

### <u>THE MASTER PLAN:</u> <u>Microbiome Applications for</u> <u>Sustainable food systems through</u> <u>Technologies and EnteRprise</u>

Project Co-ordinator: Prof Paul Cotter, Teagasc, Ireland @pauldcotter; paul.cotter@teagasc.ie





http://www.master-h2020.eu/

@MASTER\_IA\_H2020 #MASTER



#### Microbes are everywhere in the food system

Soil

-

Agriculture

against diseases.

Some bacteria help crop

growth and defend plants

Microbial communities are essential for food chain health, for food security and climate change mitigation.

Small numbers of potentially harmful microbes are a natural part of these communities.



Aquatic animals

ish have microbial communities that upport their health.

Soil microbial communities differ: fungi form spider webs in the soil to connect plants, bacteria are near plant roots.

Plants

Microbes are on and in plants, supporting plant health.



Animals and disease Potentially harmful microbes are often part of the natural host microbial community and attack when immunity is weak. The animal food chain may also spread anti-microbial resistances.

#### Microbes on food

Foods like voghurt and sauerkraut are rich in beneficial bacteria that outnumber those microbes which cause food go rotten. In food production, veasts are often used for bread and beer.





Humans Humans have many beneficial microbial communities: our gut bacteria is influenced by the food we eat.



Humans and disease Microbial infections are common when immune systems are weak.



Food waste Rotting of food means microbes recylce food leftovers so that nutrients can be released back into the environment.

Nater bodies

**Microbes in phytoplankton** communities are food for fish.

Shellfish and disease Some microbial compounds accumulate in shellfish and cause food poisoning.



ingle celled algae ingle-celled algae re a food source for us.

Plants and disease Some microbes can cause plant disease and food losses, in the right environment.

Animals Animals have microbial communities that are supportive of the animal's health.



Microbes in foods Wrong food storage causes food to go rotten as moulds take over the microbial community. These microbes often cause food poisoning.

### A revolution in Microbiome research

#### Traditional, culture-based, approaches to microbiology only reveal the 'tip of the iceberg'

The success of culture based approaches is dependent on having types of agar/culture media/growth conditions that allow all microbes to grow

Such an agar does not exist!!

Indeed only a small % of microbes are easily cultured in the laboratory.

DNA sequencing-based approaches can allow an analysis to the entire population regardless of whether it can be grown or not

All of the DNA from the microbes present in a particular environment = <u>Metagenomic DNA</u>





### **DNA-based community analysis**







### Pre-MASTER: The Back Story

### Fermented beverage microbiome





Water kefir

Marsh et al FEMS 2013



#### Kombucha

Marsh et al Food Microbiol 2014

Walsh et al. mSystems 2016

Kefir



#### Nunu

Walsh et al Appl Env Microbiol 2017



- Short shelf-life
- Trained vs Untrained producers



### Compositional analysis reveals primary source of contaminants in raw milk across different seasons





#### Doyle et al. Appl Environ Microbiol 2017

Shotgun metagenomic analysis of commercial cheese samples

 Pink discolouration defect in cheese (Swiss, Cheddar, Italian) has been a worldwide problem over many decades.
 %

 Here's Why Some Cheese Turns Pink
 Image: Cheese Turns Pink





m)aster

Defect cheese is enriched with *Thermus thermophilus in particular* 

#### Quigley et al MSystems

### Movement of microbes through the food chain













Standardised Skim Silo (2)



Tanker (11)

Whole Milk Silo (2)

Pasteurization Separation





### **Dairy chain microbes**



SMS

SMP

(m)aster



### The MASTER Plan









ABInBev

operation in the later of the second se



### Microbiome Applications for Sustainable Food Systems through Technologies and EnteRprise

MASTER takes a global approach to the development of microbiome products, foods/feeds, services or processes with high commercial potential.

This will benefit society through improving the quantity, quality and safety of food across multiple food chains. These include marine, plant, soil, rumen, meat, brewing, fruit and vegetable waste, and fermented foods.







### **Objectives of MASTER**



68 64	1. Develop microbiome-based solutions for improving important traits of fodder crops
SS SS	(grass & maize) and employ microbiome-based diagnostics (for use in real time by
UU	food operators) to improve primary production of these crops



2. Develop microbiome-based solutions to **increase bioavailability of sustainable feeds** for enhanced fish growth and employ microbiome-based **diagnostics** (for use in real time by food operators) to assess **fish health & detect pathogens** 



3. Target the rumen microbiome (increased feed efficiency, reduce methane emissions) by utilising host genomics & novel feeding technologies to beneficially alter the rumen microbiome & to develop technologies to monitor microbiome markers & other tools and models which predict ruminant phenotype



4. Optimize **fermented food microbes** and processes to add to the **value and health**promoting properties of substrates, including those resulting from waste streams.



5. Contribute to the design of **novel functional foods** on the basis of the study of available data on **food/human gut microbiome inter-relationship** and provide effective tools to test the potential of food ingredients to move the gut microbiota away from dysbiosis to that reflective of healthy individuals.

### **Objectives of MASTER**





6. Develop, optimize, standardize and validate technologies, as well as **software tools and targeted reference databases** for microbiome analysis and pathogen detection in foods and to map the microbiome of food producing facilities



7. Develop **long-term databases, tools and resources** supporting the food-chain industry after the end of the project



 Promote an understanding of, engagement and interest in, microbiome-related applications for the food industry through communication and engaging with diverse end-users



9. Create a clear **knowledge exchange plan** between all participants, developing mutual specialisation, standard operational procedures, supporting management and training, thus strengthening the complementarity and trans-disciplinarity of the partners

10. Bring to market new and cost-effective commercial applications to assist different stages and processes throughout the food chains, by 2025 and, in the process, move available solutions from TRL (Technology Readiness Level) 5/6 to TRL 7



### Plant and soil microbiomes

WP2: Plant, Soil and Marine Microbiomes – Microbiome-based improved production of fodder crops & aquaculture Lead AIT (Sessitsch), Co-Lead MATIS (Marteinsson), partners Teagasc, INOQ, Qiagen, ONT, AIT, Anadiag, CH, IFREMER, FVG in close interaction with WPs 1, 3, 5 and 6

#### **Objectives**:

Microbes to improve nutrient uptake, provide biocontrol of *Fusarium* and lower mycotoxins in fodder crops (maize and grass).

Develop microbiome-based diagnostic tools enabling detection of pathogens and prediction of the responsiveness of a system (based on fodder maize) to microbial applications



# Microbiome solutions for improving traits of fodder crops



Work plan overview

Fodder maize	Fodder grasses
<ul> <li>Biofertilizing strains:</li> <li>Rhizophagus irregularis</li> <li>Funneliformis mosseae</li> <li>Funneliformis caledonium</li> <li>Biocontrol strains (against Fusarium spp.):</li> <li>Bacillus subtilis</li> <li>Bacillus licheniformis AB12</li> </ul>	<ul> <li>Biofertilizing strains:</li> <li>Rhizophagus irregularis</li> <li>Funneliformis mosseae</li> <li>Funneliformis caledonium</li> <li>Bacillus sp. AB20</li> <li>Paraburkholderia phytofirmans PsJN</li> </ul>
<ul> <li>Field trails (2020 &amp; 2021)</li> <li>two consecutive years</li> <li>three representative locations (Italy, France, Spain)</li> </ul>	<ul> <li>Field/on-farm trails (2020 - 2022)</li> <li>two consecutive years</li> <li>Ireland</li> <li>assessment of: agronomic traits, colonization of applied bacteria &amp; fungi and effect on plant-associated microbiome</li> </ul>

Agronomic traits, colonization of applied bacteria & fungi, mycotoxin concentration, fodder/silage quality and effect on plant-associated microbiome

# Microbiome solutions for improving traits of fodder crops

- master
- Highlight: mycorrhizal colonization of the plants improved or altered depending on the bacterial strain



The presence of *B. subtilis* tends to lower the mycorrhizal colonization of the roots, whereas *Bacillus* sp. improves it. This effect is dependent on the concentration of AMF (arbuscular mycorrhizal fungi) in the substrate.

Microbiome-based diagnostics to improve primary production of fodder crops



- Kit facilitating the isolation and enrichment of microbial DNA from plant material
- Adaptation of available sequencing pipelines for detection of relevant pathogens as defined in the literature
- Microbiome markers -prediction of the response of a system to microbial inoculants
- Development and validation of sequencing and analysis pipelines for the identified markers
- Microplot trials

### Marine microbiomes

WP2: Plant, Soil and Marine Microbiomes – Microbiome-based improved production of fodder crops & aquaculture Lead AIT (Sessitsch), Co-Lead MATIS (Marteinsson), partners Teagasc, INOQ, Qiagen, ONT, AIT, Anadiag, CH, IFREMER, FVG in close interaction with WPs 1, 3, 5 and 6

#### **Objective**:

Develop microbiome-based solutions to increase bioavailability of **sustainable feeds for enhanced fish growth** and **employ microbiome-based diagnostics** (for use in real time by food operators) to assess fish health & detect pathogens.



#### Approach:

Comparative growth trials with juvenile Salmonids fed with alternative protein sources, prebiotics and probiotic strains.

Pathogen detection in aquaculture systems using on-site ONT sequencing.

### Marine and aquaculture microbiomes

#### Sustainable feeds for enhanced fish (juvenile salmonids) growth

#### Comparative growth trials



#### Gut microbiome analysis





Comparison of growth rates between diets (insect meal, SCP, prebiotics) and probiotic supplementation

m)aster

Detection of alterations in the gut microbiome based on diet

### Marine and aquaculture microbiomes

#### Pathogen detection in marine aquaculture systems – Reliable screening pipeline



Aerial view of marine aquaculture net cages. Water samples collected at 0, 10 and 100 m distance



Whole shotgun metagenomic sequencing on Nanopore MinION



Detection of fish pathogens (Flavobacterium psychrophilum and Alivibrio salmonicida).

m)aster

### Rumen microbiome

WP3: Rumen Microbiome – Improving animal production and reducing environmental impact through manipulation of the rumen microbiome Lead QUB (Huws), Co-Lead CSIC (Yanez-Ruiz), partners Teagasc, LUKE, INRA, Devenish, ICBF, AFBI,

#### **Objective**:

- Utilise host genomics to alter the rumen microbiome and animal phenotype.
- Define the rumen microbiome (different life stages) to improve efficiency, reduce greenhouse gases emissions & improve the fatty acid content of meat and milk.
- Develop microbiome-based tools to predict animal phenotype.







Define the rumen microbiome to improve efficiency, reduce greenhouse gases emissions & improve the fatty acid content of meat and milk.





m)aster

Green Feed



#### Portable accumulation chambers



Dietary manipulations of the rumen microbiome for improved animal phenotype.





pH; Fatty acid (raw and cooked meat); Oxidation - shelf life; Sensory profiling; Human intervation; Hyperspectral image; REIMS Escherichia coli O157 Thermotolerant Campylobacte Aerobic colony count ß-Glucuronidase + E. coli Salmonella Listeria spp. Utilise host genomics to alter the rumen microbiome and animal phenotype.



(m)aster

## Microbiomes & food processing, waste streams and gut health

WP4: Microbiome in Food Processing, Valorisation of Waste Streams and Human Gut Health Lead UCC (Arendt), Co-Lead UNINA (Ercolini), partners Qiagen, Teagasc, 4DC, ONT, ULE, Novolyze, FFOQSI, CSIC, Danone in close interaction with WPs 1, 3, 5 and 6

WP4 will cover a variety of areas. The first task relates to testing of microbiome mapping workflows and tools in the food industry.

WP4 will also have a number of activities relating to preventing waste, through biopreservation or fermentation, or adding value to waste streams/low value raw materials.



#### Brewery by-products and their production





### Brewery by-products and their production

#### PASTA



• Incorporation of BSG into pasta formulas proved successful

High fibre BSG and FBSG pastas superior to wholemeal control

- Effect of Fermentation on BSG
  - Causes an effect on proteins and starch

#### BREAD



m)aster

• BSG and FBSG can be incorporated into a bread

Source of fibre breads more promising than High in fibre breads

- Fermentation of BSG
  - Aids textural properties of bread (lower hardness, chewiness and gumminess)
  - Lighter colour bread produced

BSG-based ingredients can be used to enhance the fibre content of pasta and bread cereal products

#### **Food processing microbiome**



- Microbiome resident in food processing environment may impact on food quality and safety
- This community may be shaped by several factors

Bulk Bag Off (3x3) m)aster

• Develop SOPs and apply to 200 companies

#### Microbiome sampling at the food industries



To visit

Visited

**Visits targeted** Visits completed 9 Seafood Cheese / dairy Raw vegetables Cured meats 58.6 % Fermented sausages Raw meat Ice creams Raw meat Ice creams Raw vegetables Seafood 12 Fermented sausages Cured meats 3 91 Cheese / dairy



### **Global Fermented Food Initiative**



Id Sample N	Name	
1FS00a_2016	Wagashi	Che
2FS01_2016	Bread Kvass	Bev
3FS02_2016	Carrot Kimchi	Ferr
4FS03_2016	Boza	Bev
5FS04_2016	Lemon	Pres
6FS05_2016	Turnip	Ferr
7FS06_2016	Orange	Pres
8FS07_2016	Krauthehi	Ferr
9FS08_2016	Tepache	Bev
10FS09_2016	Ginger Beer	Bev
11FS10_2016	Tempeh	Ferr
12FS11_2016	Cucumber	Ferr
13FS12_2016	Milk Kefir	Bev
14FS13_2016	Water Kefir	Bev
15FS14_2017	Sushi Ginger	Con
16FS15_2017	Fukujin Zuke	Con
17FS16_2017	Tofu Chilli	Ferr
18FS17_2017	Daikon	Ferr
19FS18_2017	Pickled Bamboo	Con
20FS19_2017	Pickled vegetables	Con



Bread Kvass, Carrot Kimchi, Boza, Turnip, Orange, Krauthehi (sauerkraut), Tepache, Ginger Beer, Tempeh, Cucumber, Milk Kefir, Water Kefir, Tofu Chilli, Daikon, Pickled vegetables, Raw Sauerkraut & Juniper berries, Brown rice amazake, Beetroot Kvass, Kefir and fennel soup, Mead, Sauerkraut, Dill dearg (sauerkraut), Kimchi, Golden child (sauerkraut), Water Kefir Hibiscus, Water Kefir lemon, Water Kefir Ginger, Kombucha Vinegar, Sauerkraut, Kombucha, Apple Cider Vinegar, Water Kefir(Pear, Ginger & Honey, Water Kefir(Pear, Ginger & Sugar), Dilly Carrots, Brussel Sprout Kimchi, Kimchi, Garlic Kraut, Dukkah Kraut, Ginger Sliced in 2% Brine, Daikon Radish in 2% Brine, Okra in 2% Brine, Tomatoes & mustard seeds in 2% Brine, Kombucha, Cherry Water Kefir, Beet Kvass, Coconut Kefir, Carrot sticks, Lemon and Ginger Fizz, Scallion Kimchi



aster

Type	Origin
diment, Pickeld vegetables	England
eses, Dairy product	Cyprus
bage	Ireland
wn Rice	Japan
troot	Ireland
ry Soup	Ireland
mented honey	Ireland
bage	Ireland
rkraut	Ireland
bage	Ireland
rkraut	Ireland
erage	Ireland
erage	Ireland
erage	Ireland
uid condiment	Ireland
erage	Russia
erage	Russia
ick	Russia
eses, Dairy product	Russia
erage	Ireland

#### Leech et al Under review

## Microbial diversity across a range of fermented food



Leech et al Under review

izun zuzuresearch and innovation programme under grant agreement No 818368 This project has received running from the European onion's

#### **Fermented Foods – the Cheese Microbiome**



m)aster

#### Food and gut health





- Comparative genomics of MAGs obtained from fermented food and human gut metagenomes
- LAB strains transmission between food and gut microbiome
- Fermented foods as source of potential probiotics

			11.10 COM	LEPC MILLI	ICATIONS			-				
1			ARTS	CLE	tana separat	-	OWEN				Abrams	
			Lar	Large-scale genome-wide analysis links lactic acid bacteria from food with the gut microbiome								
			Edoard Jate Li	o Pasi Heith <sup>a</sup>	oli <sup>1,2</sup> , Francesca <sup>2</sup> , Paul D. Cotte	De Fi	ppis <mark>0 <sup>13</sup>, Italia E. Mau</mark> Nicola Segata <mark>0</mark> <sup>3</sup> & D	nelo", asilo B	Fabio Cumbe <mark>ro</mark> <sup>7</sup> , colini <mark>co</mark> Lona	Aaron	M. Watsh <sup>4,9</sup> ,	
irce	Food (Isolate)	• Food (MAG)	Other		Human	0	NA					
category	Newborn	Child	Schoolage		Adult	0	NA					
y site	Airways	Oral cavity	Skin		Stool		Vagina					
tinent	Africa	Asia	China		Europe		N. America		Oceania		S. America	

#### S. thermophilus

### Integrated microbiome technologies

WP5: Integrated Microbiome Technologies for the Food Chain Lead UNITN (Segata), Co-lead QUB (Creevey), partners WU, Teagasc, UCC, 4DC, ULE, ONT, FFOQSI, Novolyse, Qiagen, Baseclear, Danone in close interaction with WPs 1, 2, 3, 4 and 6

WP5 will provide the technological, computational, and analytical tools to (i) support the other WPs, (ii) establish standardized tools and procedures for companies in the food chain, (iii) meta-analyse the produced data, and (iv) build user- and company-friendly resources to support all the microbial tasks associated with the food chain.



**AGEN** 

BASECLEAR



### Integrated Microbiome Technologies for the Food Chain



Provide the technological, computational, and analytical tools to

- (i) **Support** the other parts of MASTER
- (ii) Establish standardized tools for companies
- (iii) Build user- and company-friendly resources to support all the microbial tasks associated with the food chain

(iv) Meta-analysis



### WP5: Integrated Microbiome Technologies for the Food Chain



## WP5: Integrated Microbiome Technologies for the Food Chain



m)aster

**Meta-analysis** of thousands of samples that will crucially highlight specific microbiome characteristics associated with environments, geography, specific foods, and safety characteristics

### **Advisory Boards**



<u>Rachel Dutton</u> <u>Bart C Weimer</u> <u>Jack Gilbert</u> <u>Mick Watson</u> <u>Linda Kinkel</u> <u>Curtis Huttenhower</u> Kelley Eversole Sandrine Miller Colette Shortt John Conroy Pamela Byrne Aine MackenWalsh







@worldmicrobiomeday

#WorldMicrobiomeDay; #DiversityMatters

Discover the diverse world of microbes...





www.worldmicrobiomeday.com







### Acknowledgements





#### Special thanks to Dr Mairead Coakley – Project Manager

#### The MASTER Consortium





This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 818368



@MASTER\_IA\_H2020 http://www.master-h2020.eu/





### Acknowledgements

**Aaron Walsh** John Leech **Raul Cabrera Rubio** Liam Walsh **Edoardo Pasolli Danilo Ercolini** Nicola Segata **Fiona Crispie Guerrino Macori** Aoife McHugh **Ben Bourrie Mairead Coakley** Laura Finnegan **Orla O'Sullivan** Marcus Claesson Paul O'Toole



microbio blochemistry

**Diarmuid Sheahan, Kieran Kilcawley** Alan Marsh, Paul Ross, Colin Hill Lisa Quigley, Ger Fitzgerald, Tom Beresford Marcel van de Wouw, Kieran Rea, John Cryan **Karen Scott (Aberdeen)** Ben Willing (Edmonton)

**Conor Feehily** Bhagya Jonnala **Cathy Lordan** Chloe Matthews Ciara O' Donovan Enriqueta Garcia Garreth Lawrence Tais Kuniyoshi Miguel Ullivari Min Yap Veronica Peterson Elhadji Seck Therese McNamee Eugenia Jimenez Paula O'Connor Arghya Mukherjee Sheila Morgan

Dara Leong **Trudy Quirke Dinesh Thapa** Flaine Lawton Grace O'Callaghan **Gwynneth Halley** Sara Arbulu Ruiz Serena Boscaini Wiley Barton Calum Walsh Katie Fala Laura Wosinska Natan Pimentel Beatriz Gomez Sala











