Isolation & evaluation of an effective probiotic for use as an alternative to in-feed antibiotics for pigs

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Outline

1. Background & objective
2. Isolation & screening of potential probiotic strains
3. Dev. of a probiotic for weaned pigs – *in vitro* work
4. Dev. of a probiotic for weaned pigs – pig-feeding trial
5. Commercialisation
6. Conclusions

1. Background

Problems:

- Emergence of antibiotic-resistant pathogens
- EU ban on routine use of in-feed antibiotic growth promoters
- ↑ diarrhoea and ↓ animal performance

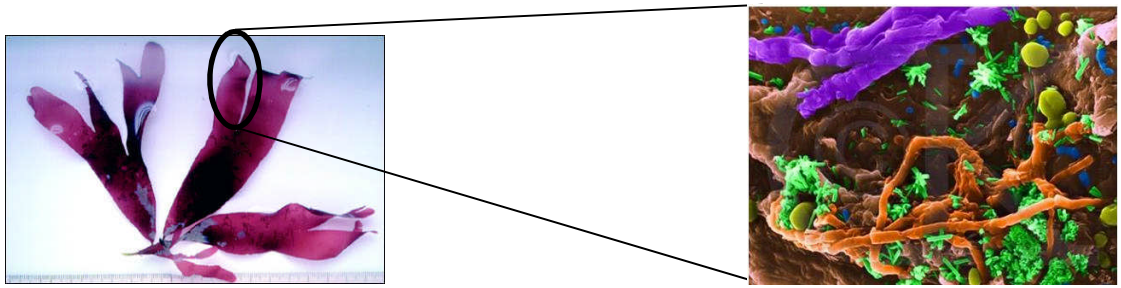
Solution: Find alternatives to antibiotics

Probiotics – 'Live microorganisms which when administered in adequate amounts confer a health benefit on the host'

Particular benefits at times of stress - e.g. post-weaning

Marine bacteria as probiotics

- Marine environment untapped source of novel microorganisms
- Antimicrobial production (probiotic trait) common amongst seaweed-associated bacteria
- Marine bacteria used as probiotics for aquaculture
- To date, unexploited as probiotics for pigs



Objective

- To isolate antimicrobial-producing bacteria and to evaluate their potential for use as probiotics in pigs

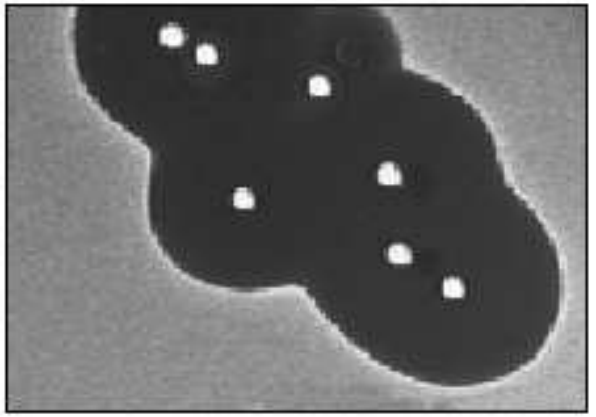
2. Isolation & screening potential probiotic strains

Seaweed, sand & seawater samples

311 bacterial isolates

15 isolates (*Bacillus licheniformis* and *Bacillus pumilus*) selected for antimicrobial activity

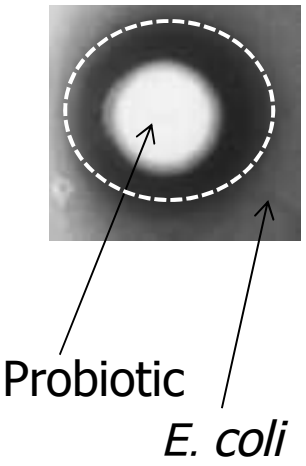
Detecting antimicrobial production



3. Dev. of a probiotic for weaned pigs – *in vitro* work

- 15 candidate probiotics
- 6 with anti-*E. coli* and/or *Salmonella* activity
- *Bacillus pumilus* selected based on probiotic properties

	E. coli						*Salmonella* Typhimurium			
	K88	F18ab	F2S2	F3P3	F1L3	F15OF3	DT104	PT12	DT17	DT104
***B. pumilus* WIT 588**	**+++**	**+++**	**++**	**+++**	**+++**	**++**	**++**	**+/-**	-	-
B. licheniformis (Bioplus 2B®)	-	-	-	-	-	-	+/-	++	-	-
B. subtilis (Bioplus 2B®)	-	-	-	-	-	-	-	-	-	-

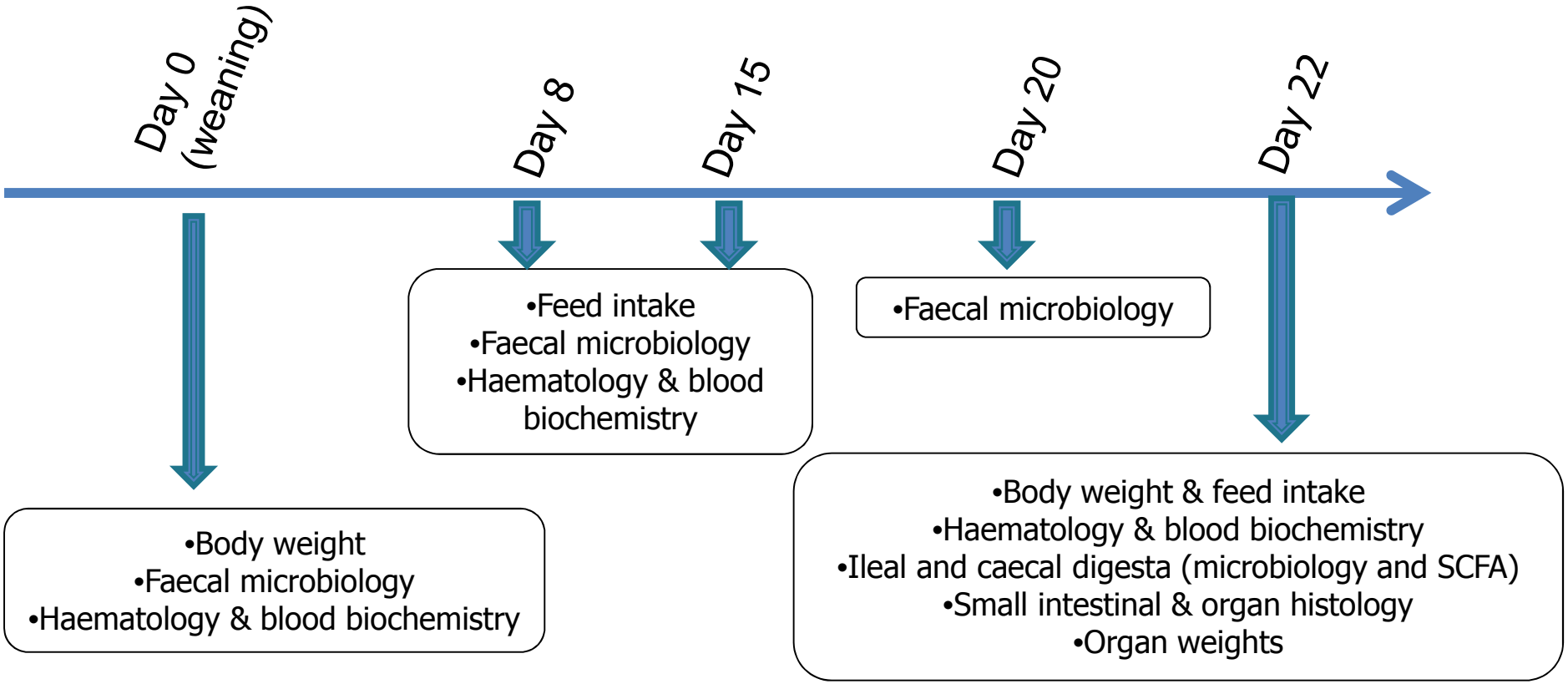


- Better anti-*E. coli* activity than commercial probiotics
- Spores resistant to intestinal juices
- Safety tested - no antibiotic resistance, no toxin genes, not toxic to red blood cells or intestinal cells

Prieto et al. (2014). Marine Drugs 12, 2422-2445

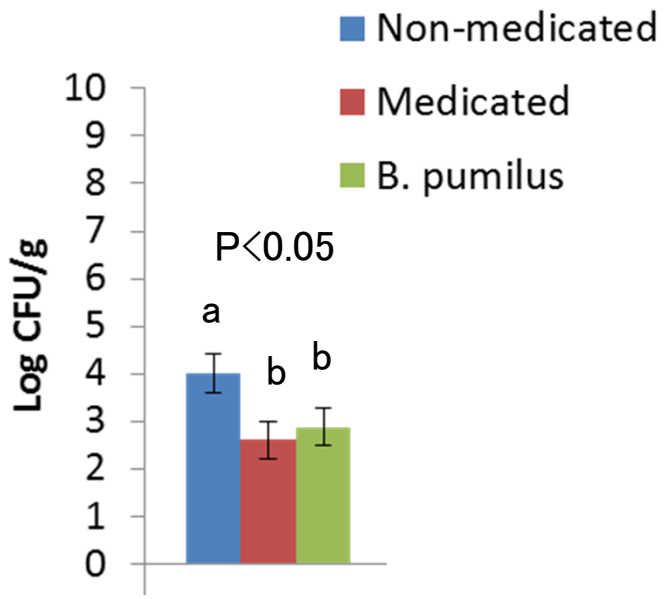
4. Dev. of a probiotic for weaned pigs – pig trial

- Pig unit with oedema disease post-weaning
- 48 pigs assigned at weaning (~ 28 d) to:
  1. Non-medicated diet (negative control)
  2. Medicated diet (apramycin + zinc oxide; positive control)
  3. Non-medicated diet + *B. pumilus* probiotic (~10^{10} spores/day)



4. Dev. of a probiotic for weaned pigs – pig trial

	Non-medicated	Medicated	*B. pumilus*	P value
Day 0 BW (kg)	8.7	8.6	8.8	NS
Day 22 BW (kg)	**18.1**	**17.6**	**18.7**	**0.07**
ADFI (g/d)	471	458	475	NS
ADG (g/d)	**427**	**405**	**455**	**0.07**
FCE	**1.11^{ab}**	**1.14^{a}**	**1.05^{b}**	**0.04**



Reduced *E. coli* in ileum

- ↓ ileal *E. coli* counts as well as medicated diet
- But without ↓ growth, ↓ caecal and faecal *Lactobacillus* counts, ↓ caecal SCFA
- and possible liver toxicity (↓ liver weight , ↑ liver enzymes) seen with medication

Prieto et al. (2014). PLoS ONE 9(2): e88599.

5. Commercialisation

- International patent application filed for probiotic strain
- Enterprise Ireland commercial feasibility study commencing
- Enterprise Ireland Commercialisation grant to fund additional animal trials
- Potential to licence technology to a company?

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6. Conclusions

- ↑ growth performance compared to in-feed antibiotic
- ↓ *E. coli* counts as effectively as in-feed antibiotic
- BUT without negative effects seen with in-feed antibiotic
- *B. pumilus* strain appears safe for use as probiotic in weaned pigs and has potential for use as alternative to in-feed antibiotics
- Lesson: If selected methodically for a particular purpose and used for that purpose, a positive response from probiotic use is likely

Acknowledgements

- Funded by Higher Education Authority/Institutes of Technology Ireland Technological Sector Research Strand III Programme

- Farm staff and technicians at Moorepark

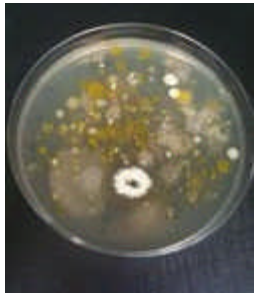
Institutes of Technology Ireland

Questions?

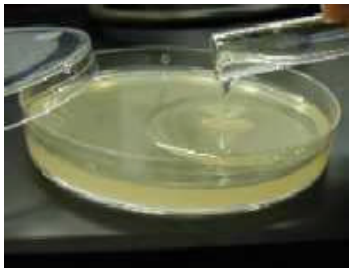
2. Isolation & screening of potential probiotic strains

Washed seaweed (7)
Sand (1)
Seawater (1)

Plate on 6 different media & select for sporeformers



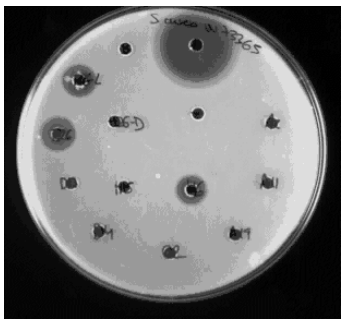
Overlay with *E. coli, Salmonella, Listeria, Staphylococcus, Bacillus* **(>6,000 colonies)**



Look for antimicrobial activity (clear zones around colonies)

Purify & stock antimicrobial-producing isolates **(8 isolates)**

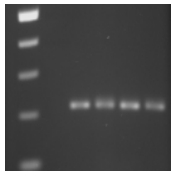
Confirm antimicrobial activity and check spectrum of activity **(311 isolates)**



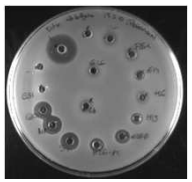
15 isolates with consistent confirmed antimicrobial activity

Purify & stock (**303 isolates**)

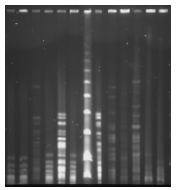
PCR for known *Bacillus* bacteriocins and lantibiotics



Physicochemical characterisation of antimicrobial compounds



Identify bacteria (16S rRNA gene sequencing) & fingerprint by PFGE



Detecting antimicrobial production in the laboratory