Turning enzymes into efficient enzybiotics

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The rising threat of antimicrobial resistance and the scarcity of new antibiotics underscore the need for innovative methods to combat pathogenic bacteria. Enzybiotics, a class of peptidoglycan hydrolases, are garnering increasing attention for their defined specificity and exceptional efficiency in eliminating bacterial cells. These enzymes target and cleave bonds within the bacterial cell envelope, leading to rapid cell lysis. Notably, enzybiotics can eliminate millions of bacterial cells in minutes, selectively sparing the natural microflora. Importantly, they exhibit a low prevalence of resistance development.

In our pursuit of enzybiotic candidates among peptidoglycan hydrolases encoded by various bacterial species, we observed that while most enzymes displayed the desired specificity, their applications were restricted by limited tolerance to environmental conditions and low stability. To overcome these limitations, we conducted several rounds of protein engineering, based on prior thorough structural and biochemical investigations.

Through the strategic application of domain shuffling and point mutagenesis, we successfully engineered several new enzybiotics.

AuresinePLUS, for instance, selectively targets staphylococci, maintains activity in high ionic conditions and a broad pH range, and displays enhanced stability. This enzybiotic demonstrates exceptional efficacy in serum and milk.

Enterines, a trio of engineered enzymes resulting from the fusion of the EnpA catalytic domain with various SH3b binding domains, shows expanded specificity, increased stability, and increased tolerance for ionic and pH conditions. They primarily target staphylococci and selected enterococcal species, such as *Enterococcus faecalis*.

FaecineE, a chimeric enzybiotic, exhibits improved activity against *E. faecium*.

ZoocineA, a truncated variant of M23 peptidase which displays enhanced efficacy towards streptococci across a wide range of environmental conditions (ionic and pH).

These engineered enzymes' improved features have opened new avenues for practical applications. They can be combined with bacteriocins and other antibacterials. Innovative products developed in our research are currently tested for the prevention and treatment of bovine mastitis and infected wounds.

Our findings emphasize that transforming an enzyme into effective enzybiotic requires time and effort. However, a solid foundation of structural and biochemical data enables successful engineering, tailoring the enzymes to meet specific conditions required in particular applications.