



XLVI Congresso Nazionale

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AMCLI

11 - 14 Novembre 2017

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Palacongressi di Rimini



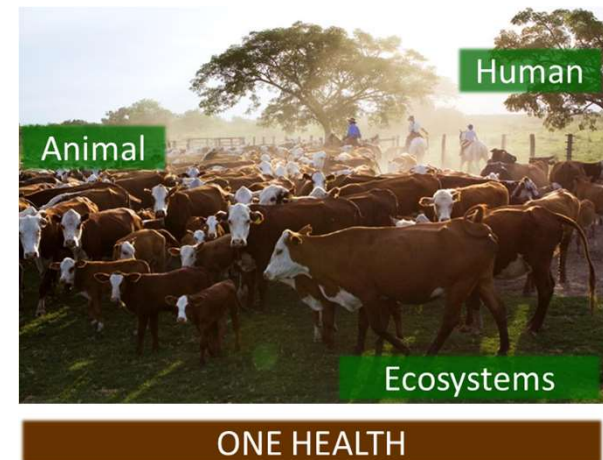
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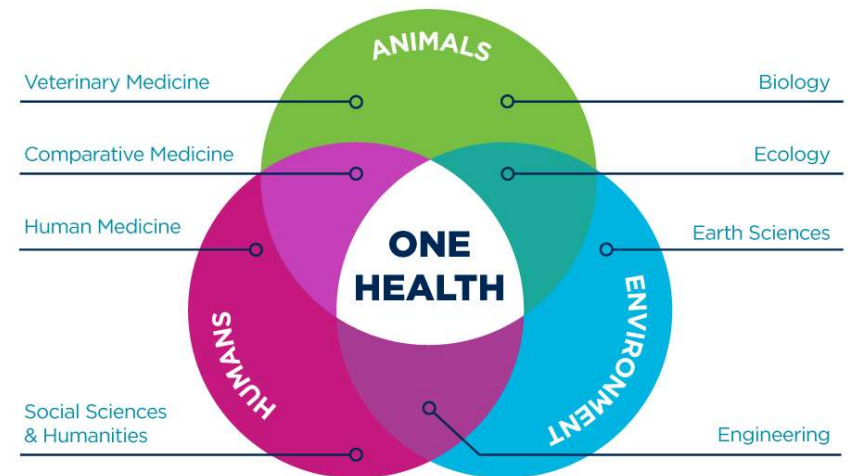
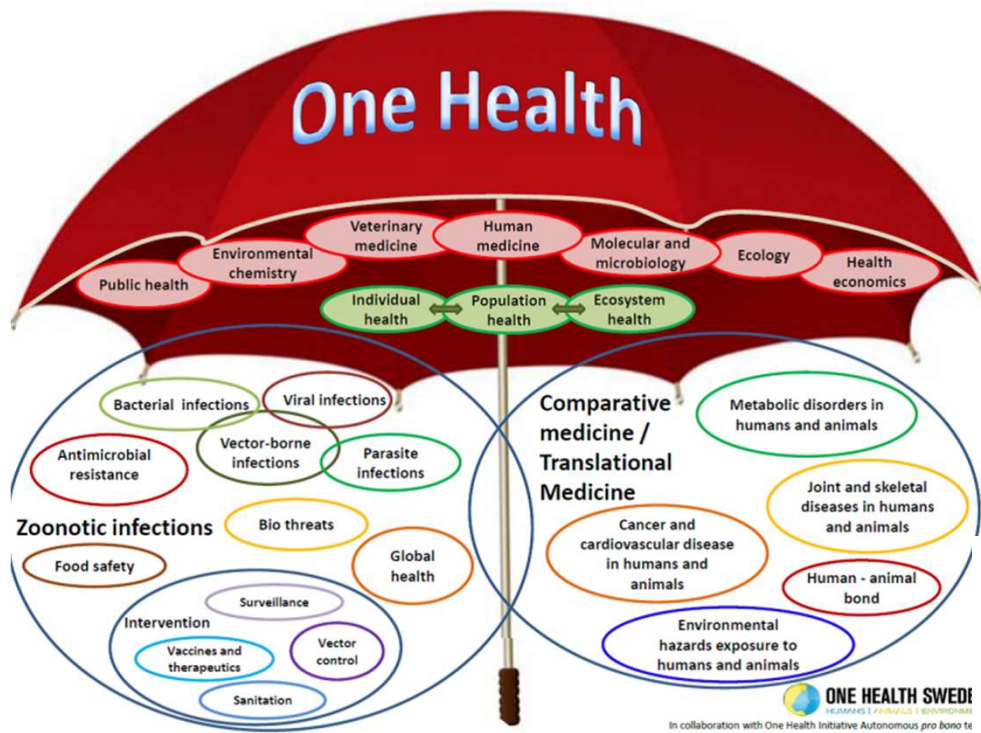
# Dalla salute animale alla sicurezza alimentare: la proteomica nell'approccio *One Health*

- Alessio Soggiu , PhD
- Dipartimento di Medicina Veterinaria, Università degli Studi di Milano



Il concetto "One Health" è stato introdotto all'inizio del 2000. questo riassume un'idea che è conosciuta da più di un secolo; che la salute umana e la salute degli animali siano interdipendenti e vincolati alla salute degli ecosistemi in cui esistono.







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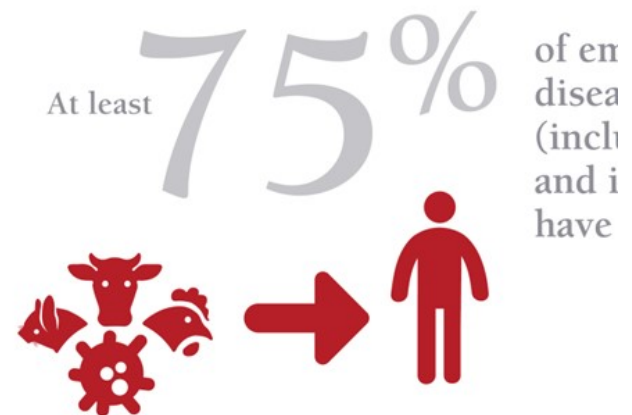
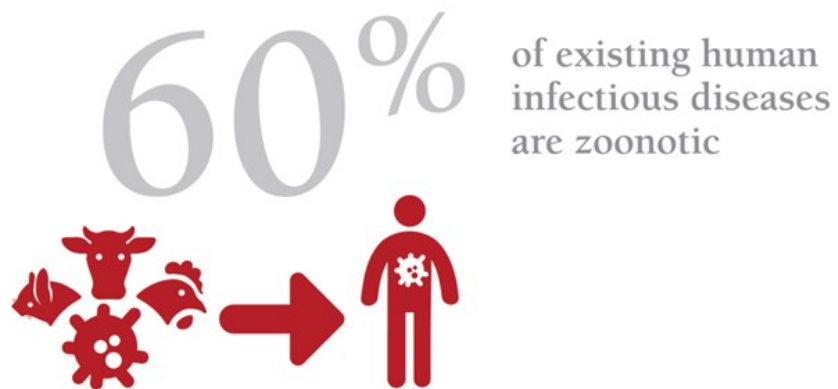
Roma, 14 settembre 2017, firma del Ministero della salute e dell'Organizzazione Mondiale della sanità animale (OIE) di un Memorandum of Understanding per la collaborazione nella promozione dell'approccio One Health alla sanità pubblica e per la sicurezza alimentare



Firma dell'accordo, sottoscritto dal delegato OIE italiano, Capo dei servizi veterinari, Silvio Borrello e dal Direttore Generale dell'Organizzazione Mondiale della sanità animale (OIE) Monique Eloit



# worldwide



## WORLD HEALTH

- **60%** of pathogens that cause human diseases come from domestic animals or wildlife.
- **75%** of emerging human pathogens are of animal origin.
- **80%** of pathogens that are of concern for bioterrorism originate in animals.



## FOOD SECURITY



- **More than 70%** <sup>[1]</sup> additional animal protein will be needed to feed the world by 2050.
- Meanwhile, **more than 20%** of animal production losses in the world are linked to animal diseases.

## ENVIRONMENT



Understanding the **connections** between biodiversity, ecosystems and infectious diseases is crucial.

## ECONOMY



- Animal diseases pose a direct threat to the **incomes** of rural communities that depend on livestock production.
- **More than 75%** <sup>[2]</sup> of the billion people in the world who live on less than \$2 per day depend on subsistence farming and raising livestock to survive.

<sup>[1]</sup> FAO, 2011. World Livestock 2011 - *Livestock in food security*.

<sup>[2]</sup> FAO & OIE, 2015. *Global control and eradication of peste des petits ruminants Investing in veterinary systems, food security and poverty alleviation*.



# ONE HEALTH

BY PROTECTING ANIMALS, WE PRESERVE OUR FUTURE

Animal and human sectors work together to protect health and ensure food safety and security

60%

of human pathogens are of animal origin

5

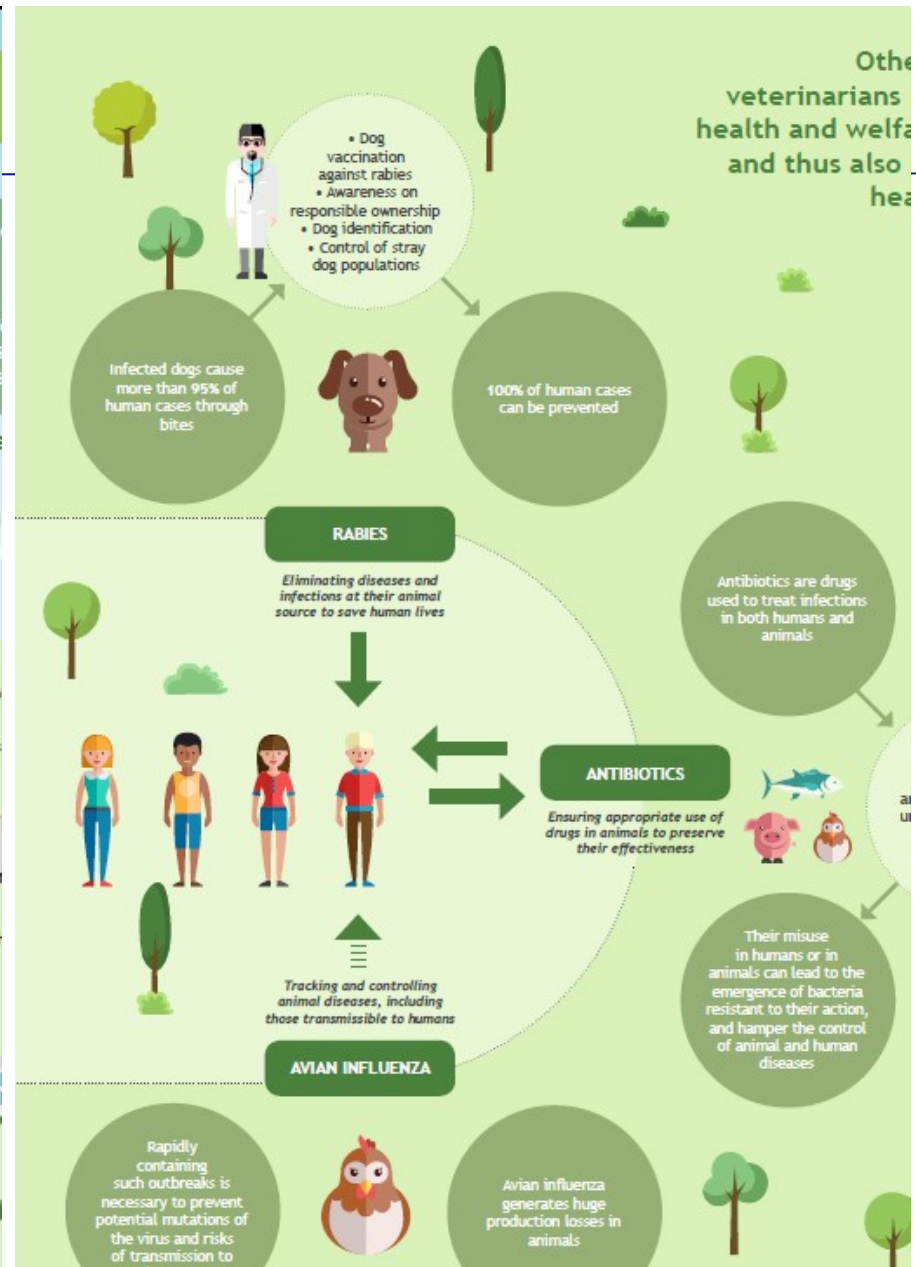
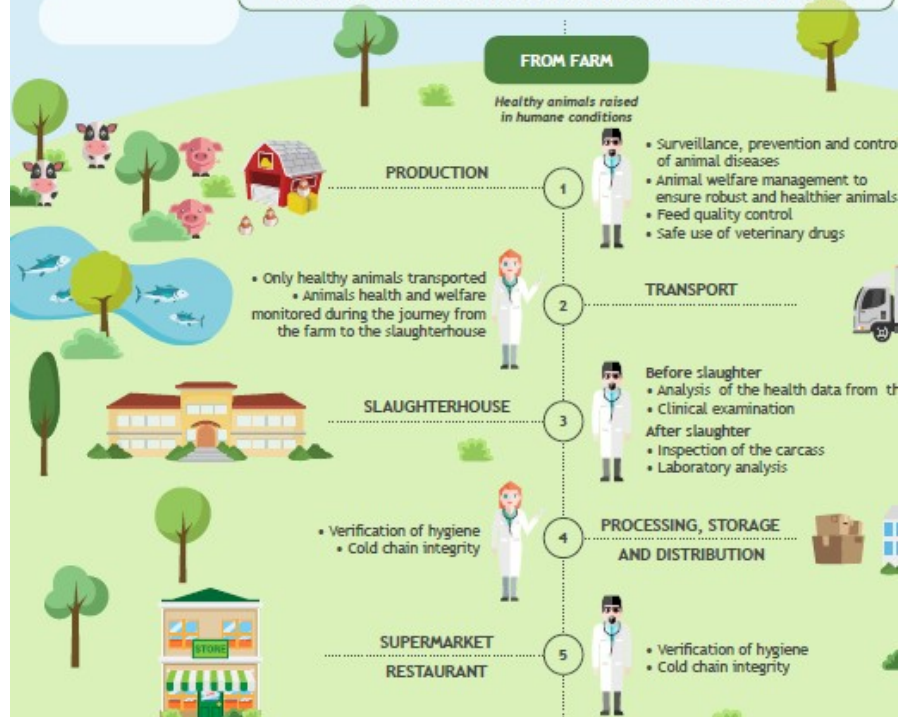
new human diseases appear each year

20%

of lost

With regards to animal health, veterinarians are key players of the 'One Health' concept

Early detection of diseases and infections at animal source can prevent their transmission to humans or introduction of pathogens into the food chain

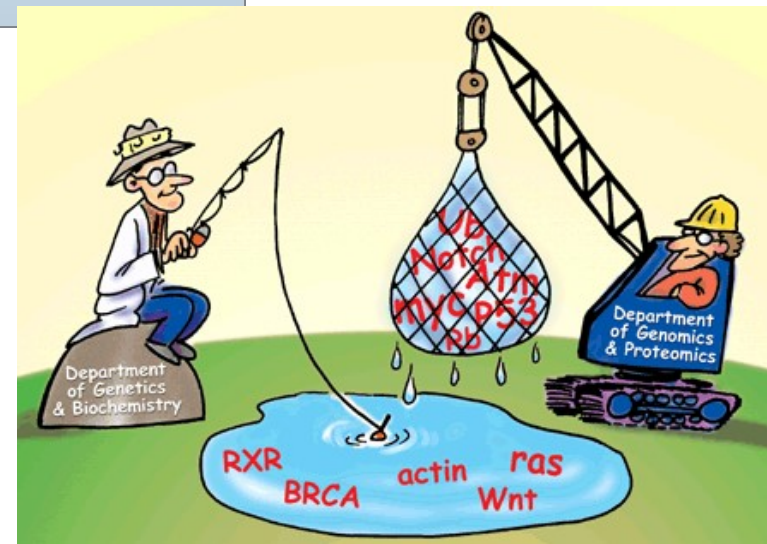
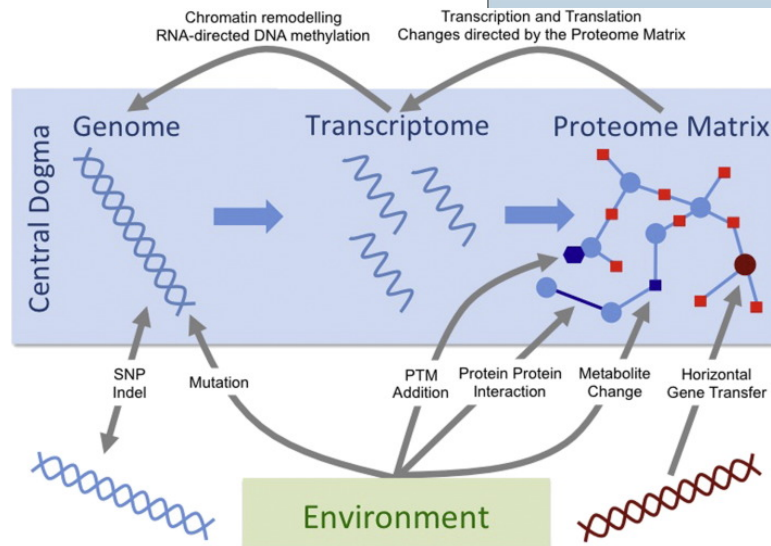
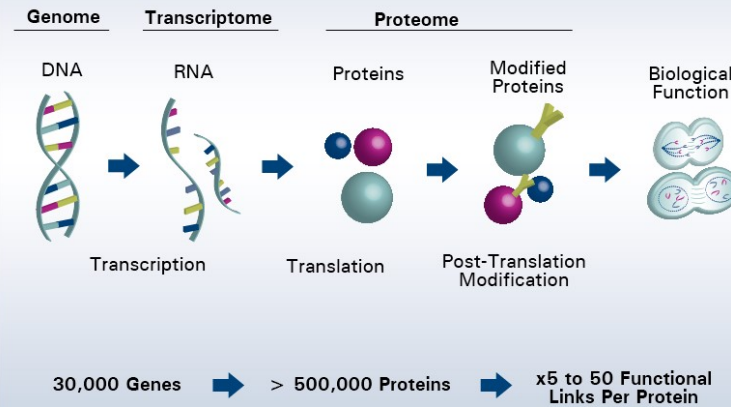


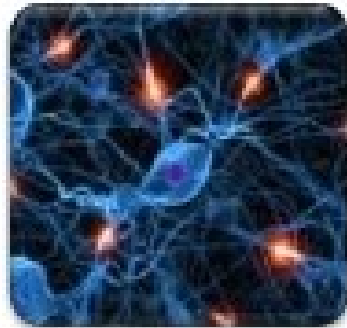
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# Proteine e Proteomica

## Proteins Are Critical to Understanding Disease





Cellular &  
Genetic Targets

Genomics

**Proteomics**

Bioinformatics

# Proteomics:

It is the study of the proteome, the complete set produced by a species, using the technologies of large – separation and identification.

It is becoming increasingly evident that the complexity systems lies at the level of the proteins, and that genome not suffice to understand these systems.

It is also at the protein level that disease processes become and at which most (91%) drugs act.

Therefore, the analysis of proteins (including protein-protein, nucleic acid, and protein ligand interactions) will be utmost to target discovery.



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Proteomics is a key piece that helps to complete the puzzle.

## Microbial proteomics



Molecular  
BioSystems

EDITORIAL



**One medicine – one health – one biology and many proteins: proteomics on the verge of the One Health approach**

Cite this: *Mol. BioSyst.*, 2014, 10, 1226

Paola Roncada,<sup>a</sup> Alessandra Modesti,<sup>b</sup> Anna Maria Timperio,<sup>c</sup> Luca Bini,<sup>d</sup> Massimo Castagnola,<sup>ef</sup> Mauro Fasano<sup>g</sup> and Andrea Urbani<sup>\*hi</sup>



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# Immunoproteomica e zoonosi

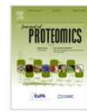
- Paratubercolosi
- Mastite
- Brucellosi



Journal of Proteomics

Volume 75, Issue 14, 19 July 2012, Pages 4412–4428

Special Issue: Farm Animal Proteomics



Proteomics of inflammatory and oxidative stress response in cows with subclinical and clinical mastitis ☆

Romana Turk<sup>a</sup>, Cristian Piras<sup>b</sup>, Mislav Kovačić<sup>c</sup>, Marko Samardžija<sup>d</sup>, Hany Ahmed<sup>e</sup>, Michele De Canio<sup>f</sup>, Andrea Urbani<sup>g</sup>, Zlata Flegar Meštrić<sup>h</sup>, Alessio Soggiu<sup>e</sup>, Luigi Bonizzi<sup>g</sup>, Paola Roncada<sup>i</sup>



BrucMedNet



Improvement of epidemiological and serological tools for diagnosis and control of *Brucella* in the Mediterranean region  
(2016 – 2019)



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# PROTEOMICS

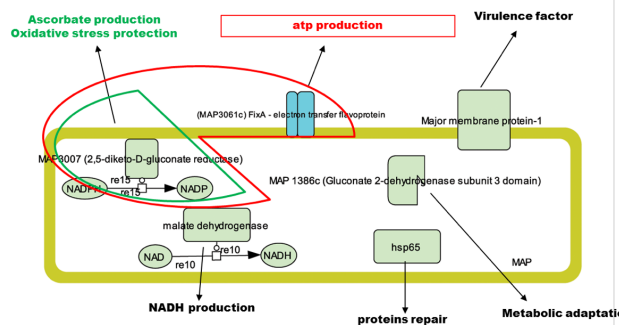
Proteomics 2015, 15, 813–823

DOI 10.1002/pmic.201400276

RESEARCH ARTICLE

## Identification of immunoreactive proteins of *Mycobacterium avium* subsp. *paratuberculosis*

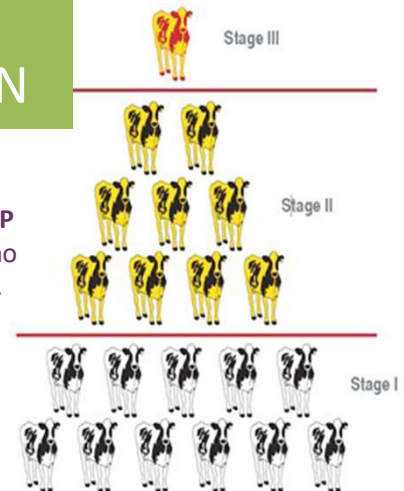
Cristian Piras<sup>1</sup>, Alessio Soggiu<sup>1</sup>, Luigi Bonizzi<sup>1</sup>, Viviana Greco<sup>2,3</sup>, Matteo Ricchi<sup>4</sup>, Norma Arrigoni<sup>4</sup>, Anna Bassols<sup>5</sup>, Andrea Urbani<sup>2,3</sup> and Paola Roncada<sup>1,6</sup>



## THE ICEBERG PHENOMENON

9

Infected, shedding MAP but showing no clinical signs.



11

Infected but showing no clinical signs and not shedding MAP

EUPA OPEN PROTEOMICS 3 (2014) 48–59



Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

ScienceDirect

journal homepage: <http://www.elsevier.com/locate/euprot>



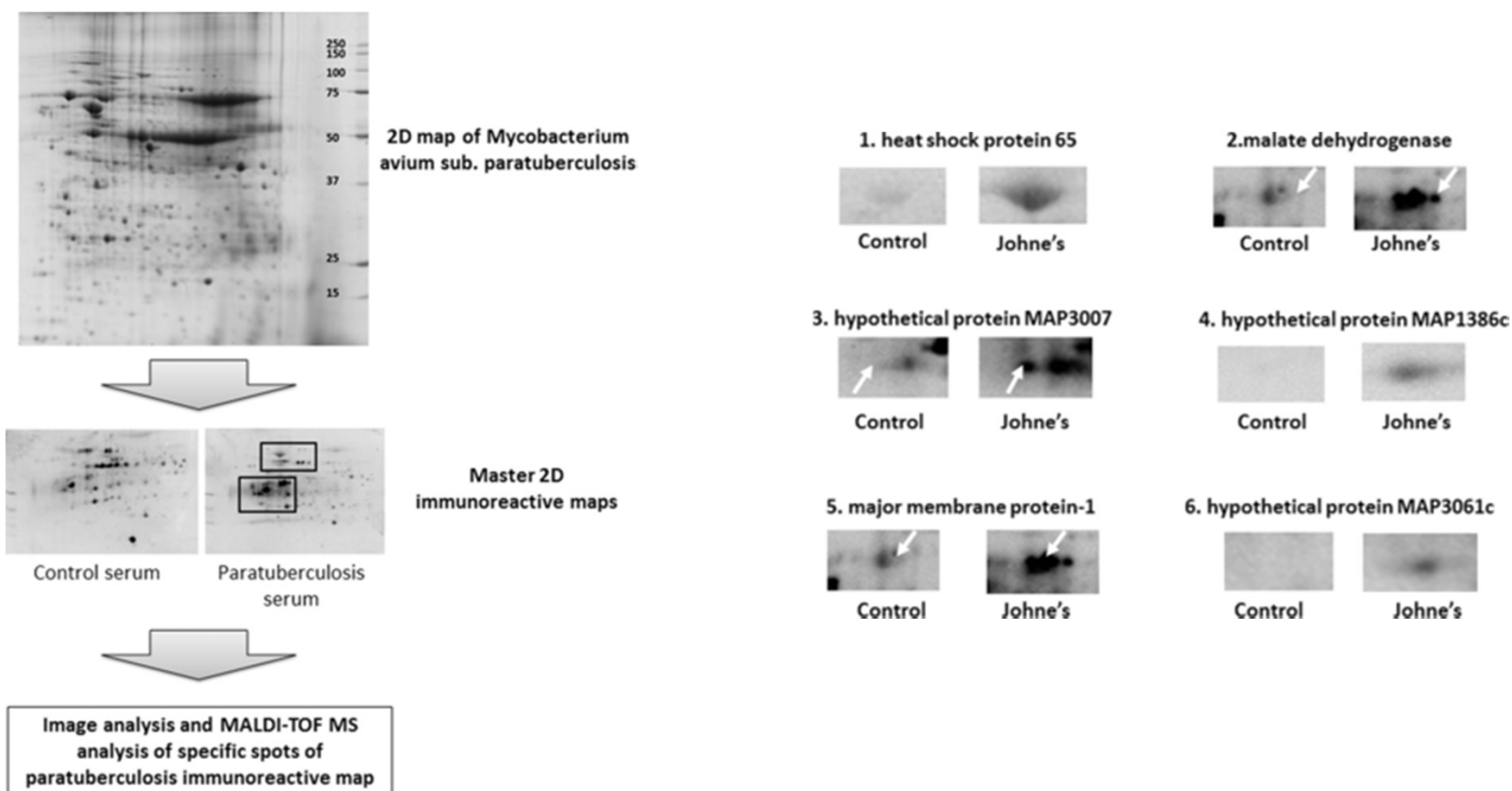
### Serum protein profiling of early and advanced stage Crohn's disease

C. Piras<sup>a</sup>, A. Soggiu<sup>a</sup>, V. Greco<sup>b</sup>, A. Cassinotti<sup>c</sup>, G. Maconi<sup>c</sup>, S. Ardizzone<sup>c</sup>, A. Amoresano<sup>d</sup>, G. Bianchi Porro<sup>c</sup>, L. Bonizzi<sup>a</sup>, P. Roncada<sup>a,e,\*</sup>



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## Identification of immunoreactive proteins of *Mycobacterium avium* subsp. *paratuberculosis*



### PROTEOMICS

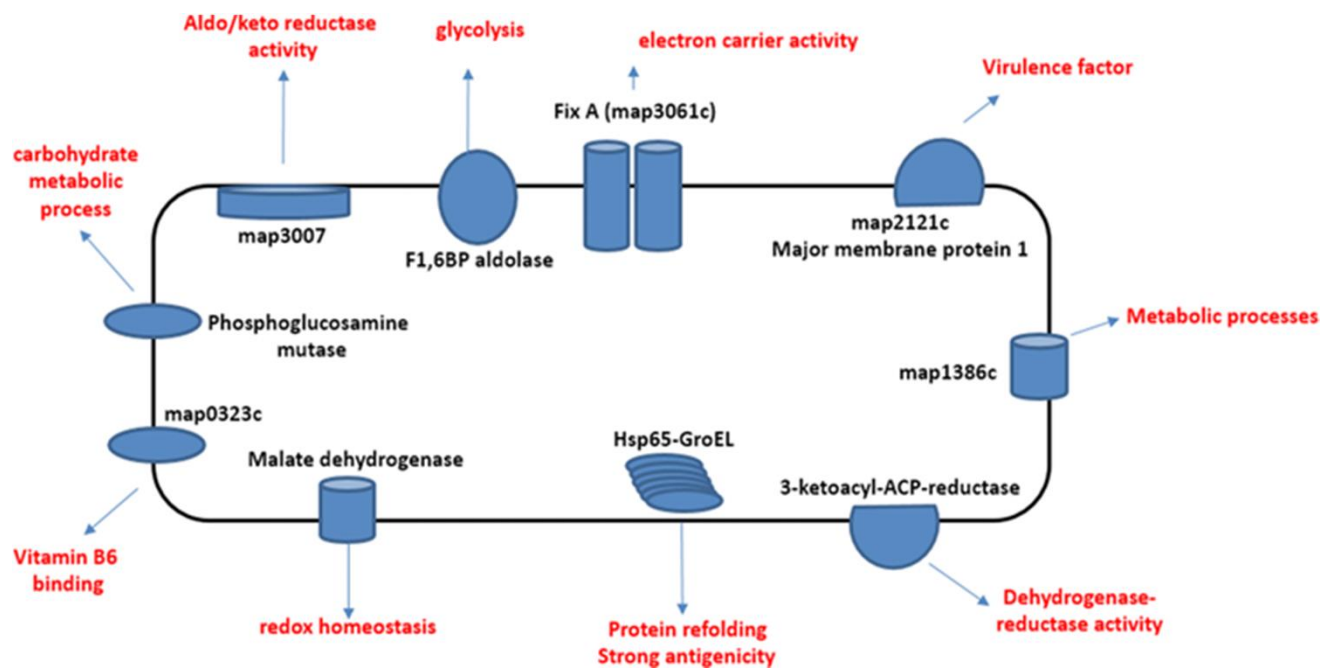
Volume 15, Issue 4, pages 813-823, 14 JAN 2015 DOI: 10.1002/pmic.201400276

<http://onlinelibrary.wiley.com/doi/10.1002/pmic.201400276/full#pmic7977-fig-0001>



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## Identification of immunoreactive proteins of *Mycobacterium avium* subsp. *paratuberculosis*



Novel immunogenic proteins for diagnosis and vaccination

### PROTEOMICS

Volume 15, Issue 4, pages 813-823, 14 JAN 2015 DOI: 10.1002/pmic.201400276

<http://onlinelibrary.wiley.com/doi/10.1002/pmic.201400276/full#pmic7977-fig-0004>



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# Antimicrobial Resistance: Commission steps us the fight with new Action Plan

Brussels, 29 June 2017



**Pillar 1: Making the EU a best-practice region**

**Pillar 2: Boosting research, development and innovation**

**Pillar 3: Shaping the global agenda**

Today the Commission adopted a new Action Plan to tackle Antimicrobial Resistance (AMR) – a growing threat that is responsible for 25,000 deaths and a loss of €1.5 billion in the EU every year.

The **Action Plan** is underpinned by a **One Health approach** that addresses resistance in both humans and animals. In parallel, the Commission adopted the first deliverable of the plan: **EU Guidelines** on the prudent use of antimicrobials in human health.

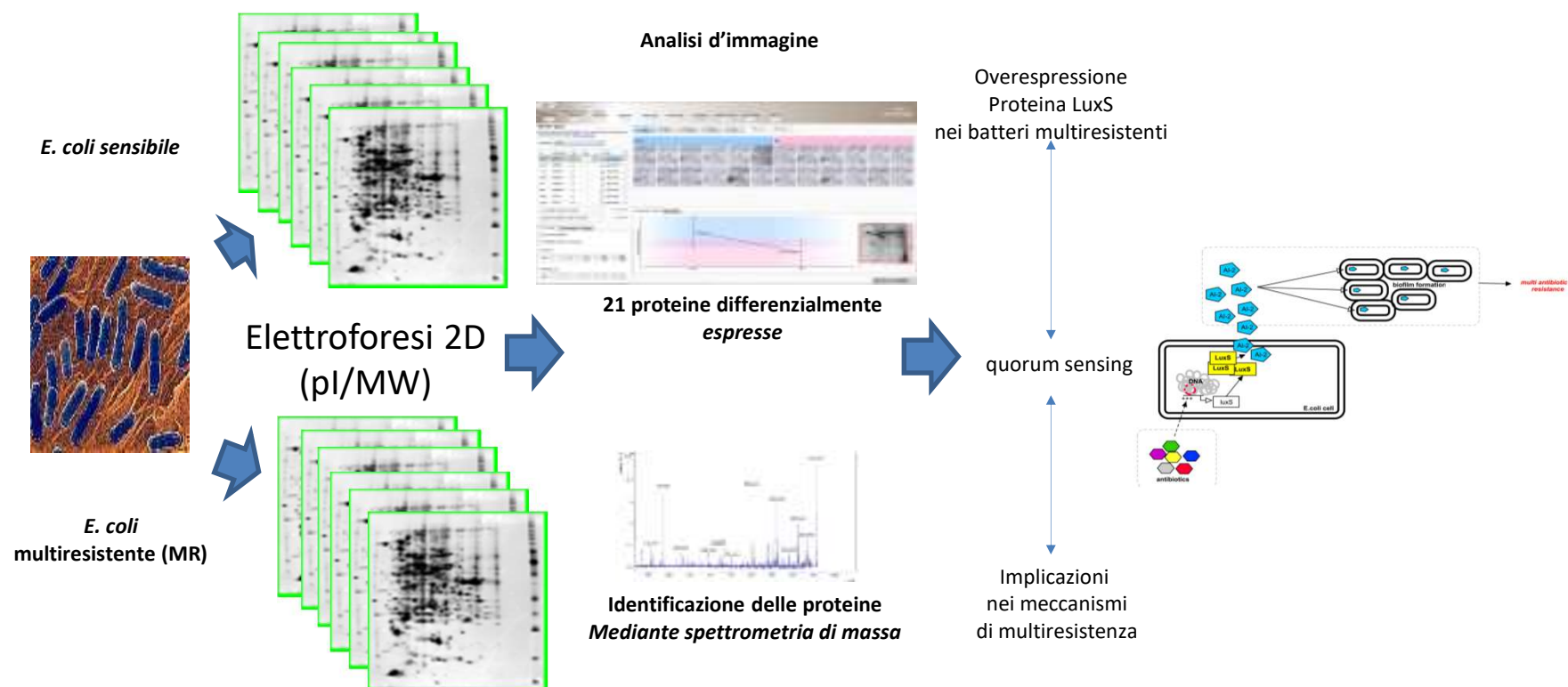


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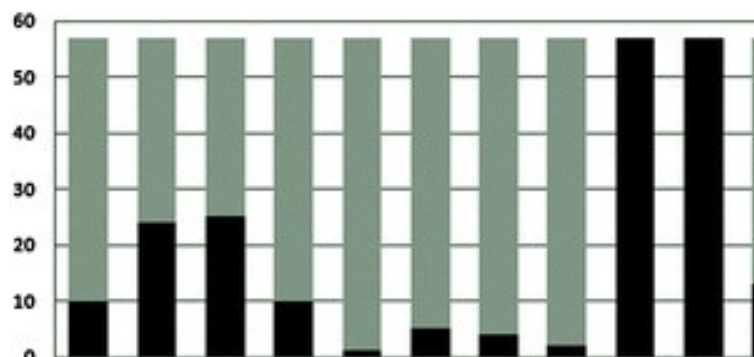
## Comparative proteomics to evaluate multi drug resistance in *Escherichia coli*†

Cristian Piras,<sup>a</sup> Alessio Soggiu,<sup>b</sup> Luigi Bonizzi,<sup>c</sup> Alessandro Gaviraghi,<sup>c</sup> Francesca Deriu,<sup>c</sup> Luisa De Martino,<sup>d</sup> Giuseppe Iovane,<sup>d</sup> Angela Amoresano<sup>e</sup> and Paola Roncada<sup>a,f</sup>

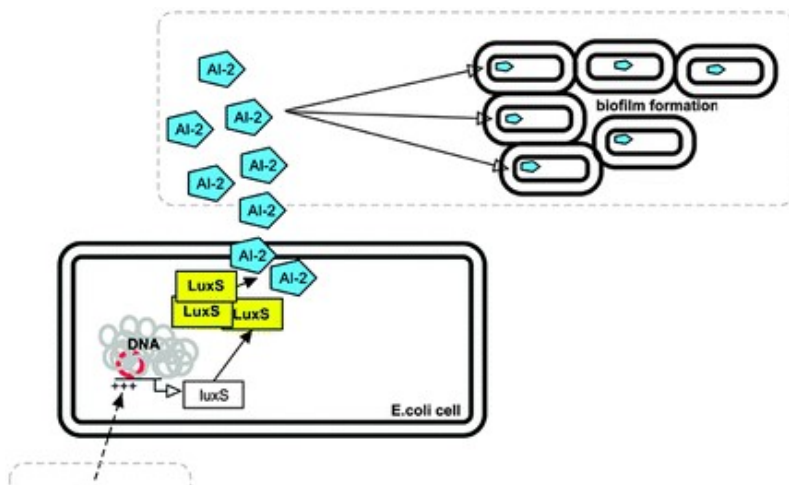


# Targeting QS for alternative antibiotic drugs

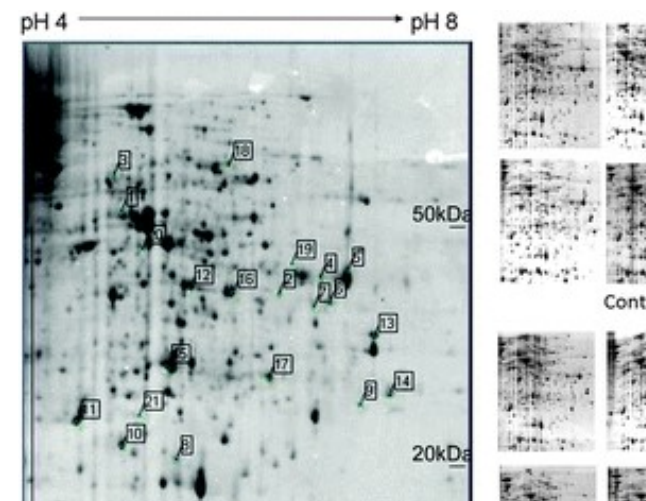
*E.coli* – 57 campioni



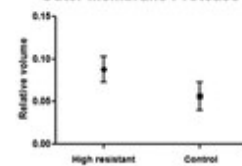
Estrazione  
Separazione  
proteine



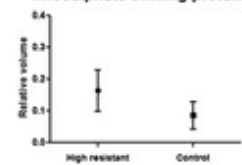
Elettroforesi-2D



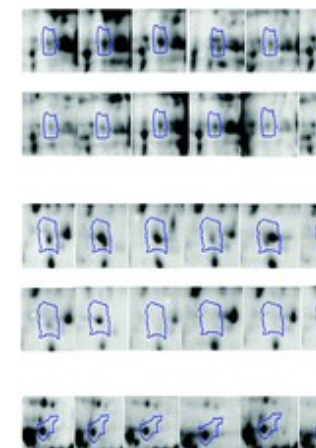
Outer Membrane Protease



Thiosulphate binding protein



Zinc transport protein



Piras C, Soggiu A, Bonizzi L, Gaviraghi A, Deriu F, De Martino L, Iovane G, Amoresano A, Roncada P.  
Comparative proteomics to evaluate multi drug resistance in Escherichia coli. Mol Biosyst. 2012 Apr;8(4):1060-7



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# Antibiotic resistance in UPEC *e.coli*: a proteomic approach



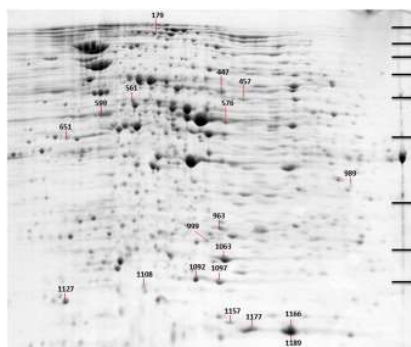
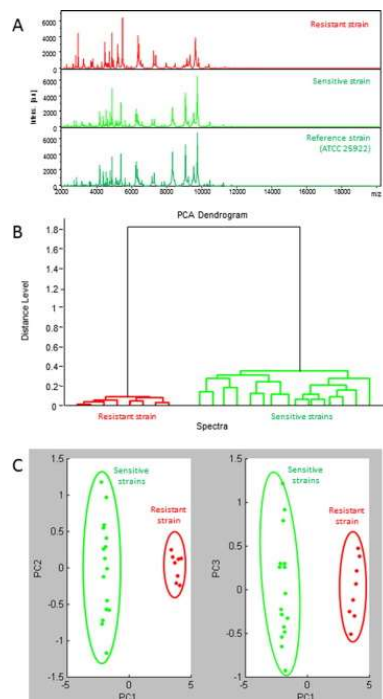
## Mechanisms of antibiotic resistance to enrofloxacin in uropathogenic *Escherichia coli* in dog ☆

Cristian Piras<sup>a</sup>, Alessio Soggiu<sup>a</sup>, Viviana Greco<sup>a,c</sup>, Piera Anna Martino<sup>a</sup>, Federica Del Chierico<sup>d</sup>, Lorenza Putignani<sup>a</sup>, Andrea Urbani<sup>b,c</sup>, Jarlath E. Nally<sup>d</sup>, Luigi Bonizzi<sup>a</sup>, Paola Roncada<sup>a,b</sup>    

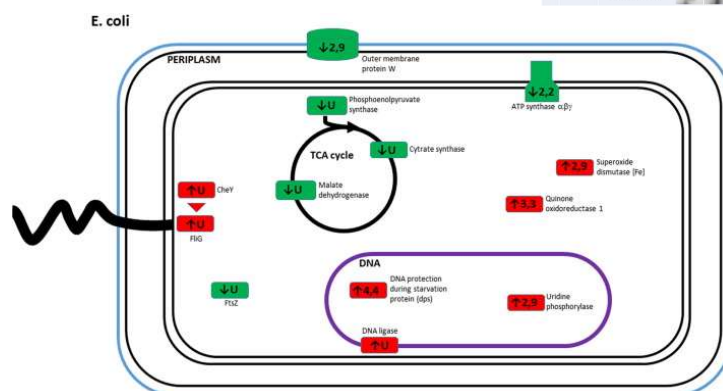
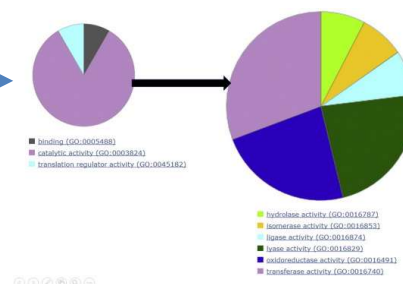
[Show more](#)

<http://dx.doi.org/10.1016/j.jprot.2015.05.040>

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Spot Number	Protein name	Control	Enrofloxacin
1	179 biodegradative arginine dihydrolase		
2	447 alpha-galactosidase		
3	457 Tryptophanase		
4	561 beta-phosphogluconate dehydrogenase, decarboxylating		
5	599 Sulfate adenylyltransferase subunit 1		
6	576 separate/serine carboxyltransferase protein		
7	651 Transcriptional regulatory protein DnaB		
8	989 glucokinase		
9	963 Uridine phosphorylase		
10	999 Putative DNA-invertase from lambdoid prophage Qn		
11	1063 Flavoprotein WbA		
12	1092 Outer membrane protein W		
13	1097 Superoxide dismutase [Fe]		
14	1108 Glucuronate dehydratase		
15	1127 Glucose-specific phosphotransferase enzyme IIA component		
16	1157 ATP synthase subunit b		
17	1177 DNA protection during starvation protein		
18	1166 DNA protection during starvation protein		
19	1189 DNA protection during starvation protein		

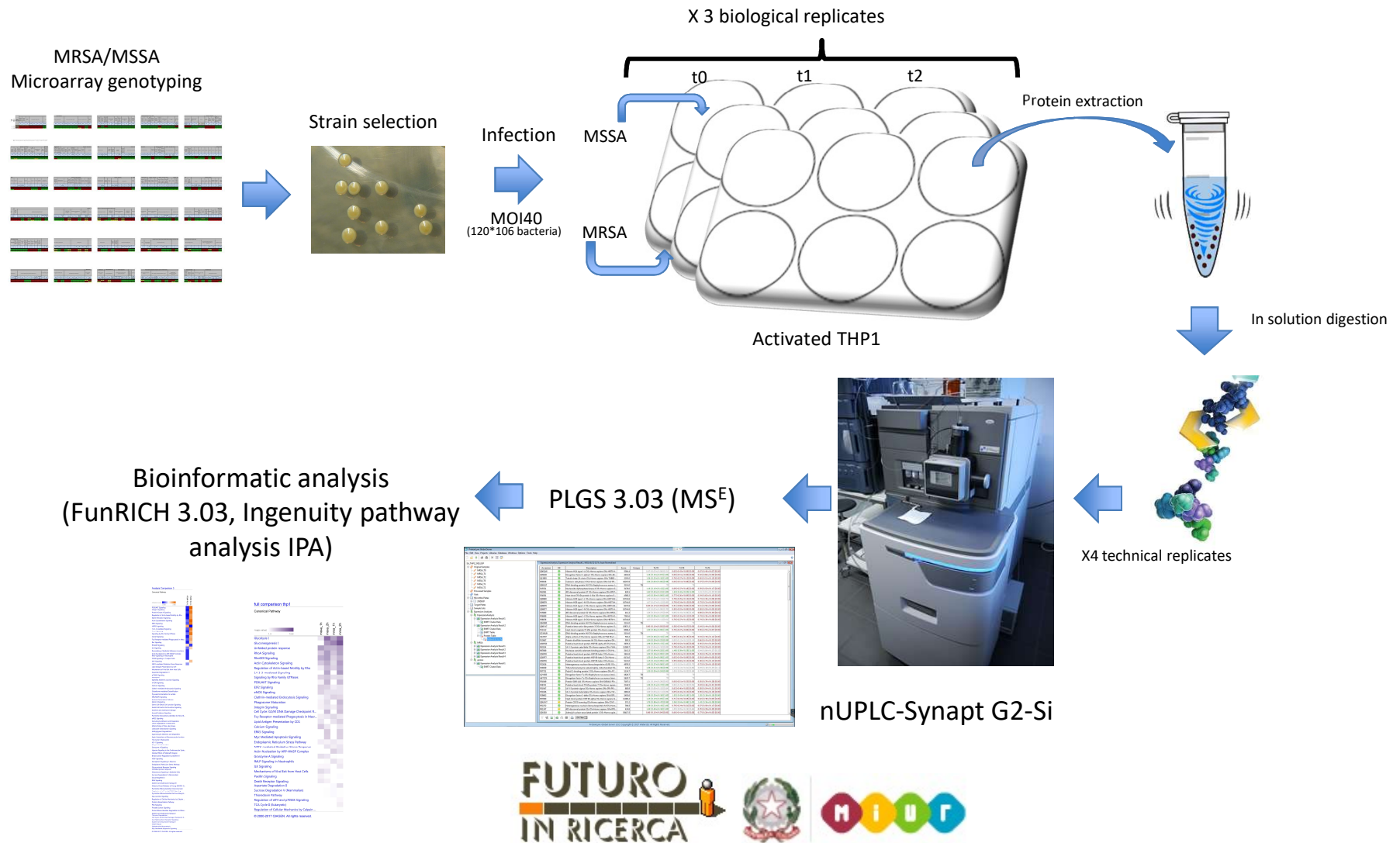


Mechanisms of antibiotic resistance to enrofloxacin in uropathogenic *Escherichia coli* in dog  
Journal of Proteomics Volume 127, Part B, 8 September 2015, Pages 365–376



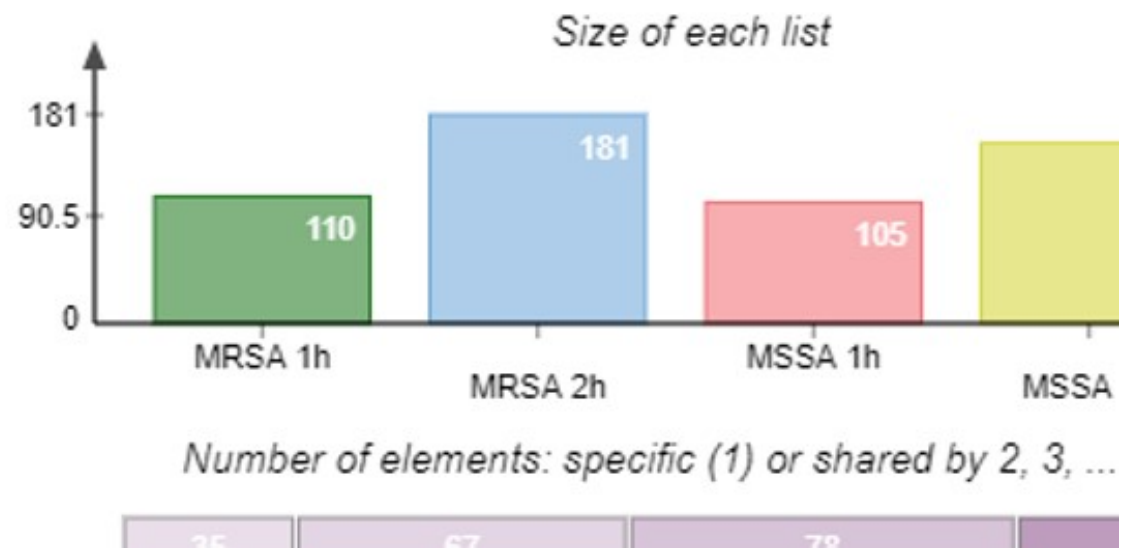
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# Host-pathogen interaction: mssa e mrsa vs human activated macrofages



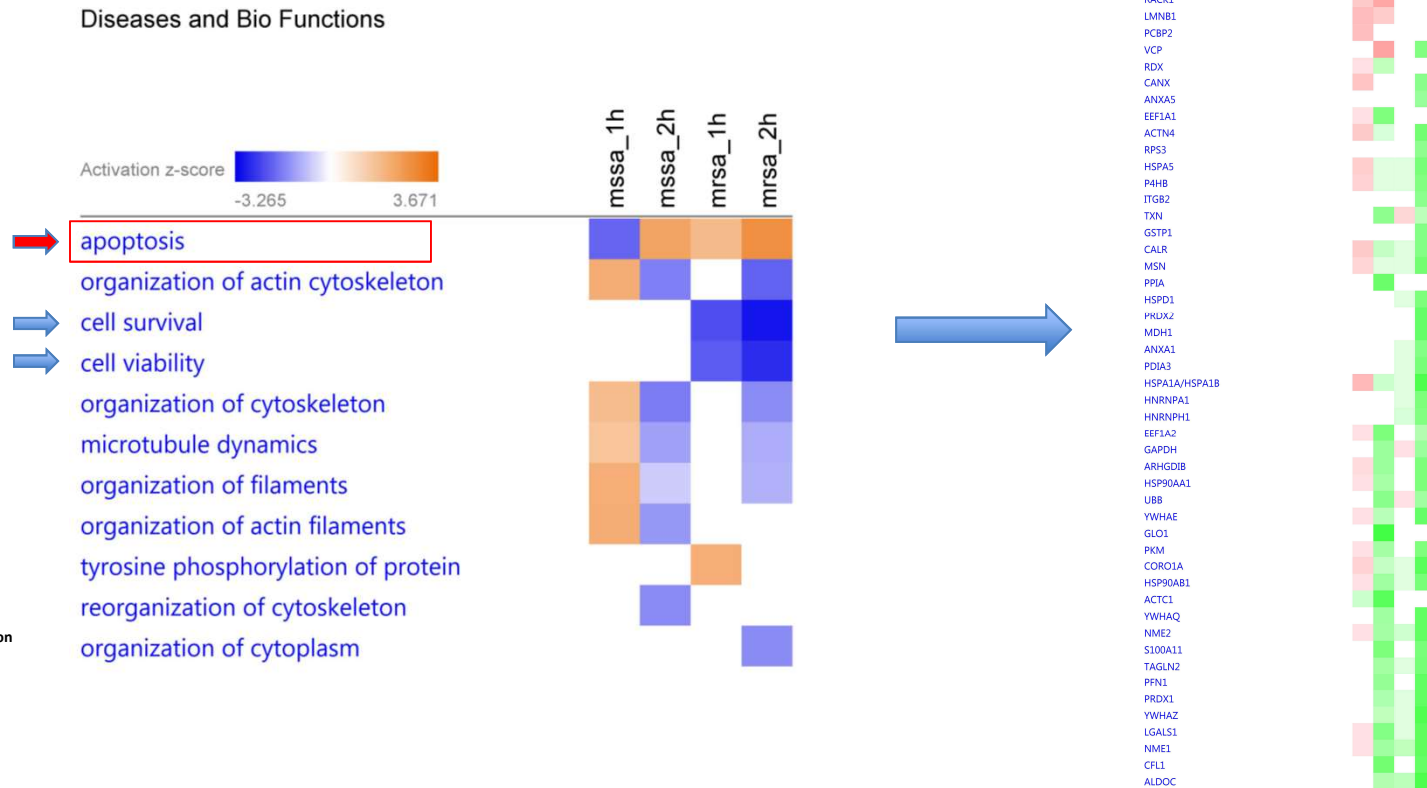


Differential protein expression evaluated among 400-500 identified proteins



# Dynamics of bio-functions in a-THP1 cells during the infection

Genes in the apoptosis network



Soggiu et al. , in preparation

- MSSA strains induce (or delay) apoptosis and/or different types of cell death
- Several mitochondrial proteins are important in phagocytic cell fate control



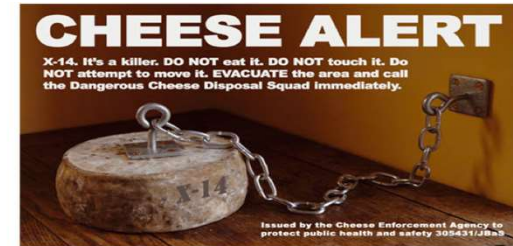
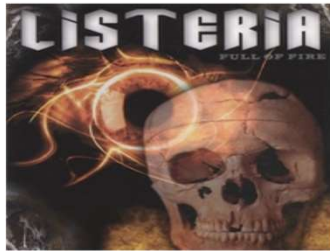
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## Food Safety in Dairy Products

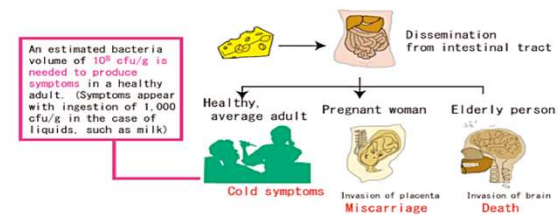


# COMPETIZIONE BATTERICA e FOOD SAFETY

Proteomics in Food safety: monitoring competition between *Listeria monocytogenes* and *Lactococcus lactis* by Imaging Mass Spectrometry



If *Listeria* food poisoning occurs--



## *Different factors can contribute to the outcome of microbial competition*

- *Efficiency of nutrient acquisition*
- *Strategies for surface attachment*

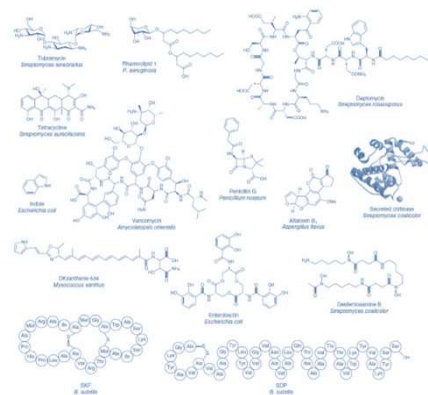
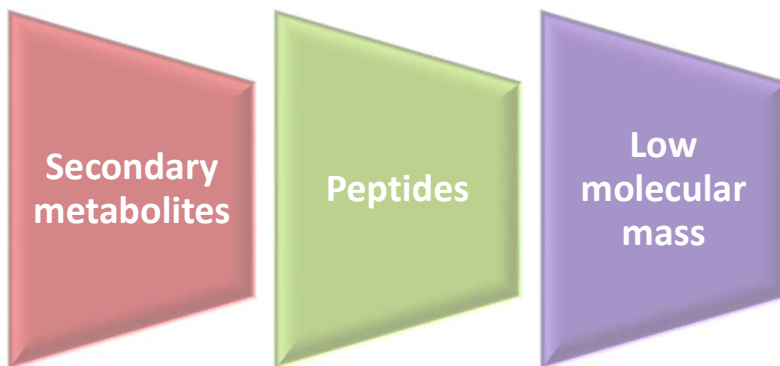


**Microbial niche survival**

- *Production of molecules to kill the niche competitors, limit their growth or modulate their metabolism*

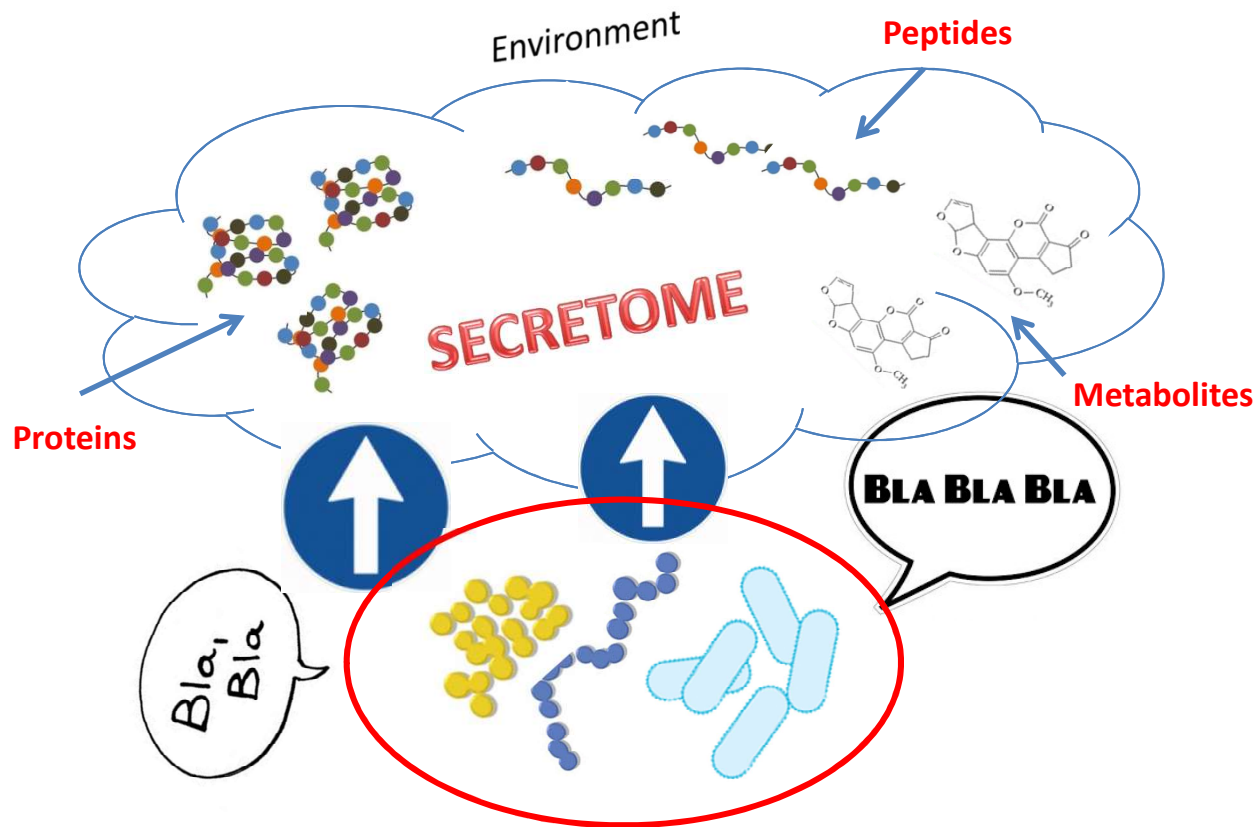


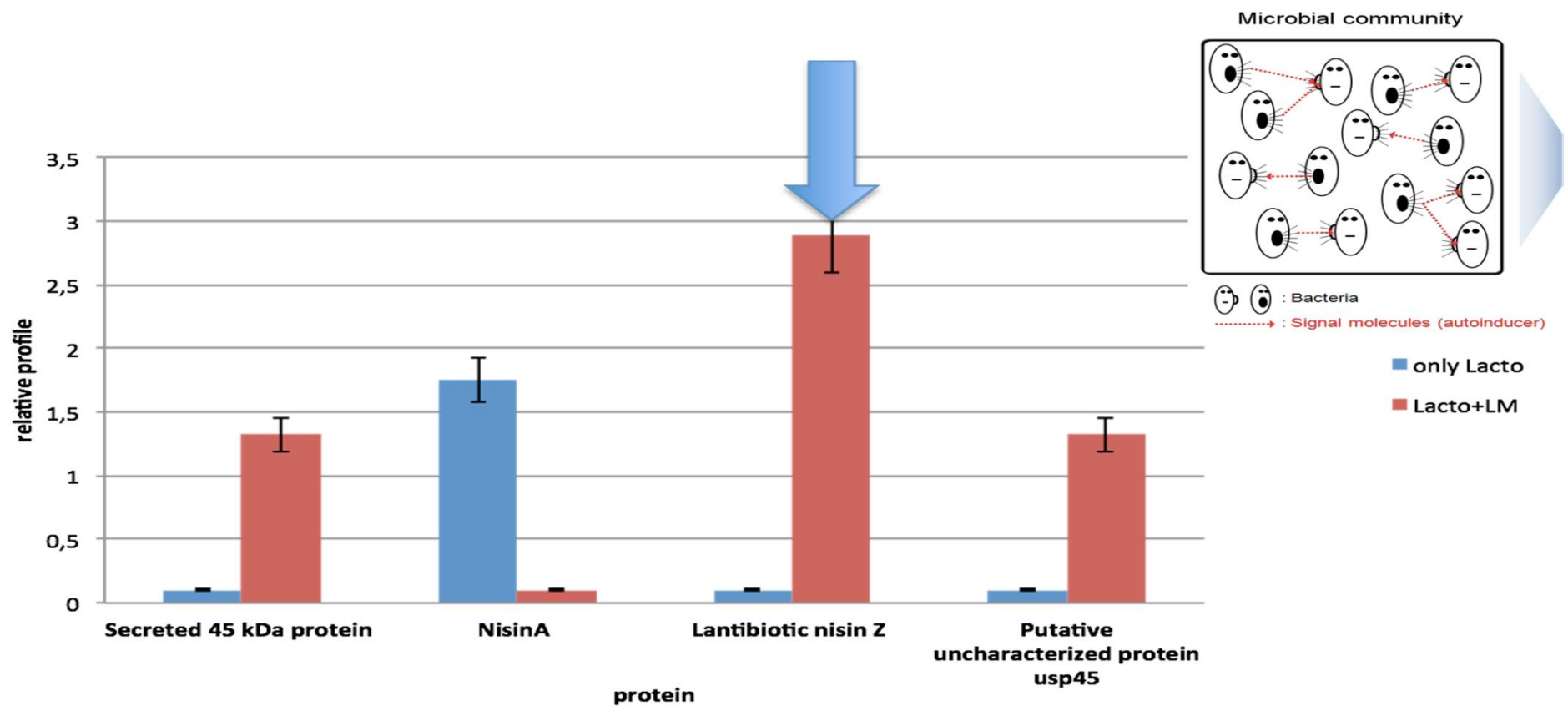
**Niche monopolization**

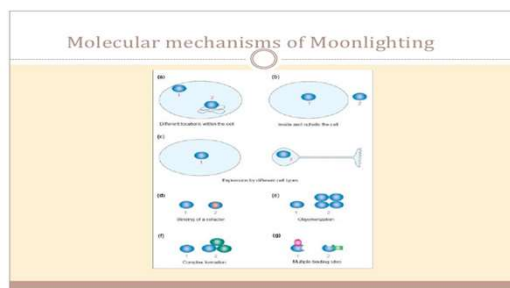
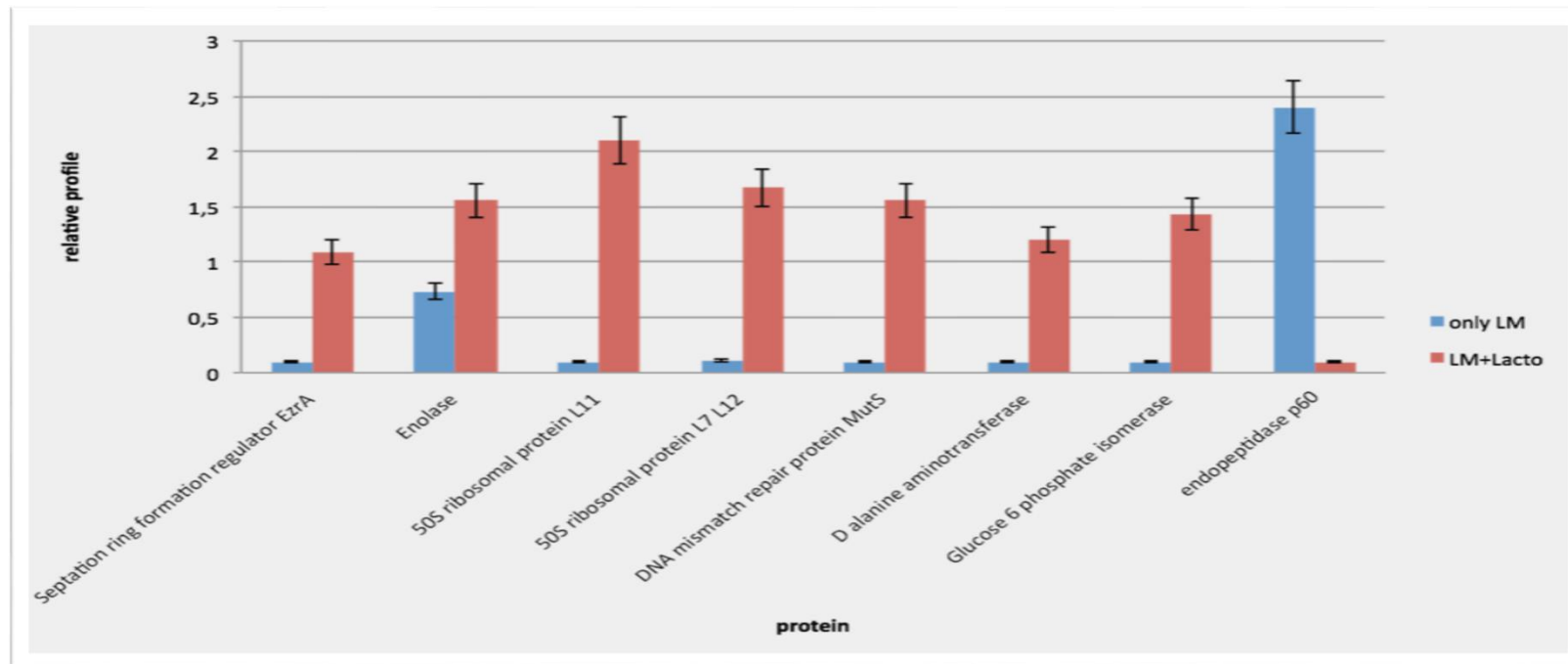




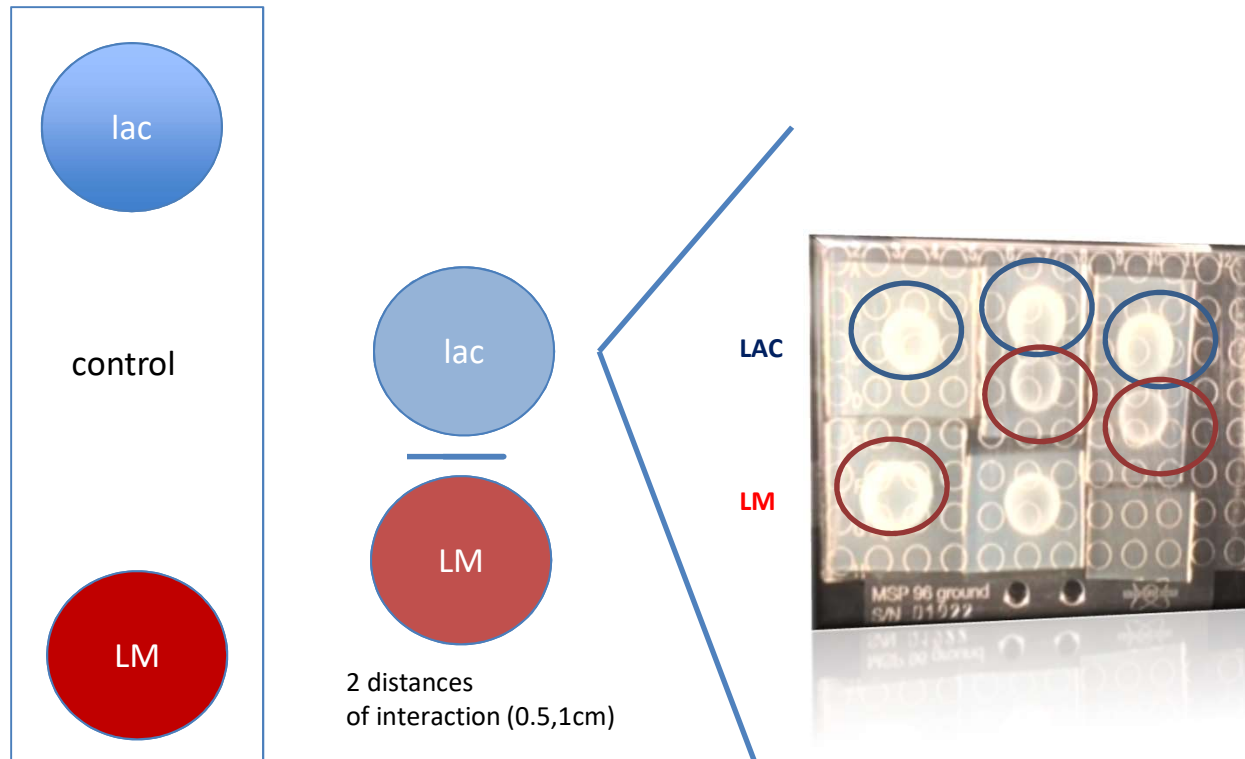
## Bacterial communication



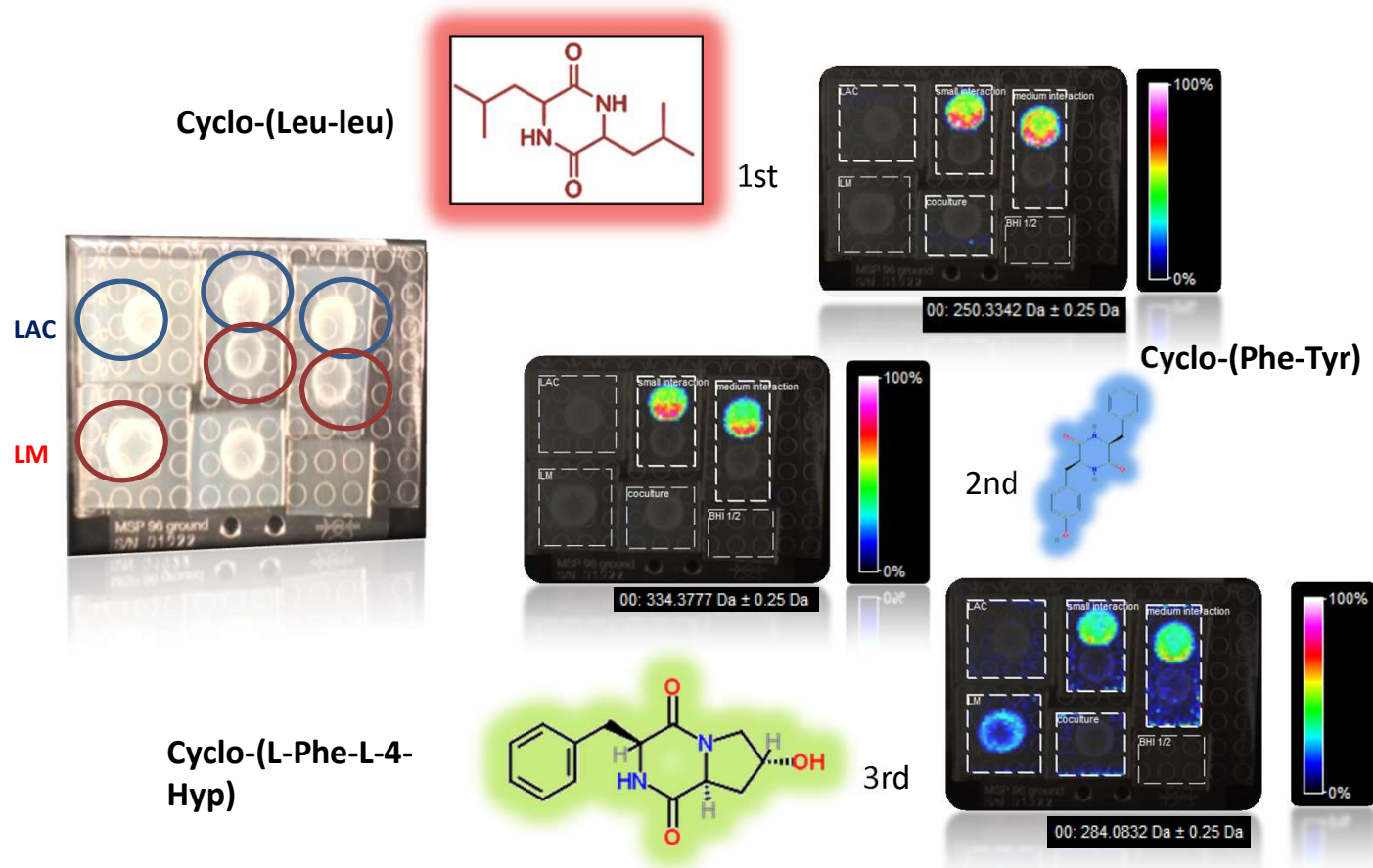




## Experimental setup



# Live colony IMS of microbial interaction between *L. monocytogenes* and *Lactococcus lactis*





## Cyclo-(Leu-leu)

1st



- **Diketopiperazines**: smallest cyclic peptides known with different bacterial function: **antibiotic biosynthesis**, **production of virulence factors**, **exopolysaccharide biosynthesis**, **bacterial aggregation**, **plasmid conjugal transfer** and transition into the **stationary phase**.
- Novel family of **signalling compounds** identified in cell-free supernatants of several gram-positive cultures.
- The precise role played by diketopiperazines in **bacterial cell-to-cell communication** has yet to be established, but their potential to act as **auto-inducer antagonists**, preventing bacterial biofilm formation
- Compound purified from **Lb. plantarum AF1**

A.M. Dallagnol, C.A.N. Catalan, M.I. Mercado, G.C. Rollan

J Biotechnol. 2001 Oct 4;91(2-3):197-209.

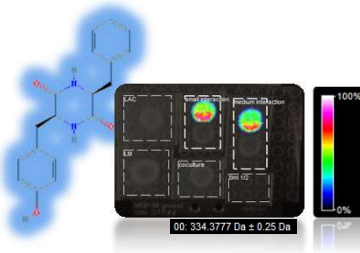
Brelles-Marino G.I., Bedmar E.J.

E.J. Yang, H.C. Chang

International Journal of Food Microbiology, 2010

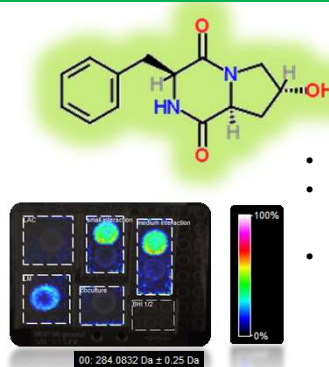
## Cyclo-(Phe-Tyr)

2nd



- Compound produced by **Lactobacillus plantarum CRL 778**
- **active secondary metabolites**
- Lysozyme activity, phagocytic activity and **bactericidal activity**

## Cyclo-(L-Phe-L-4-Hyp)



- Compound produced by **L. Plantarum**
- Cyclic dipeptides possess **antibacterial** activities
- Due to their chiral, rigid, and functionalized structures, they bind to a large variety of receptors with high affinity, giving a broad range of biological activities.

3rd

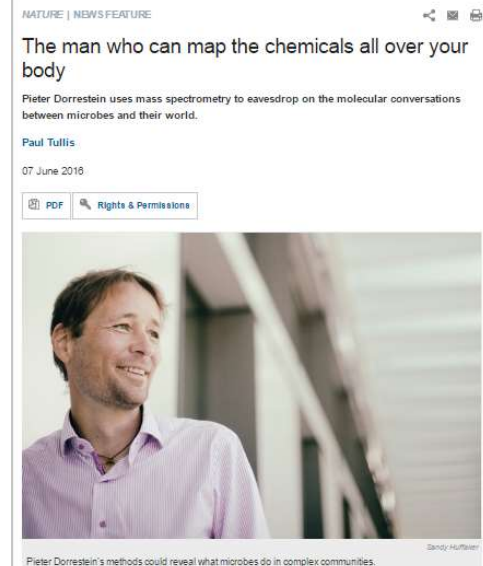


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**Imaging Mass Spectrometry** could  
be well validated method to  
investigate “online molecular  
conversation between bacteria” to  
increase knowledge and strategy in  
**food safety**

Skaggs School of Pharmacy – The Dorrestein Lab

**Professor Pieter Dorrestein**



JUN  
**12**  
2017



**FaN**

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Food and  
Nutrition  
Proteomics

# ONE HEALTH MICROBIO

**1st WORKSHOP OF FaN  
PROTEOMICS INITIATIVE**

**HP** Human Proteome Project

info@ [www.itpa.it](http://www.itpa.it)  
follow us

**LECCE, ITALY 2017**  
**14.00-18.00**

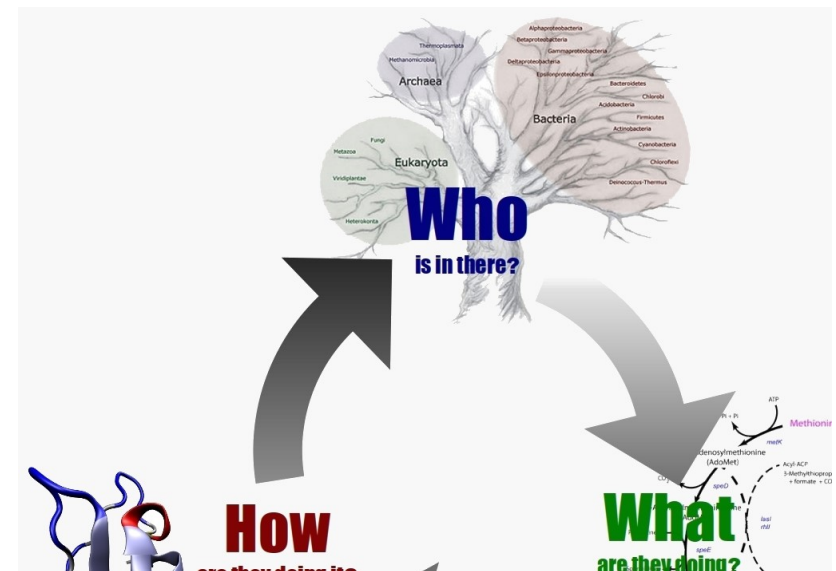
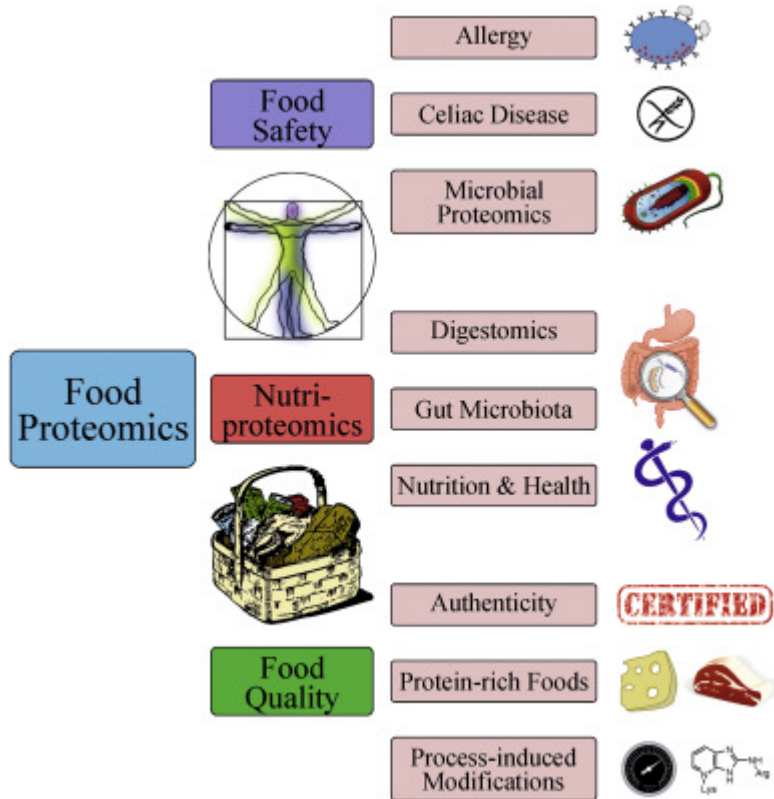
hosted by Italian Proteomics Association Annual Meeting

Paola Roncada, Istituto Spallanzani, Milano , Italy



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# Food safety and quality: the microbiota of food



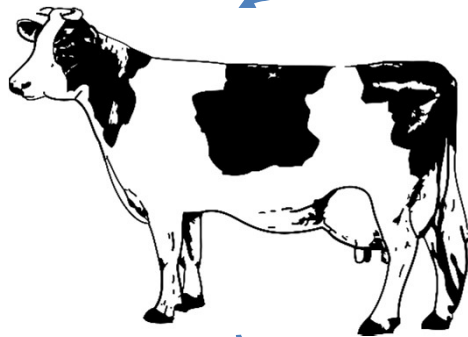
**METAPROTEOMICS**  
(on FUNCTIONAL MICROBIOME)  
To understand microbiome dynamics



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# Cheese microbiota dynamics : insights from Grana Padano PDO



- GRANA PADANO IS A HARD COOKED CHEESE
- THE COMMERCIAL VALUE INCREASE WITH AGING
- DURING AGING, MORE THAN 20% OF CHEESES SHOW SEVERE DEFECTS DUE TO “LATE BLOWING”,
- LYSOZIME additive



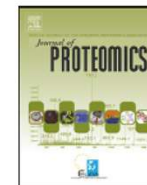




Contents lists available at ScienceDirect

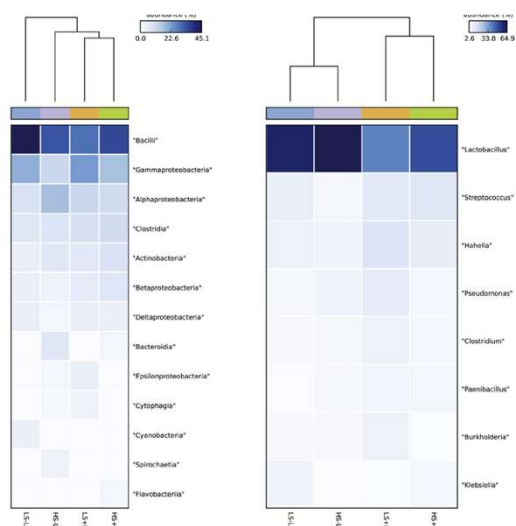
Journal of Proteomics

journal homepage: [www.elsevier.com/locate/jprot](http://www.elsevier.com/locate/jprot)



## Unravelling the effect of clostridia spores and lysozyme on microbiota dynamics in Grana Padano cheese: A metaproteomics approach

Alessio Soggiu<sup>a</sup>, Cristian Piras<sup>a</sup>, Stefano Levi Mortera<sup>b,c,1</sup>, Isabella Alloggio<sup>a</sup>, Andrea Urbani<sup>b,c</sup>, Luigi Bonizzi<sup>a</sup>, Paola Roncada<sup>a,d,\*</sup>



a. Heatmap plot representing the core microbiome of analysed samples from the most abundant to the less abundant. All the classes account for roughly 95% of the total bacterial faunation in each group analysed. b. Analysis on relative abundances of the eight most abundant genera present in all experimental groups.



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# METAPROTEOMICS: complete flowchart

4 exp. Groups ( 3 samples group / 3 rep)

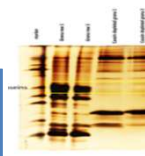
Low\_spore\_no\_lysozyme  
Low\_spore\_plus\_lysozyme  
High\_spore\_no\_lysozyme  
High\_spore\_plus\_lysozyme



Experimental samples



CASEIN DEPLETION



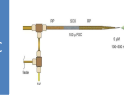
PROTEIN EXTRACTION



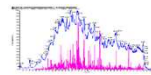
Amazon ETD ion trap



NANO 2D-LC



Protein digestion



Peptide (FDR 1%)  
Multiple search engines

Blast 2.2.30+

custom\_DB selection

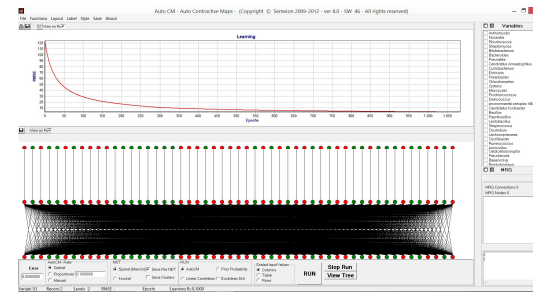
taxa classification  
(MEGAN 5.9.1)

Global taxonomic profile  
LCA algorithm

functional analysis using  
SEED

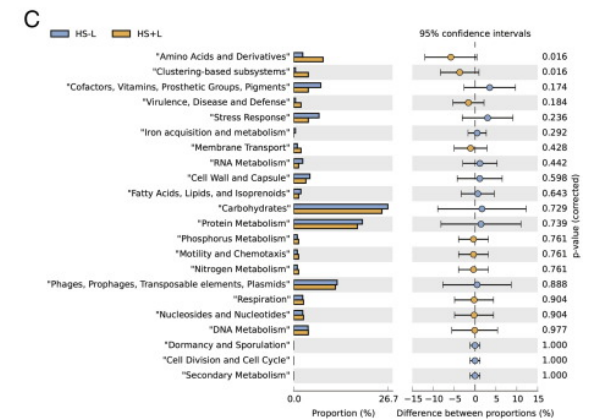
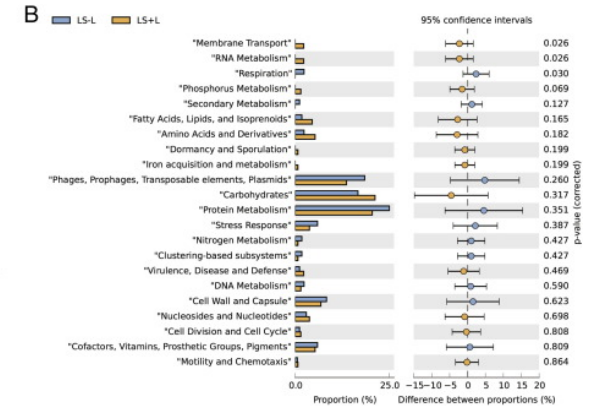
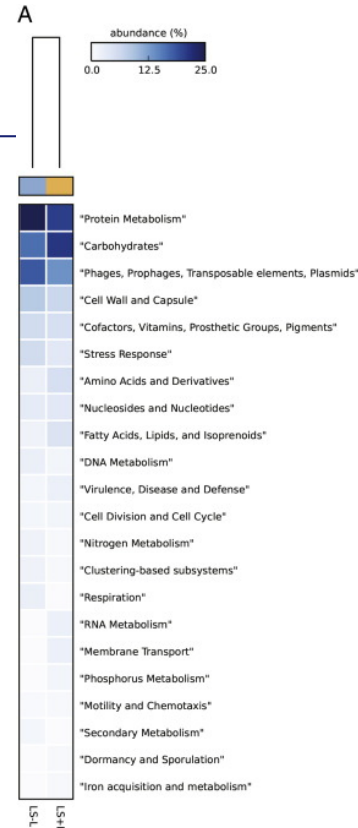
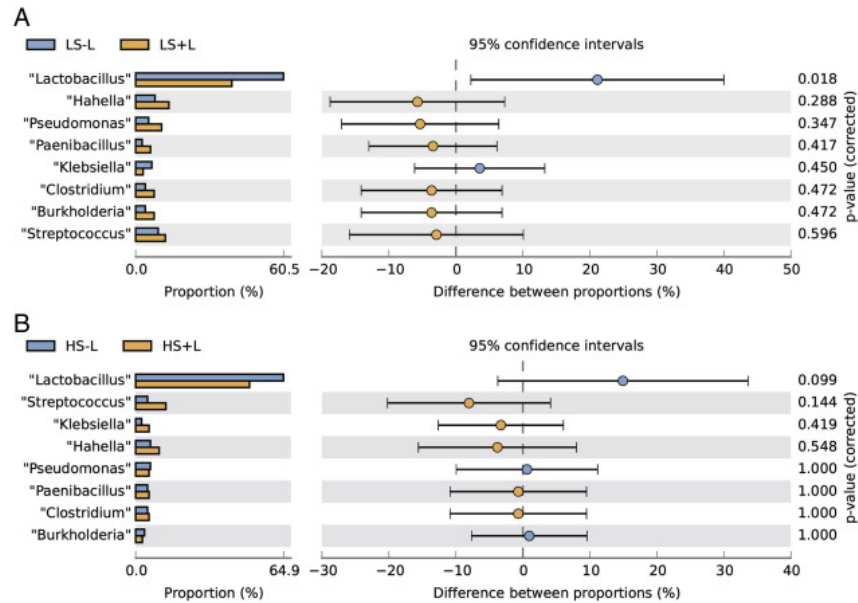


## Artificial NN Auto-CM

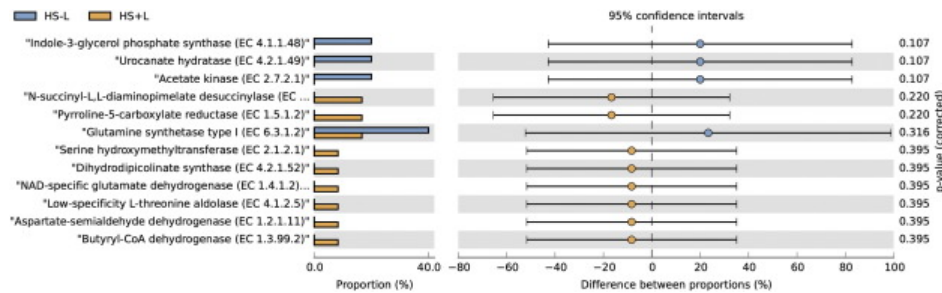


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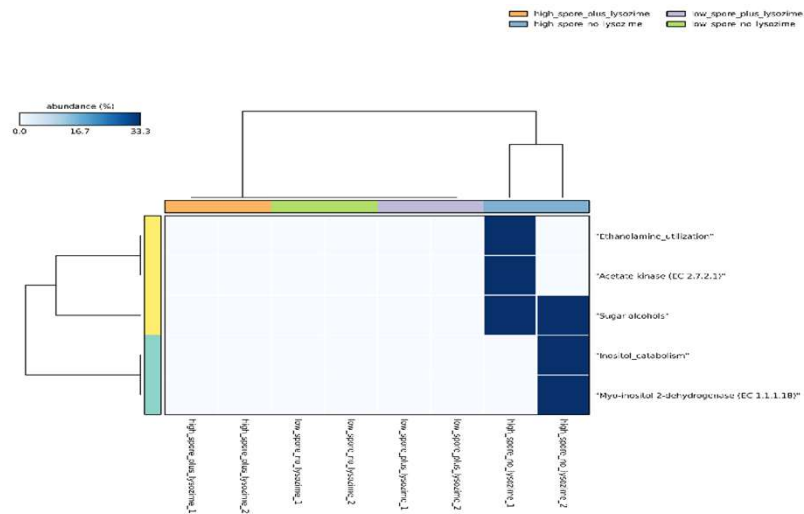
## taxonomy



## functions



## Acetate kinase (Ack)



```

Acetate kinase (EC 2.7.2.1) [1]
  ISK [100]
    DATA[length=21, complexity=1.00]
    Clostridium score=63.8
      >acetate kinase [[Clostridium] spiroforme] >gi|169292037|gb|EDS74170.1| acetate kinase [
        [[Clostridium] spiroforme DSM 1552]
          Length = 396
          Score = 63.8 bits (143.0), Expect= 1.525140e-10
          Identities = 19/19 (100%), Positives = 19/19 (100%), Gaps = 0/19 (0%)
        Query:      1  SVNISMGFTPLAGIMAGIR  19
                   SVNISMGFTPLAGIMAGIR
        Sbjct:    220 SVNISMGFTPLAGIMAGIR  238
    
```





# NETWORK



The Farm Animal Breeding and Reproduction Technology Platform (FABRE TP) is an industry-led forum. It provides a framework for stakeholders to define research priorities and action plans for the farm animal and reproduction sector in order to achieve EU growth, competitiveness and sustainability through major research and technological advances in the medium and long term.



Improving Allergy Risk Assessment Strategy for new food protei



COST  
ACTION  
FA1308

DairyCare is a COST Action focused on dairy animal health and welfare. [Read more.](#)



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# Sommario

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- La cooperazione multi-disciplinare a livello locale fino a quello globale è fondamentale per mettere in pratica un approccio One Health a 360°
- in questo contesto servono nuovi strumenti analitici adatti a monitorare e analizzare lo stato di salute di animali uomo e ambiente e le loro interazioni (e.g. zoonosi , AMR, food safety)
- In quest'ottica la proteomica si sta rivelando un ottimo strumento per l'analisi e l'implementazione di nuove strategie diagnostiche e preventive in un contesto altamente complesso quale quello one health



# Ringraziamenti

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Prof. Luigi Bonizzi  
Dr. Cristian Piras



UNIVERSITÀ  
CATTOLICA  
del Sacro Cuore



Prof. Andrea Urbani  
Dr. Viviana Greco  
Dr. Luisa Pieroni



Dr. ssa Paola Roncada



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