

Peculiarities of the interaction of the bacteriolytic protease B1p from *Lysobacter capsici* XL1 with the cell wall of *Staphylococcus aureus* 209P

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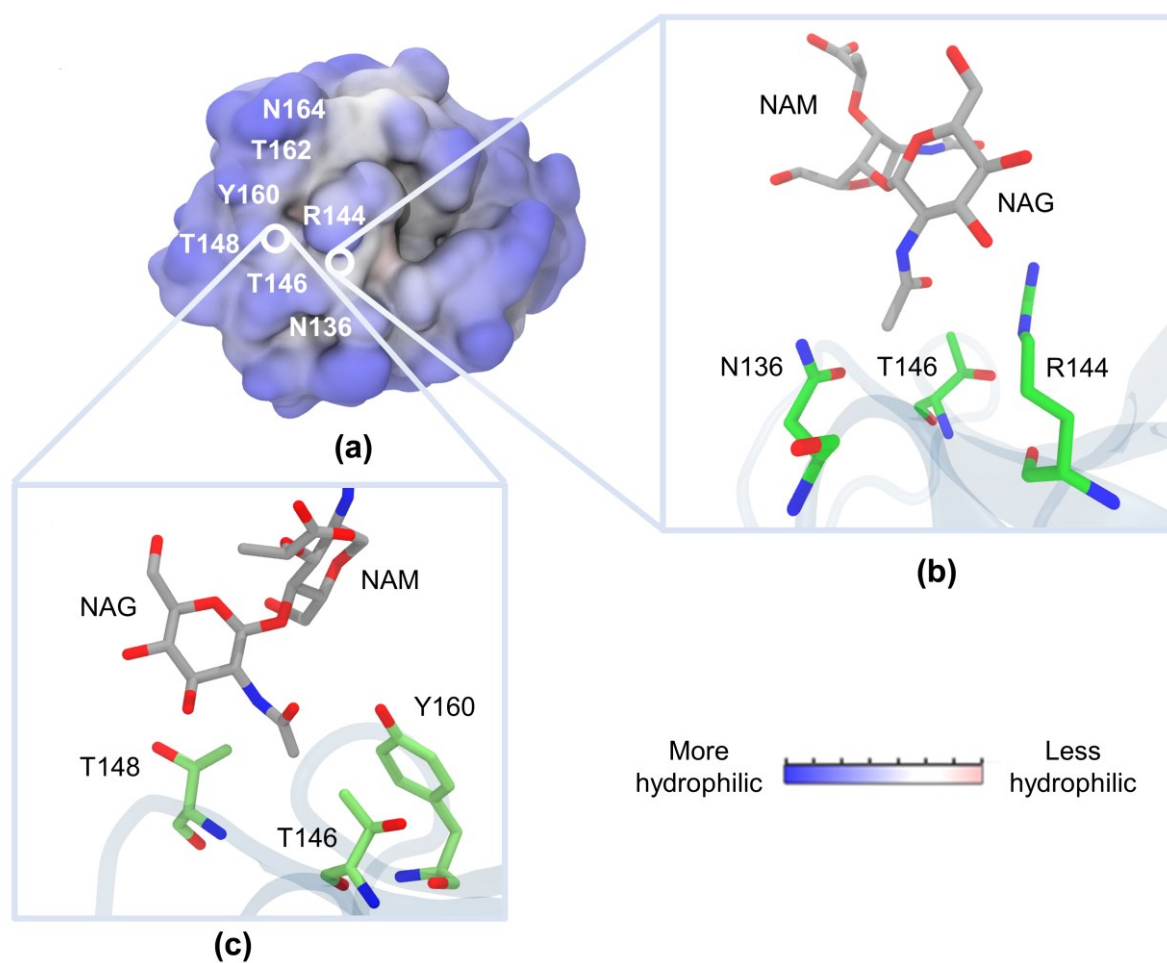


Figure S1. Superficial and structural organization of peptidoglycan binding site within C-terminal subdomain of the bacteriolytic protease Blp. Hydrophobicity/hydrophilicity map **(a)** indicates the location of less hydrophilic pocket-like structures, which interact with acetamide groups of (NAG–NAM)₂ ligand **(b, c)**.

Table S1. Content of the secondary structure elements of the Blp protease and its mutant forms (%).

Bacteriolytic enzymes	Helix	Antiparallel	Parallel	Turn	Other
Blp	0.6	47.3	0	10.9	41.2
Y160A	1.1	45.3	0	11.6	42
Y160R	0.2	47.2	0	10.9	41.7
N136A	0.5	47.2	0	11.8	40.5
N136R	2	41.3	0	13.3	43.4
R144A	0	47.8	0	11.4	40.8

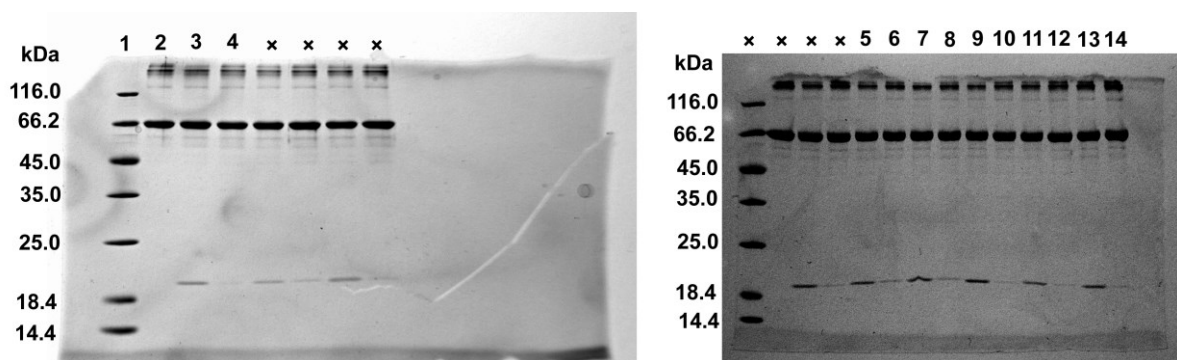


Figure S2. Original gel images for Figure 6a. Lane 1 corresponds to M of Figure 6a; lane 2, to the negative control of Figure 6a; lanes 3 and 4, to the reaction mixture with the BIp of Figure 6a; lanes 5 and 6, to the reaction mixture with Y160R of Figure 6a; lanes 7 and 8, to the reaction mixture with N136R of Figure 6a; lanes 9 and 10, to the reaction mixture with R144A of Figure 6a; lanes 11 and 12, to the reaction mixture with Y160A of Figure 6a; lanes 13 and 14, to the reaction mixture with N136A of Figure 6a.

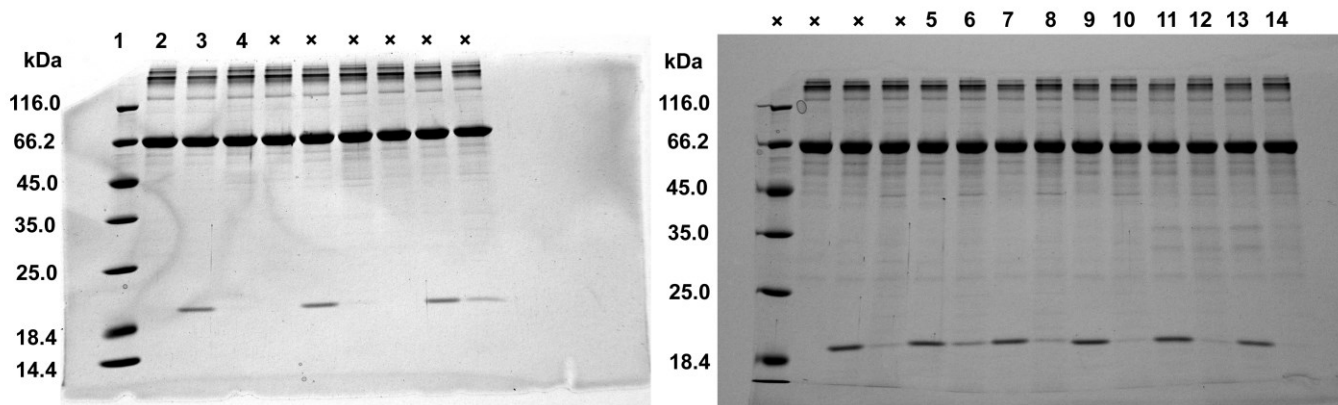


Figure S3. Original gel images for Figure 6b. Lane 1 corresponds to M of Figure 6b; lane 2, to the negative control of Figure 6b; lanes 3 and 4, to the reaction mixture with the BIp of Figure 6b; lanes 5 and 6, to the reaction mixture with Y160R of Figure 6b; lanes 7 and 8, to the reaction mixture with N136R of Figure 6b; lanes 9 and 10, to the reaction mixture with N136A of Figure 6b; lanes 11 and 12, to the reaction mixture with R144A of Figure 6b; lanes 13 and 14, to the reaction mixture with Y160A of Figure 6b.

Table S2. Oligonucleotides used in the work for knockout of the *blp* gene.

Oligonucleotides	Purpose
CTCGAGCCGATCCGCGTTG CCCGGGCCAACAGCGTGATCCT	Amplification of a 1036-bp fragment (5' end of the <i>blp</i> gene and its flanking upstream region).
CCCGGGAAGTGGGTCGGCGCG TCTAGACATGGAAACGGGCGTG	Amplification of a 963-bp fragment (3' end of the <i>blp</i> gene and its flanking upstream region).
GAATTCTCATGTTTGACAGCTTATCATCGA CCCGAGATGCGCCG	Amplification of a 1433-bp Tc cassette from plasmid pBR322.
GCGAGGGGCGGATTATGC CAGCTCCGATGCGATGACG	Validation of a deletion in the <i>blp</i> gene, marked with a 1430-bp Tc ^R cassette.

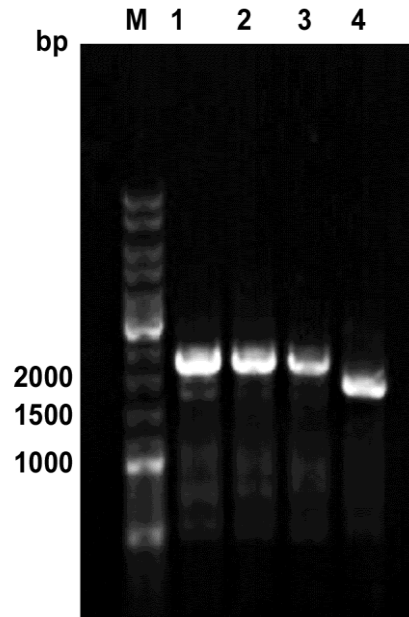


Figure S4. Electropherogram of PCR products. As a template, use was made of selected clones with the Suc^RTc^RGm^S phenotype (lanes 1, 2), plasmid DNA pJQ200SKΔblp::tet (lane 3), and DNA of *L. capsici* XL1 (lane 4). M, markers.

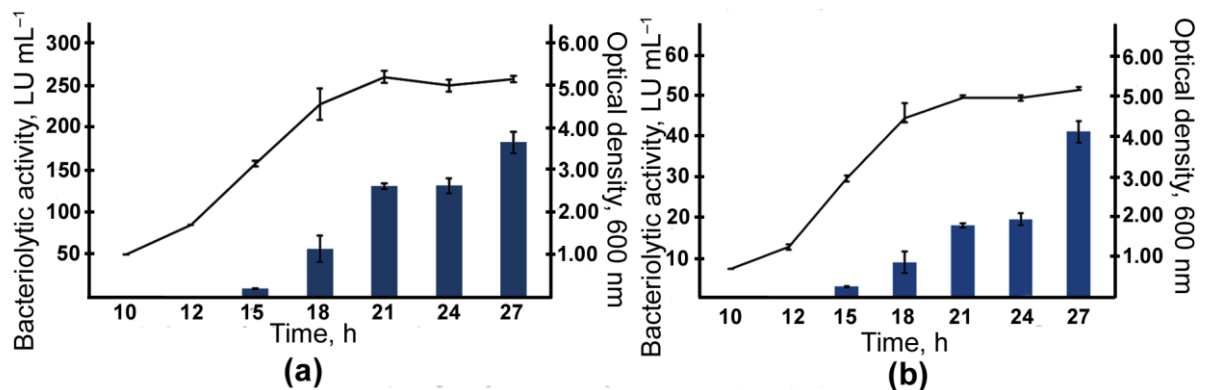


Figure S5. Dynamics of growth and bacteriolytic activity of wild-type strain *L. capsici* XL1 (a) and mutant strain *L. capsici* XL1Δblp (b) in relation to autoclaved cells of *Staphylococcus aureus* 209P.

The growth dynamics and development of the bacteriolytic activity of mutant strain *L. capsici* XL1Δblp and wild-type strain *L. capsici* XL1 did not differ (Figure S5). However, the total bacteriolytic activity in the culture fluid of the mutant strain was 7 times lower than that of the wild-type strain. The bacteriolytic activity against living cells of *Micrococcus luteus* Ac-2230^T and *Kocuria rosea* Ac-2200^T also decreased by 9 and 4 times, respectively (Figure S6). Mutant strain *L. capsici* XL1Δblp completely lost bacteriolytic activity against living cells of *S. aureus* 209P.

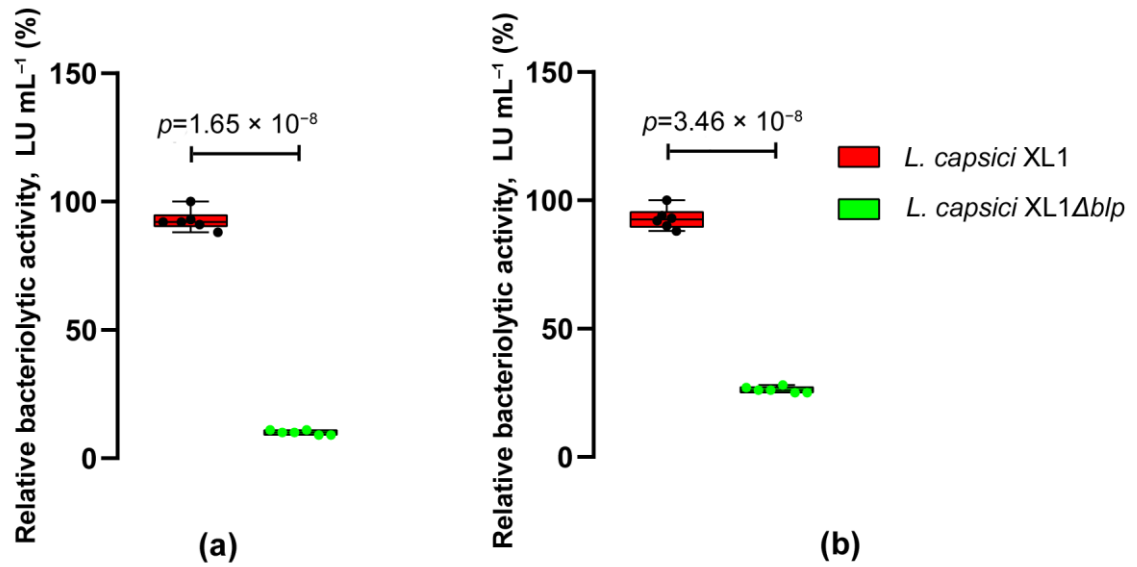


Figure S6. Comparative bacteriolytic activity of the culture fluid of *L. capsici* XL1 and *L. capsici* XL1Δ*blp* strains against living cells of *Micrococcus luteus* Ac-2230^T (a) and *Kocuria rosea* Ac-2200^T (b).

The mean values were obtained from two biological replicates, each performed in three technical replicates. Statistical analysis was performed using an unpaired two-tailed Student's *t*-test with Welch's correction: $t = 38.14$, $df = 5.8$ (a); $t = 49.60$, $df = 5.5$ (b). Data were considered statistically significant at $p < 0.05$.