Discovering Inhibitors of Rumen Methanogens Using High-Throughput X-ray Crystallography and Enzyme Screening Techniques

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Methane is a potent greenhouse gas and methane emissions from ruminants are widely recognized as a major contributor to global warming and climate change. One strategy for mitigating these emissions is to develop compounds that specifically inhibit the growth of archaeal methanogens. To accelerate inhibitor discovery, we targeted enzymes involved in specialized biochemical pathways of rumen methanogens (biochemically distinct from bacteria, humans and ruminant animals), including methanogenesis, cofactor biosynthesis and archaeal lipid synthesis. Over three hundred enzymes were identified as candidates for structural determination, producing in excess of one hundred soluble proteins for crystallographic screening. Over 50 different enzymes have produced crystals and 29 structures have been solved to date. A subset of these enzymes were then the focus of *in vitro* inhibitor screening (5 enzymes) utilising large commercial compound libraries and *in silico* screening (5 enzymes) to identify possible inhibitors for use in ruminants.

Keywords: X-ray crystallography, methanogenesis, rumen, Greenhouse gas, inhibitors