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MabS: The use of Marine derived antibacterial agents to combat the prevalence of Salmonella in pork products

Key external stakeholders:

The key stakeholders are the indigenous animal feed industry, pork producers and producers of pork-derived products and consumers of pork products.

Practical implications for stakeholders:

Infections caused by foodborne pathogens, such as *Salmonella* spp. are a major public health concern worldwide and the consumption of pork products containing salmonellae is a significant cause of food poisoning. Thus, there is a clear need to identify and control the threat both to human health and the pig industry in Ireland. Previous studies on the genus *Pseudovibrio* identified the presence and production of potential antimicrobial products and secondary metabolites with anti-*Salmonella* properties. Genome sequencing and subsequent *in silico* analysis of the *Pseudovibrio* strains isolated from Irish marine sponges aids in the identification of potential antimicrobial products or secondary metabolites which are capable of inhibition of the pathogen *Salmonella.*

- These compounds may find application as antibacterial agents, either as single molecules or in synergy with other compounds, in the control of the growth and spread of foodborne pathogenic *Salmonella.*
- The antimicrobials have the potential to be incorporated directly into the product, coated on its surface or incorporated into the packaging in an effort to inhibit the growth of pathogenic and spoilage microorganisms.
- The physicochemical shelf life of food products and in particular porcine-based products may be improved, leading to reduced net losses and enhanced public health.

Main results:

Salmonella is currently one of the leading causes of food poisoning worldwide. Control of this pathogen is very desirable to the food industry. Numerous strategies are already employed but the discovery of novel antimicrobial agents produced by the marine sponge-derived *Pseudovibrio* spp. has the potential to further aid in the inhibition of this bacterium and other pathogens. *In silico* analysis, LC-MS/MS (Liquid Chromatography tandem Mass Spectrometry) and NMR (Nuclear Magnetic Resonance Spectroscopy) characterisation played a key role in identifying the gene clusters of interest and metabolites such as tropodithietic acid (TDA), which exhibited anti-*Salmonella* properties.

Opportunity / Benefit:

Food: Assure and improve the microbial quality and safety status of Irish pork and its derivatives.

Food industry: Control over *Salmonella* and other pathogenic and spoilage organisms in the food chain will result in less waste and net losses to the key stakeholders.

Food Industry Development: To provide Technology Development support for food SMEs and start up food businesses.

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Collaborating Institutions:

Teagasc (Moorepark and Ashtown) and UCC

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1. Project background:

Infections caused by food borne pathogens, such as *Salmonella* spp., are a major public health problem worldwide and the consumption of pork products containing salmonellae continues to be a significant source of food poisoning. There is a clear need to identify novel products to control the threat both to human health and the pig industry in Ireland.

Marine sponge-derived *Pseudovibrio* species were previously identified as a novel source of anti-*Salmonella* activities but the isolation and characterization of these anti-*Salmonella* bioactive molecules has proved difficult using standard approaches. This project was designed to build upon previous findings, using an approach combining genomics, molecular microbiology and natural product chemistry, to fully characterise anti-*Salmonella* compounds from these *Pseudovibrio* isolates.

This project aimed to determine the draft genome sequences of three selected bioactive *Pseudovibrio* species. These genomes were analysed for the presence of genes involved in the biosynthesis of known families of antibiotics. Anti-*Salmonella* molecules were purified using a bioassay-guided strategy with novel compounds being rapidly identified using a mass spectrometry approach.
This novel approach aimed to result in the identification of compounds with anti-*Salmonella* activity and an improved means to produce them for commercialization and field applications.

2. Questions addressed by the project:

- Can we identify the biosynthetic gene clusters *in silico* which may be responsible for the antimicrobial activity exhibited by the *Pseudovibrio* isolates?
- Can we determine a range of inhibition for the *Pseudovibrio* strains?
- Can we use a variety of analytical techniques to help decipher the bioactive compounds being produced and their structures?

3. The experimental studies:

- DNA extraction and genome sequencing of the *Pseudovibrio* strains of interest.
- *In silico* analysis of the sequencing data to elucidate the anti-*Salmonella* biosynthetic gene clusters.
- Deferred antagonism assays to ascertain complete spectrum of inhibition.
- Natural chemistry analysis to determine what bioactive compounds are being produced and their proposed chemical structures.

4. Main results:

The use of genome sequencing and the subsequent *in silico* analysis allowed for the identification of biosynthetic gene clusters implicated in the production of the bioactive molecule tropodithietic acid (TDA) and other antimicrobial compounds. As our search for new antibiotics continues, TDA, or some of its analogues produced by marine sponge-derived *Pseudovibrio* isolates, has the potential to be a template for clinical development. Indeed, synthetic modification focussing on structure-activity relationships resulting in analogues with enhanced antimicrobial activity can result in the discovery of antibiotic-like molecules, thereby helping to combat the emergence of bacteria resistant to common antibiotics. These studies clearly

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indicate that the *Pseudovibrio* genus holds the potential to be a source of new antimicrobial compounds other than TDA.

5. Opportunity/Benefit:

The inhibition of *Salmonella* spp. in the food chain would be of enormous benefit to the food industry as a whole. The bioactive molecules produced by members of the *Pseudovibrio* genus exhibit a promising alternative to current methods.

6. Dissemination:

Main publications:

Marine *Pseudovibrio* sp. as a Novel Source of Antimicrobials
Crowley, S.P., O' Gara, F., O'Sullivan, O., Cotter, P.D. and Dobson , A.D.W. (2014) Marine Drugs12(12):5916-5929.

Characterisation of non-autoinducing tropodithietic acid (TDA) production from marine sponge *Pseudovibrio* species.
Harrington, C., Reen, J.F., Mooij, M.J., Stewart, F., Chabot, J-B., Antonio Fernandez Guerra, A.F., Glockner, F.O., Nielsen, K.F., Gram, L., Dobson, A.D.W., Adams, C. and O'Gara, F. (2014) Marine Drugs 12, 5960-5978.

Comparative genomic analysis reveals a diverse repertoire of genes involved in prokaryote-eukaryote interactions within the *Pseudovibrio* genus.
Romano, S., Fernandez-Guerra, A., Glöckner, F., Crowley S.P, O'Sullivan, O., Cotter, P., Reen, J.F., Adams, C., Dobson, A.D.W. and O'Gara, F. (2016). Frontiers in Microbiology. Mar 30; 7:387.

HPLC-ESI-MS/MS Characterisation of metabolites produced by *Pseudovibrio* sp. W64, a marine sponge-derived bacterium isolated from Irish waters.
Choudhary, A., Naughton, L.M., Dobson, A.D.W. and Rai, D.K. (2018) *Rapid Communications in Mass Spectrometry* 32(19): 1737-1745.

Current status and future prospects of marine natural products (MNPs) as antimicrobials.
Choudhary, A., Naughton, L.M., Montánchez, I., Dobson, A.D.W. and Rai, D.K. (2017) *Marine Drugs* 15(9):E272.

Identification of secondary metabolite gene clusters in the *Pseudovibrio* genus reveals a promising potential toward the discovery of novel bioactive compounds.
Naughton, L.M., Romano, S., O'Gara, F. and Dobson, A.D.W. (2017) Frontiers in Microbiology; 8: 1494.

TResearch article titled "Marine antimicrobials for the food industry" by Choudhary, A., Rai, D., Cotter, P.D. and Dobson A.D.W. was published in Summer 2018, 13(2):24-25.

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