Title	**Targeting hydrogen and methyl-compound production in the rumen to prevent methanogens using these compounds to make methane**
Project Timeframe	Aug 2017 – Dec 2020
Countries Involved	New Zealand (AgResearch, University of Otago, Donvis Ltd.) Japan (Hokkaido University) USA (University of Illinois)
Aims	This project targets the main microbial groups producing hydrogen and methyl-compounds and aims to lower their activities to reduce substrate supply to methanogens.
Research Highlights	• Determined the main hydrogen-producing microbes in the rumen, their hydrogenase types, the abundance of their hydrogenase genes and the level of hydrogenase gene expression in low and high methane yield sheep. • Determined the most abundant rumen microbes responsible for releasing methyl-compounds from plant material (mainly methanol and trimethylamine), the types of enzymes involved, the abundance of genes encoding these activities and their level of gene expression in the sheep rumen. • The screening of five target rumen bacteria (Clostridiales R-7, Ruminococcus albus 7, Selenomonas ruminantium HD4, Prevotella bryanti C21a and Olsenella umbonata) using growth assays against 1800 Pfiser FDA-approved drugs identified 14 compounds which were tested in rumen in vitro assays, and six compounds have shown promising reductions in methane formation. • The work in the project complements and supports the current PGgRc and NZAGRC methane mitigation programmes through targeting alternative pathways and identifying new compounds as potential inhibitors of methane formation. • Collaborations were formed with international experts in hydrogenases and their expertise has been used to elucidate hydrogenase function in several important hydrogen-producing rumen bacteria (in the USA, Japan, and Australia).
Future Work	• The six inhibitors identified from the screening and rumen in vitro studies will be tested further to establish their minimum inhibitory concentrations that have an effect in vitro, and then will be tested in combination with other methane inhibitors to identify synergistic effects. • Inhibitors with low minimum inhibitory concentrations will be made available to the PGgRc Inhibitor programme for further testing and development. • The co-culture studies between methanogens and either hydrogen-producing or methyl-compound-producing bacteria will

	be used to identify the specific interactions whereby hydrogen and/or methyl-compounds are produced and consumed and will identify new opportunities for disrupting or controlling these processes that can lead to reduced methane emissions.