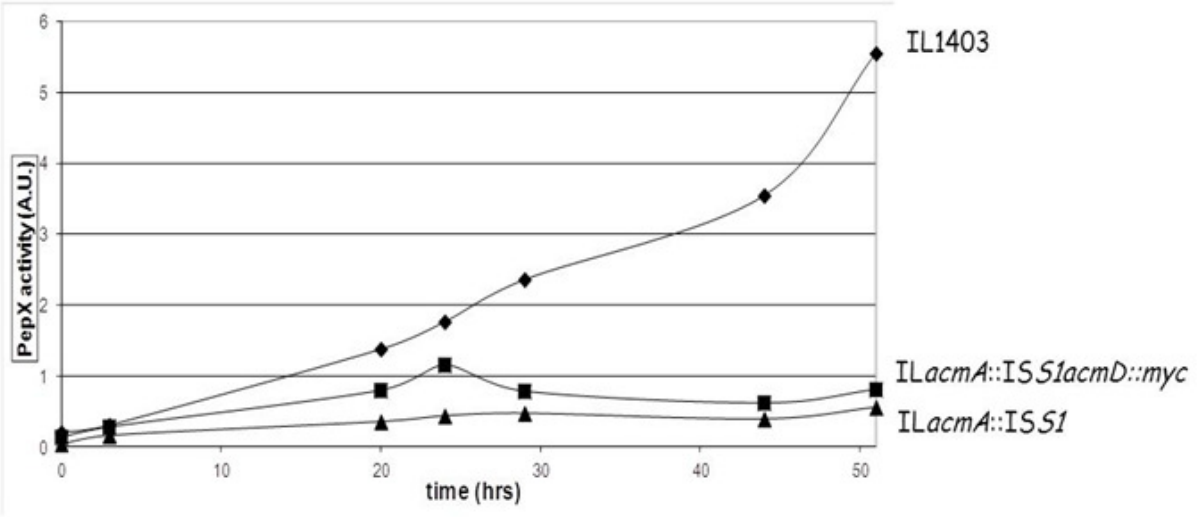

Figure S2.

Deletion of *acmD* does not affect cell lysis during growth. Release of intracellular X-prolyl dipeptidyl aminopeptidase (PepX) from *L. lactis* IL1403 (♦), IL1403*acmA*::IS*S1* (▲) and IL1403*acmA*::IS*S1acm*D::*myc* (■). Samples were taken at the indicated time points from the bacterial cultures incubated in GM17 broth. Upon removal of the cells by centrifugation the PepX-activity (in arbitrary units) released into the medium due to autolysis was determined using a chromogenic substrate, as described in the Materials and Methods section

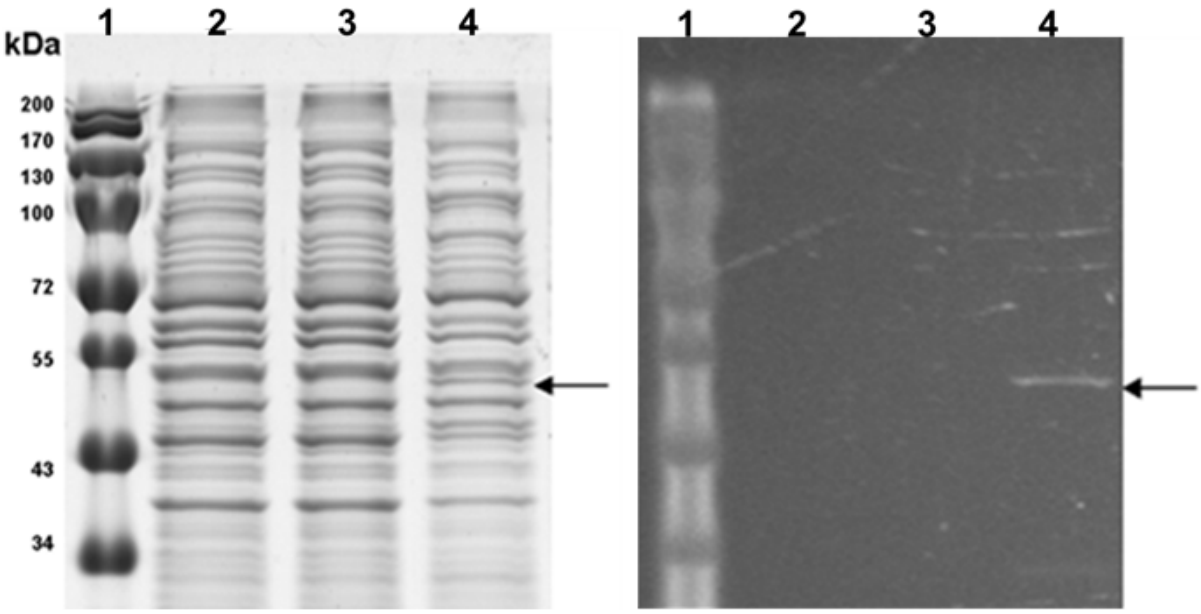

Figure S3.

Expression of $LysM_{AcmD}$-GFP-His_{10} in *E. coli*. Coomassie brilliant blue-stained SDS- (15%) PAA-gel (left) and *in-gel* GFP- fluorescence (right) showing the expression of $LysM_{AcmD}$-GFP-His_{10} on SDS- PAGE with a 15% PAA gel. *E. coli* MC1061 bearing the pBADcLIC-$LysM_{AcmD}$ was grown at 37° C until OD_{600} of 0.8 and induced with 0.2% arabinose for 2 h (see Materials and Methods section). The cell extracts of control and test samples were loaded on PAA gel for the identification of specific protein band. For the latter figure, the PAA gel is exposed to UV-light prior to coomassie staining for imaging the fluorescent bands. Prestained protein marker lane 1, cell extracts of empty vector control strain, un-induced control and 0.2%-arabinose induced test samples in lanes 2, 3 and 4, respectively. Arrows indicate $LysM_{AcmD}$-GFP-His_{10} protein/activity bands.

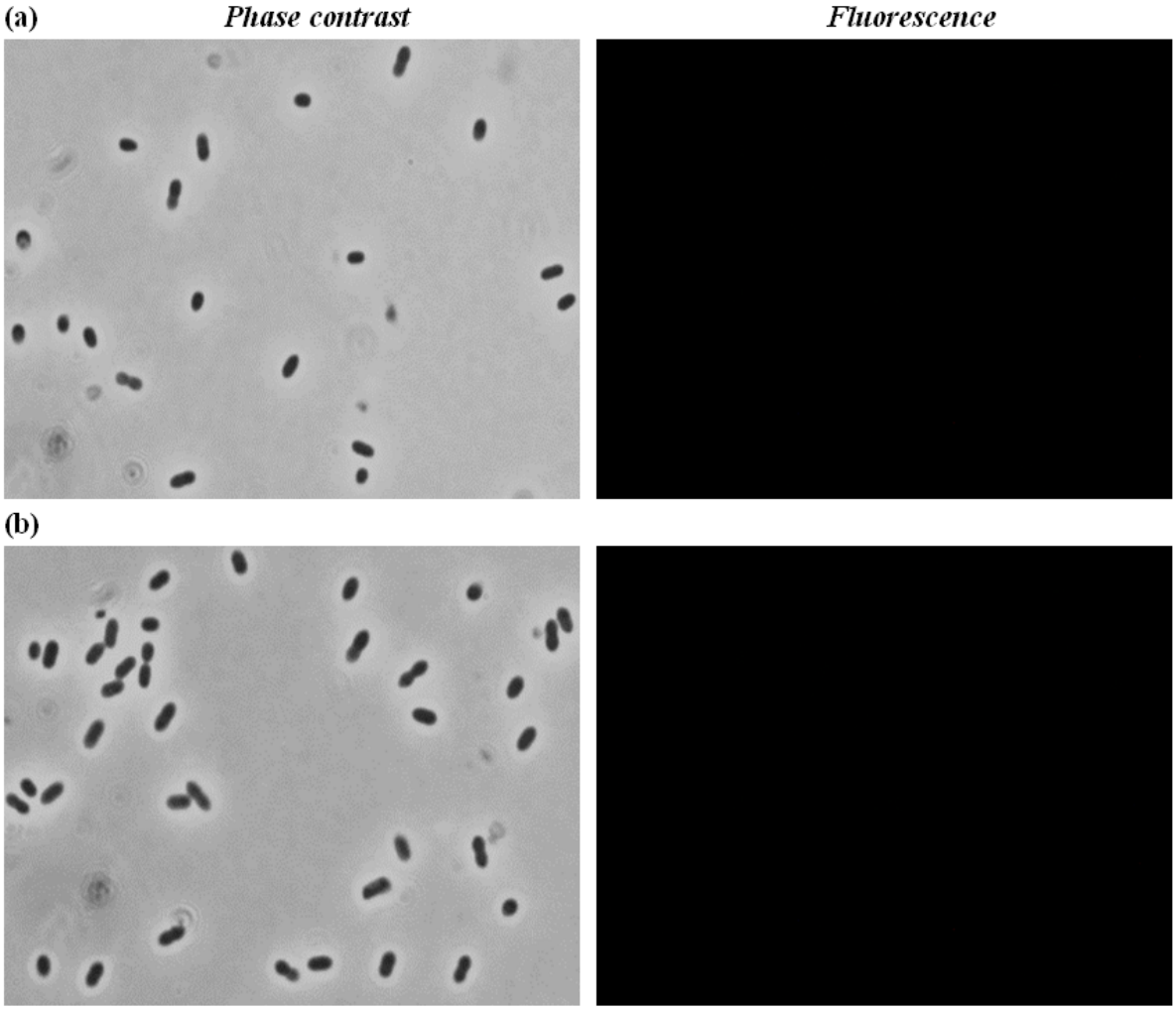

Figure S4.

Negative and autofluorescence controls Phase-contrast and fluorescence microscopy of *L. lactis* NZ9000 cells incubated at pH 4.0 with HIC-purified GFP (**a**) and without addition of any recombinant protein (**b**). Original magnification: 1250-fold in all frames.

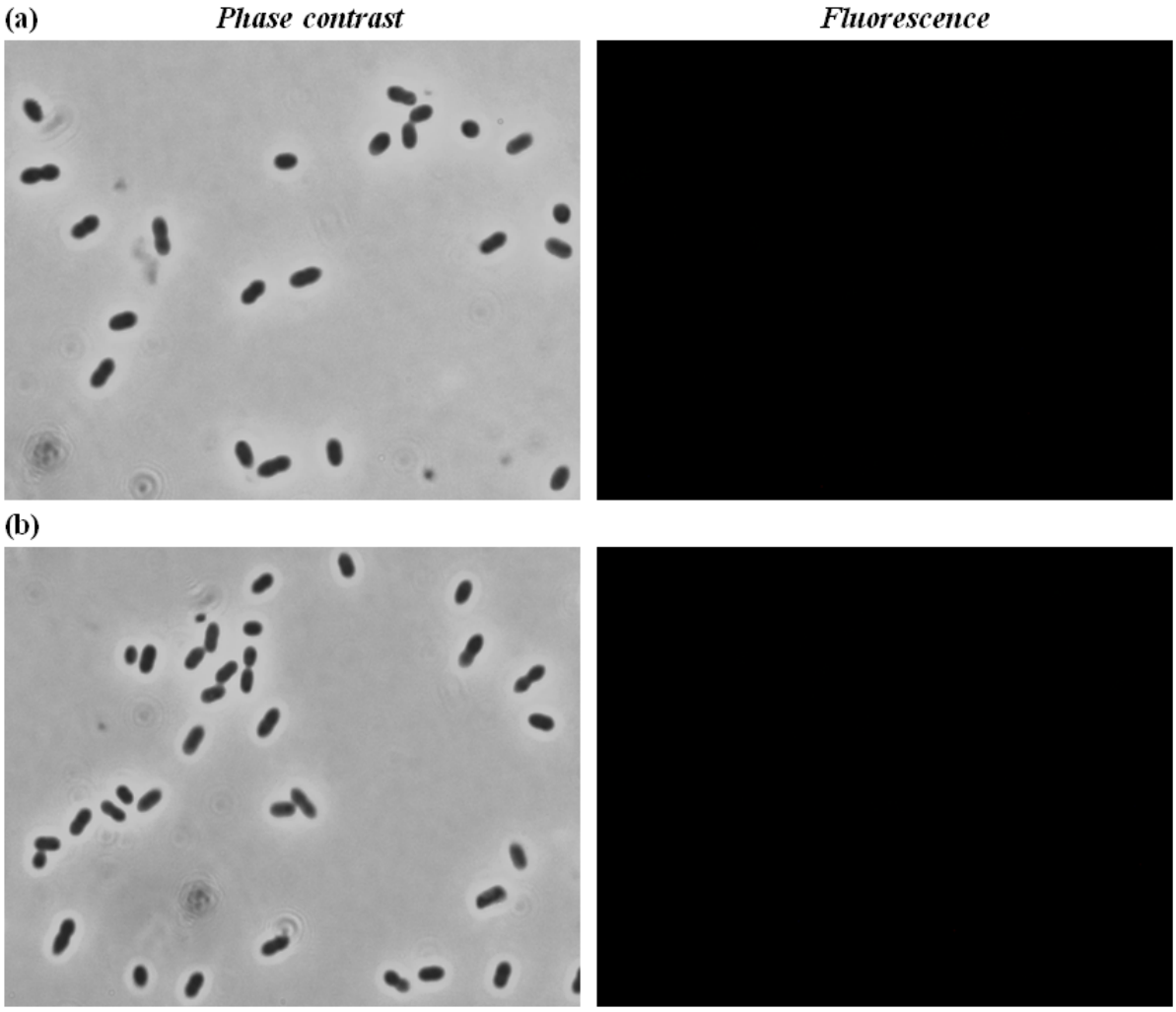

Figure S5.

Binding of $LysM_{AcmD}$-GFP-His_{10} to *L. lactis* NZ9000 cells at pH 6.0 and 8.0. Phase-contrast and fluorescence microscopy of *L. lactis* NZ9000 cells incubated with $LysM_{AcmD}$-GFP-His_{10} at pH 6.0 (**a**) and 8.0 (**b**). Original magnification: 1250-fold in all frames.