REPORT OF FEMS CONGRESS
9-13 July 2017

This year, the 7th congress of European microbiologists was held in Valencia, Spain.

List_MAPS participated to this major international event from the 9th to the 13th of July 2017, along with more than 2 000 participants from all over the world. What a nice opportunity to network with scientists from 90 countries.

This event, organised by the Federation of European Microbiological Societies was a real opportunity for the ESRs to meet internationally renowned experts. Many aspects and current hot topics in the field of Microbiology were addressed during morning plenary sessions and many topical parallel sessions. As such, it was a major opportunity for the ESRs to have an overview of the latest developments in this field of research.

As a network, List_MAPS has taken this opportunity to disseminate results and achievements. Indeed, List_MAPS members presented in total 10 posters and 2 oral presentations.

All ESRs and PIs enjoyed science and social gatherings in sunny Valencia. Indeed, a nice way to start summertime!

Pascal Piveteau, coordinator of List_MAPS

POSTERS

On Monday 10th

Impact of dietary fat content on host susceptibility to *Listeria monocytogenes* infection

V. de Las Heras, K. Govindarajan, S. Joyce, P. Casey, R. Rubio, P. Cotter, C. Hill, C. Gahan

Backgrounds

Currently the world is facing an escalation in cases of obesity, influenced by an increase in consumption of elevated levels of saturated fats and sugar, the so-called Western-diet. However the impact of these dietary changes on host susceptibility to foodborne pathogens such as *Listeria monocytogenes* is currently unclear.

Objectives

In this project, using an in vivo systems biology approach, we explore the host response to oral *Listeria monocytogenes* infection following murine exposure to defined rodent diets that mimic low-fat or high-fat (Western) diets.

Methods

Mice were fed a control chow diet, a low-fat diet (10% of caloric intake from fat) or a high-fat diet (45% of caloric intake from fat) throughout the experiment. The total bacterial load from the organs at 3 days post-infection was used to characterize *L. monocytogenes* infection. Microbiota analysis (16S DNA sequencing) and host transcriptome analysis were performed to explore the impact of the dietary fat content on the host.

Conclusions

Total bacterial loads from the organs harvested 3 days post-infection show an increased susceptibility to *L. monocytogenes* in mice receiving a high fat diet. This increased susceptibility to infection was associated with significant changes to the composition of the gut microbiota, physiological changes in the gut barrier and immune suppression caused by increased dietary fat content. The findings indicate that diet mediates physiological changes to the host, that can significantly influence susceptibility to infection with *L. monocytogenes*, suggesting that diet should be considered as an important factor in future models of *L. monocytogenes* pathogenesis.

The effects of growth conditions and secondary environmental stresses on the response of *Listeria monocytogenes* to visible light

A. Dorey, B. O'Donoghue, K. NicAogáin, C. O'Byrne

Backgrounds

Research investigating the response of *L. monocytogenes* to visible light has identified the Lmo0799 protein in the sensing of visible light, and the activation of the SigB signalling cascade. Triggering of the σB signalling cascade leads to the transcription of the general stress response gene regulon via the activation of σB. Growth of *L. monocytogenes* in alternating periods of light and dark results in a ringed colony morphology, preventable by the deletion of sigB or lmo0799 from the genome. The inhibitory

effect of visible light on *L. monocytogenes* is due to accumulation of reactive oxygen species, and exposure to visible light induces a protective response against a challenge by ROS.

Objectives

The aim of our study is to elucidate the role of σB in the resistance of *L. monocytogenes* to visible light using transcriptomic and phenotypic experiments.

Methods

L. monocytogenes wild-type and ΔsigB deletion mutant cultured at 30°C or 37°C were challenged with visible light at exponential and stationery growth phases. Cells were pre-exposed to sub-lethal doses of environmental stresses to investigate cross-resistance between visible light and alternative stresses. RT-PCR targeted at σB-dependent genes was used to determine the optimum dose and exposure time for the activation of σB by visible light.

Conclusions

Our research shows that growth phase and temperature affect susceptibility of *L. monocytogenes* to visible light, with cells being more susceptible at exponential compared to stationery phase and at 37°C compared to 30°C, and the role of σB in the protective response. Some environmental stresses convey resistance to visible light.

Glycerol metabolism induces biofilm formation at the air-liquid interphase in *Listeria monocytogenes*

N. Crespo Tapia, M.W. den Besten, T. Abee

Backgrounds

Listeria monocytogenes is a microaerophilic food-borne pathogen that can grow as a biofilm on the surface of food-processing equipment. Bacterial biofilms vary greatly in structure and characteristics, and can be influenced by a wide range of environmental conditions.

Objectives

Although motile aerobic bacteria have been described to form biofilms at the air-liquid interphase of cell cultures, to our knowledge, this type of biofilm has never been described in *L. monocytogenes*. In this study we report *L. monocytogenes* biofilm formation at the air-liquid interphase of aerobically grown cultures, and that this phenotype is specifically induced when the media is supplemented with glycerol as a carbon/energy source.

Methods

A link with aerobic glycerol metabolism was confirmed by the analysis of *L. monocytogenes'* performance under anaerobic conditions, where biofilm production was reduced to the same level as the non-supplemented control, and it was located at the bottom of the well. Planktonic growth performance, metabolic activity assays and HPLC measurements of glycerol consumption over time showed that glycerol utilization in *L. monocytogenes* is restricted to growth under aerobic conditions. Additional motility assays revealed the induction of aerotaxis in the presence of glycerol. Gene expression analysis will provide further insight in parameters involved in glycerol metabolic pathway(s), aerotaxis and biofilm formation.

Conclusions

We hypothesize that the formation of biofilms at the air-liquid interphase is dependent on glycerol-induced aerotaxis towards the surface of the culture, where *L. monocytogenes* has access to higher concentrations of oxygen, and is therefore able to utilize this compound as a carbon source.

On Tuesday 11th

SigB and AgrA regulation in *Listeria monocytogenes*: effect on survival in soil/rhizosphere under biotic and abiotic conditions

C. Marinho, D. Garmyn, L. Gal, C. O'Byrne, P. Piveteau

Backgrounds

Listeria monocytogenes is the agent of listeriosis, a life-threatening condition in at-risk people. Complex transmission routes between outdoor environments and the food chain result in foodstuff contamination. Sensing of environmental changes can trigger regulation of gene expression, allowing bacteria to adapt their physiology and survive. The Agr cell-cell communication system transcription regulator AgrA is triggered during several environmental conditions including soil, an important reservoir of L. monocytogenes. The RNA polymerase σB factor aids survival in several stress conditions and may be required for *L. monocytogenes* survival in the soil environment.

Objectives

This study aims to investigate the involvement of AgrA and σB in the regulatory network of *L. monocytogenes* during saprophytic life in soil and rhizosphere according to the background biotic environment.

Methods

A collection of in-frame deletion mutant strains (ΔagrA, ΔσB and ΔagrA+ΔσB) was constructed from parental *L. monocytogenes* EGD-e. Strains were inoculated into clay soil mesocosms at different water holding capacities and with or without background microbiota. Kinetics of strains survival was followed during incubation for 14 days. Growth was investigated in the rhizosphere of Festuca arundinacea plants in vitro. One-week kinetics of strains survival was performed during incubation into climatic chamber.

Conclusions

Depending on the incubation conditions, the fitness of the deletion mutants were affected. During its saprophytic life in soil habitat, *L. monocytogenes* have to cope with ever-changing environmental conditions and adapt in order to sustain life. Integration of various stimuli results in a coordinated response including communication and stress response systems through AgrA- and σB-mediated regulation.

Cold stress induced biofilm formation of *Listeria monocytogenes* strains

B. Lee, T. Bernardi, M. Herbraud

Backgrounds

Listeria monocytogenes can efficiently survive even in extreme conditions where many other bacteria cannot withstand. Under the assumption that biofilms in food premises leads to food contamination of *L. monocytogenes*, studies have primarily compared the effect of temperature on biofilm formation by growing the bacteria at different temperatures most frequently from 4°C to 37°C and showed that *L. monocytogenes* strains are able to survive at low temperature and form biofilms. However, it is still difficult to directly compare the quantity of biofilms formed at different temperatures since the optimal growth temperature around 37°C involves higher cell growth and enzymatic activities involved in cellular physiology, even though various stress factors including low temperature are known to trigger biofilm formation of *L. monocytogenes*.

Objectives

In this study, we used 22 *L. monocytogenes* strains, of diverse origins and molecular serotypes, to investigate (i) effect of incubation temperatures on cell surface property and its influence on biofilm formation and (ii) effect of cold stress on biofilm formation by comparing biofilms formed upon cold stress and after cold adaptation.

Methods

We applied crystal violet staining to quantify the total biomass and an innovative technique called BioFilm Ring Test® to measure early step of biofilm formation, namely 'adhesion' to surfaces. To confirm the biofilm formed at different conditions, we employed scanning electron microscopy.

Conclusions

We confirmed that cold stress enhanced the biofilm formation of all 22 strains. However, there was no correlation between cell surface characteristics and total biomass or adherence of *L. monocytogenes.*

On Wednesday 12th

Effect of habitat disturbances on the population dynamics of allochthon *Listeria monocytogenes* in soil

A.R. Ortiz Camargo, A. Spor, S. Gaba, D. Garmyn, L. Gal, P. Piveteau

Backgrounds

Soil is in many cases the first stage in the routes of transmission of foodborne pathogens to plant, farm animals, foodstuff and humans as final consumers. Soil is a complex, heterogeneous environment which shelters many organisms such as allochthon bacteria. The persistence of these organisms depends on abiotic factors (temperature, humidity, texture, chemistry) and on biotic interactions, for example competition with native microflora.

Objectives

In this study, we used the foodborne pathogen *Listeria monocytogenes* as model system to investigate how disturbances affect habitat invasion by allochthon organisms.

Methods

Two soils with contrasting abiotic and microbiome characteristics were used. Inoculated soil microcosms were submitted to two cycles of temperature shifts (either increase to 42°C or freezing at -20°C) separated by 20 days of incubation at 20°C. Control microcosms were kept at 20°C. In order to investigate the impact of the indigenous microbiota, similar experiments were run in γ-irradiated, sterilized soil microcosms. Cultivable *L. monocytogenes* were evaluated by plate counts throughout the 40 days incubation. Additionally, soil samples were taken at the start of the experiment and after 20 and 40 days for DNA extraction and subsequent 16SrDNA diversity analysis. Variations of diversity were assessed in non-inoculated microcosms to evaluate the influence of *L. monocytogenes* invasion on the native soil microbiota.

Conclusions

While growth was observed in sterilized soil, the population of *L. monocytogenes* decreased in the other experimental conditions. Results showed that the fate of allochthon *L. monocytogenes* depended on the disturbance regimen. Higher survival was observed in when soil underwent cycles of freezing

Ecology of *Listeria monocytogenes* in soil: effect of the biotic environment on survival and transcriptome reshaping

L. Gal, A. Ortiz Camargo, D. Garmyn, **P. Piveteau**

Backgrounds

Listeria monocytogenes is a bacterium found in many habitats such as soil, plants, animals, foodstuff and food processing facilities. Circulation between habitats is a source of its transmission to food and eventually to the consumer. Contaminated foodstuff is indeed the vector of listeriosis, a life-threatening disease mainly to immunocompromised people and pregnant women. One of the intriguing facets of *Listeria monocytogenes* is its ability to adapt its physiology to complex, heterogeneous habitats. With its complex chemistry, texture, dense microbiota and overall biotic fraction, soil is a nice example of such heterogeneous habitat. Persistence in many habitats suggests that *L. monocytogenes* is able to integrate a range of environmental cues in the circuitry of regulation of transcription.

Objectives

This study aimed at assessing in one hand extrinsic factors that shape the fate of *L. monocytogenes* in soil, and in the other hand the response of *L. monocytogenes* to the biotic environment found in soil.

Methods

The response of *L. monocytogenes* EGD-e to the biotic fraction of soil was investigated in irradiated and untreated microcosms through a combination of transcriptomic approaches and population dynamics.

Conclusions

The fate of *L. monocytogenes* is dependent on both abiotic and biotic characteristics and the latter have a major impact on the dynamics of the populations of *L. monocytogenes* in soil. Major transcriptome reshaping was observed where L. monocytogenes recruits its repertoire of transporters and specific pathways to access and utilise the available substrates. The biotic environment further affects transcriptome and triggers further regulation.

The expression of the chitinolytic system of *Listeria monocytogenes* is subject to different regulation depending on carbon source utilisation including cellobiose and glucose

M. Villoria Recio, M. Halberg Larsen, H. Ingmer

Backgrounds

Listeria monocytogenes is a food-borne pathogenic bacterium which can cause fatal infections. Outside the host, this bacterium inhabits terrestrial and marine environments where chitin polymer is abundantly found and it serves as carbon and nitrogen source to chitinolytic bacteria. In *L. monocytogenes*, the chitinolytic system comprises two chitinases, ChiA and ChiB and a lytic polysaccharide monooxygenase (LPMO10). The chitinases are expressed during growth in soil supporting their role in environmental survival. Despite the absence of chitin in mammalian hosts, the chitinolytic system is also important for infection. The regulation of chitinases is complex and includes several central regulators, namely PrfA, σB, agr and Hfq.

Objectives

The objective of our study is investigating the regulatory mechanisms and induction cues of the chitinolytic system in order to better understand its role in the different life modes of Listeria.

Specifically, the role of different carbon sources on the expression of the chitinolytic system was studied and related to virulence.

Methods

The expression of chiA, chiB, LmLPMO10 and actA was measured quantitatively by qRT-PCR upon addition of chitin, glucose and cellobiose to wildtype cells and mutants lacking the chitinases or prfA. Bacterial cells were grown in microtiter wells and growth was followed by OD measurements.

Conclusions

Chitin is a strong inducer of the chiB gene in a low-carbon content medium. Whilst glucose can only support chiB induction in stationary phase in a chemically defined media, this study reveals that the addition of cellobiose reverts this effect and gives a new insight on the regulation of the chitinases.

Development of a MALDI Imaging Mass Spectrometry approach to bacterial proteomics: first application to *Listeria monocytogenes* biofilms exposed to dessication

T. Santos, D. Centeno, C. Chambon, D. Viala, M. Hébraud

Backgrounds

Human listeriosis cases are due to the ingestion of contaminated foods with *Listeria monocytogenes* and most cases are connected with food contamination in industries. The control of *L. monocytogenes* is difficult to achieve in processing environments due to its survival capabilities. Water availability has particular biological importance and bacteria are submitted to variations in air relative humidity (RH) in food processing plants. This bacterium is also able to grow as a biofilm but the underline features as how biofilms adapt to desiccation are not well-known. Matrix-assisted laser desorption/ionization time-of-flight imaging mass spectrometry (MALDI-TOF IMS) is a surface-sampling technology that can determine spatial information and relative abundance of analytes directly from biological samples. Spectra are collected and each peak intensity in the spectra is used to generate an ion intensity map.

Objectives

This study aims to develop an IMS approach to explore the protein expression and in situ distribution of proteins within *L. monocytogenes* biofilms exposed to desiccation.

Methods

L. monocytogenes biofilms were grown in MCDB medium during 48h before being exposed to a moderate desiccation environment (24h at 10ºC/ 75% RH), mimicking the food workshop conditions. After matrix spraying, mass spectra were acquired on a MALDI-TOF/TOF MS and processed through SCiLS software.

Conclusions

Data analyses allowed to distinguish protein localization patterns between the two conditions and chose target mass peaks for further analysis. These data demonstrate how imaging can be used to dissect the spatial proteome of an intact bacterial biofilm giving a new insight into protein regulation relating to biofilm adaptation.

A family of sRNAs plays a role in the response of *Listeria monocytogenes* to heme toxicity

P. T. dos Santos, D. Sabharwal, P. Menendez-Gil, E.M. Sternkopf Lillebæk, B. H. Kallipolitis

Backgrounds

At present, over 200 putative small non-coding regulatory RNAs (sRNAs) have been identified in Listeria monocytogenes. Interesting, several sRNAs have been identified as being induced in human blood. *L. monocytogenes* has the ability to lyse erythrocytes, remove heme from hemoglobin and liberate Fe2+ from heme. Even though iron is essential for life, it is highly toxic under aerobic conditions, as it reacts with oxygen species forming free radicals. Thus, *L. monocytogenes* needs to find a way to overpass the unfavorable conditions it encounters in the presence of excess heme.

Objectives

To understand why some sRNAs are highly induced in human blood, we hypothesized that this induction could be caused by high levels of heme in this environment. Therefore, the aim was to investigate the role of heme-induced sRNAs in response to excess heme and look for their putative targets in *L. monocytogenes*.

Methods

Wild-type cells were subjected to increasing concentrations of hemin and sRNAs levels were determined via Northen Blot analysis. To verify their role in the prevention of heme toxicity, growth of a strain lacking sRNAs was compared to the wild-type strain. Finally, a search was performed to identify possible targets of the sRNAs under hemin stress.

Conclusions

A family of sRNAs was greatly induced by hemin, and a strain lacking the sRNAs showed impaired growth in the presence of hemin, suggesting a fine-tuning role for these sRNAs in the prevention of heme toxicity. Putative target genes were identified and the mechanisms underlying the regulation by the sRNAs are under investigation.

ORAL PRESENTATIONS

On Monday 10th

Improved promoter sequence models for de novo transcription factor binding site prediction in bacteria

I. Sultan, S. Schbath, V. Fromion, P. Nicolas

Backgrounds

Unravelling gene regulatory networks helps understanding many features about an organism. Over the past decades, powerful algorithms and approaches have been developed for the discovery of transcription factor binding sites (TFBSs) but these tools are still unable to automatically identify de novo the main regulons of a bacteria from genomic and transcriptomic data.

Objectives

In this work, we present a new statistical model and Markov Chain Monte Carlo (MCMC) algorithm that attempt to overcome some limitations of the available approaches. In particular, available algorithms for motif discovery based on mixture models do not take into account the overlapping of

TFBSs. Whereas, available data on Escherichia coli suggest that overlapping is a major feature of bacterial promoter architecture (more than one third of the binding sites overlap with at least one other site). Moreover, with the advent of precise transcription start site (TSS) maps derived from RNA-Seq protocols targeting transcript 5'-ends, it is now relevant to examine the exact position in the promoter region when searching for TFBSs.

Methods

Our statistical model of promoter sequences accounts for the overlapping between TFBSs as well as their exact positioning with respect to the TSS. All the parameters are estimated in a Bayesian framework using a dedicated MCMC algorithm. The algorithm is trans-dimensional to allow adjusting the width of position weight matrix describing each motif and the number of parameters to describe its preferred position.

Conclusions

Results on *Listeria monocytogenes* regulatory network will be presented and strategies to incorporate expression data across conditions will be discussed.

On Tuesday 11th

Adaptive response of *Listeria monocytogenes* biofilms to a dehumidification stress

J. Esbelin, C. Chambon, D. Viala, **M. Hébraud**

Backgrounds

Listeria monocytogenes is a foodborne pathogen able to adhere and form biofilms on various surfaces. Associated with a high mortality rate, it is one of the major biological concerns in food hygiene. Since few years, industries attempt to reduce the environmental impact of hygiene operations in the workshops of refrigerated food processing, through optimized use of dehumidification after cleaning disinfection treatments

Objectives

Our study was focused on the adaptive response of *L. monocytogenes* cells, growing in biofilms, to a dehumidification stress mimicking food plants conditions.

Methods

The intracellular and surfaceome subproteomes were analyzed by shotgun proteomics through three complementary extraction methodologies (enzymatic shaving, biotinylation and cell fractionation). *L. monocytogenes* EGD-e and L028 biofilms were grown on stainless steel discs at 25°C during 24h, pre-adapted to 10°C and placed in a desiccation chamber where the air Relative Humidity was stabilized to 75%. The different subproteomes were analyzed after 3h and 24h dehumidification by comparison with non-stressed biofilms.

Conclusions

Among the surface proteins identified, 21 and 29 were differentially expressed during dehumidification for EGD-e and L028, respectively. Three of them were common to both strain, an autolysin, Iap and an ABC transporter. Among intracellular proteins, 38 and 65 were differentially expressed in EGD-e and L028 respectively. Five proteins were common to the two strains. The majority of these proteins belongs to information pathway (35%) and intermediary metabolism (25%) functional categories. These results could contribute to a better comprehension of mechanisms involved in the resistance and persistence of this pathogen in food plants despite the daily hygiene procedures.

TESTIMONIES OF ESRs

ESR 8: Catarina MARINHO, *University of Burgundy and National University of Ireland, Galway*

“ FEMS Microbiology Conference 2017 was **one of the biggest international conferences I've ever attended**, though its size was not the only reason why I considered this a special event. Indeed, due to its massive dimension it gathered **thousands of scientists** from all over the world that **share the same passion for microbiology**.

Therefore, it was a great opportunity to present some of my results as a poster there and discuss ideas about my research with several peers.

Besides networking, **it was also an incredible opportunity** for attending numerous interesting talks and update my knowledge on what has been done recently in microbiology worldwide. Moreover, on a professional level, FEMS also represented an important landmark on my early career since **I was awarded as one of the 5 European Best Papers**.

Overall, I consider that attending FEMS was very profitable for my career that boosted my enthusiasm and commitment to science. ”

ESR 5: Tiago SANTOS, *INRA unit MEDIS*

“ **I had a great scientific experience** attending 2017 congress from The Federation of European Microbiological Societies. It was very exciting to be surrounded by the sheer number of posters and top-notch microbiologists from across the world.

It was also **a great occasion to present and discuss my most recent data with colleagues**. Having the whole List_Maps network attending the congress it was important to stay in touch and also to improve our networking abilities. Since the venue was held in Valencia, besides the fulfilling scientific talks, we had a chance to profit from the sunny and gastronomically rich city!

If I have the chance, for sure I will attend FEMS 2019 in Glasgow. ”

ESR 7: Patrícia DOS SANTOS, *University of Southern Denmark*

"FEMS Conference 2017 was held in Valencia, from July 9th-13th. I was very excited about attending a FEMS conference because **I knew that there would be a great number of people from around the world** and a wide variety of scientific and social backgrounds. And of course, the warm Spanish summer would be the perfect igniter for the many discussion in different topics on Microbiology.

The venue had immediately a great impact on me: the beauty of the building did announce a major event. The first day was full of excitements, from getting the name tag and look for our name in the wall, to the opening session in a room completely packed with people! In the following days, **I had the chance of listening inspiring talks from renowned speakers**.

I must confess that this kind of events can be a bit overwhelming as too many things happen at the same time and it is difficult to choose the right talks to attend. Still, **it was fantastic to witness and discuss the ongoing research done** by the inspiring scientists we have around the world. This was definitely a great experience, and I believe all PhD students should have the possibility of experiencing such an event to become aware of the different Microbiology Research Areas and the current "hot" topics."

ESR 6: Natalia Crespo Tapia, *Wageningen University*

"FEMS 2017 was the second conference that I have attended so far during my career. It was a different experience compared to the ISOPOL conference, which was *Listeria*-based and therefore more specific. In FEMS **I had the opportunity to learn about a wide variety of topics in Microbiology**, that I probably wouldn't otherwise touch upon during my PhD research.

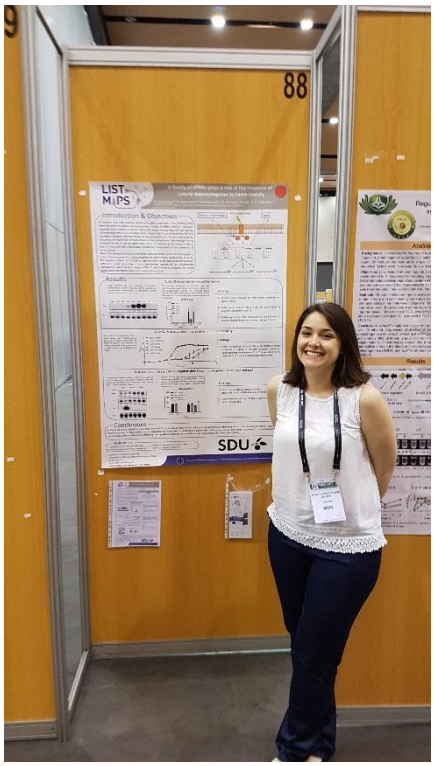
As a Medical Microbiologist by career who is now working closer to the Food Microbiology area, **it is very important for me to keep on track with the research going on in other fields that I find interesting, even if they are not directly related to my current work.**

Having said that, **I was very glad to find two full sessions that focused mainly on bacterial biofilms.** These talks were particularly helpful for me, as I they helped me come up with some ideas that I could apply to my work later on.

Finally, even though FEMS is an international event, the fact that this year's conference was based on Valencia also gave me **the chance to learn about the current research going on in my country.**"

PHOTOS OF THE EVENT

Some selected photos of the event:



ESR7 Patrícia Dos Santos

ESR3 Miguel Villoria Recio

From left to right: Vanessa Las Heras (ESR2), Marianne Halberg Larsen, Tiago Santos (ESR5), Catarina Marinho (ESR8), Laurent Gal, Patrícia Dos Santos (ESR7), Michel Hébraud, Amber Dorey (ESR4), Miguel Villoria Recio (ESR3), Natalia Crepo Tapia (ESR6), Bohyung Lee (ESR11)

ESR9 Ibrahim Sultan

See all the pictures on the [Google plus account]

LIST OF PARTICIPANTS

University of Burgundy:

1. Pascal Piveteau
2. Laurent Gal (AgroSup Dijon)
3. Dominique Garmyn

University College Cork:

4. Vanessa Las Heras

University of Copenhagen:

5. Marianne Halberg Larsen
6. Miguel Villoria Recio

National University of Ireland, Galway:

7. Catarina Marinho
8. Amber Dorey

INRA unit MEDIS:

9. Tiago Santos
10. Michel Hébraud

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11. Islam Ibrahim Sultan

Wageningen University:

12. Natalia Crespo Tapia

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