Influence of bacterial culture medium on peptidoglycan binding of cell wall lytic enzymes

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ABSTRACT The bacteriolysin lysostaphin (Lst) and endolysin PlyPH are potent modular lytic enzymes with activity against clinically-relevant Gram-positive Staphylococcus aureus and Bacillus cereus, respectively. Both enzymes possess an N-terminal catalytic domain and C-terminal binding domain, with the latter conferring significant enzyme specificity. Lst and PlyPH show reduced activity in the presence of bacterial growth-supporting conditions, such as complex media. Here, we hypothesize that Lst and PlyPH bind poorly to their targets in growth media, which may influence their use in antimicrobial applications in the food industry, as therapeutics, and for control of microbial communities. To this end, binding of isolated Lst and PlyPH binding domains to target bacteria was quantified in the presence of three increasingly complex media – phosphate buffered saline (PBS), defined growth medium (AAM) and undefined complex medium (TSB) by surface plasmon resonance (SPR) and flow cytometry. Evaluation of binding kinetics by SPR demonstrated that PlyPH binding was particularly sensitive to medium composition, with 8-fold lower association and 3.4-fold lower dissociation rate constants to B. cereus in TSB compared to PBS. Flow cytometry studies indicated a decrease in the binding-dependent fluorescent pop-ulations of S. aureus and B. cereus, for lysostaphin binding domain and PlyPH binding domain, respectively, in TSB compared to PBS. Enzyme binding behavior was consistent with the enzymes' catalytic activity in the three media, thereby suggesting that compromised enzyme binding could be responsible for poor activity in more complex growth media.