Enzymatic Inactivation of the Veterinary Antibiotic Florfenicol

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Background: The therapeutic use of antibiotics is an important milestone in medicine. However, increased use of antibiotics in humans as well as in animal husbandry has resulted in an alarming rise of antimicrobial resistances.[1] The antibiotic florfenicol, a synthetic derivative of chloramphenicol, is used worldwide in large quantities for aquaculture, poultry, and livestock. Most of the antibiotic is not metabolized and enters the environment through waste products, feces, or milk of treated cows contributing to the development of antibiotic-resistant microbes.[2] Florfenicol-resistant bacteria in particular often activate export mechanisms that result in resistance to various structurally unrelated antibiotics.[3] We devised a novel strategy for enzymatic inactivation of florfenicol.

Methods: Using a combinatorial library approach[4] combined with increasing selective pressure, we adapted a hydrolase enzyme (EstDL136)[5] for optimized cleavage of florfenicol. Reaction kinetics were followed by time-resolved nuclear magnetic resonance (NMR) spectroscopy. Different environmentally friendly application strategies for florfenicol inactivation were developed using our optimized hydrolase.

Results: To date, no efficient method for florfenicol inactivation exists. The only known enzymes capable of cleaving florfenicol, the hydrolase EstDL136, is not very efficient as judged by its inability to confer bacterial resistance to florfenicol. We optimized the enzyme activity by an evolutionary approach combining random mutations with selective pressure and were able to enrich a mutant that showed significantly improved catalysis rate as judged by time-resolved NMR measurements following florfenicol cleavage. As potential filter device for cost-effective treatment of florfenicol-contaminated waste milk or aquacultural wastewater, the optimized hydrolase was immobilized on carrier materials, added as inexpensive cell extract, or was encapsulated with a semi-permeable membrane and could be repeatedly used for florfenicol inactivation in different media. Importantly, the hydrolase was found to be very robust and active in various solutions such as salt water or cow milk.

Conclusion: Large quantities of antibiotics are used in animal farming and enter the environment contaminating the ecosystem with antibiotic residues and promoting antimicrobial resistance, ultimately leading to untreatable multidrug-resistant pathogens. We have optimized a hydrolase enzyme and devised a new strategy for enzymatic inactivation of the antibiotic florfenicol before it enters the environment. For cost-effective use, the hydrolase enzyme was immobilized on carrier materials validated for repeated use as antibiotic filter. Florfenicol inactivation was demonstrated in salt water and cow milk demonstrating potential use scenarios, such as florfenicol inactivation in waste milk from medicated cows, which enables feeding this milk to calves without the risk of inducing antibiotic resistances. Such an enzymatic inactivation of antibiotics in general enables therapeutic intervention without promoting antibiotic resistances.

References

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