

# Characterization and physiological impacts of *Enterococcus faecalis* extracellular vesicles

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**MICROBES 2025** 24-26 septembre

PALAIS DES CONGRÈS DE BORDEAUX

« Microbes dans un monde en transition »



Société Française  
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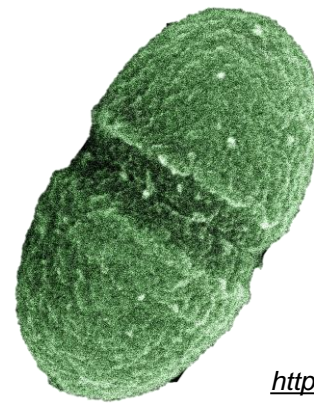
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## Déclaration de conflit d'intérêt

Pour cette présentation :

- je déclare n'avoir aucun conflit d'intérêt ;
- j'ai utilisé des images libres de droit.

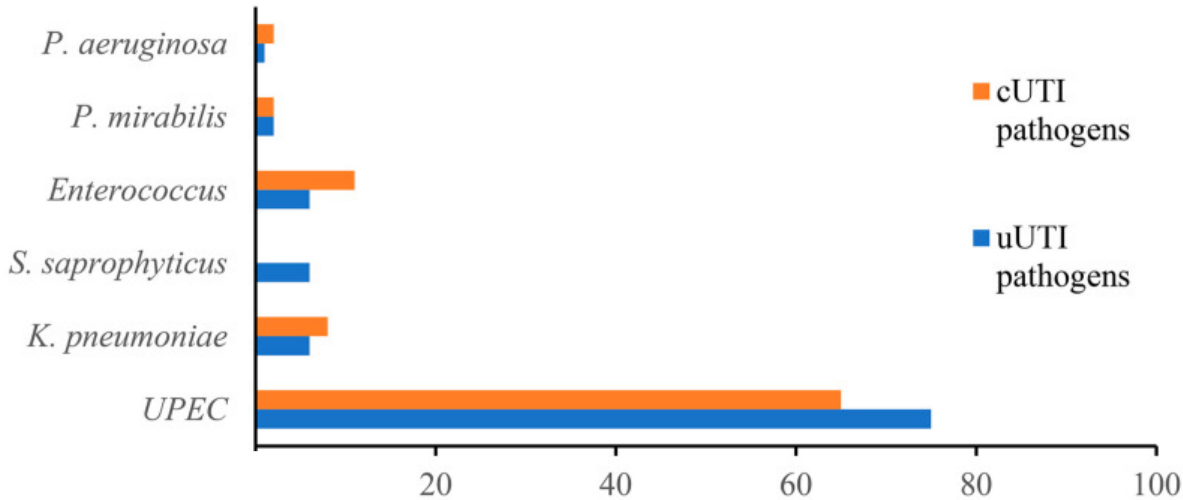
# Enterococcus faecalis



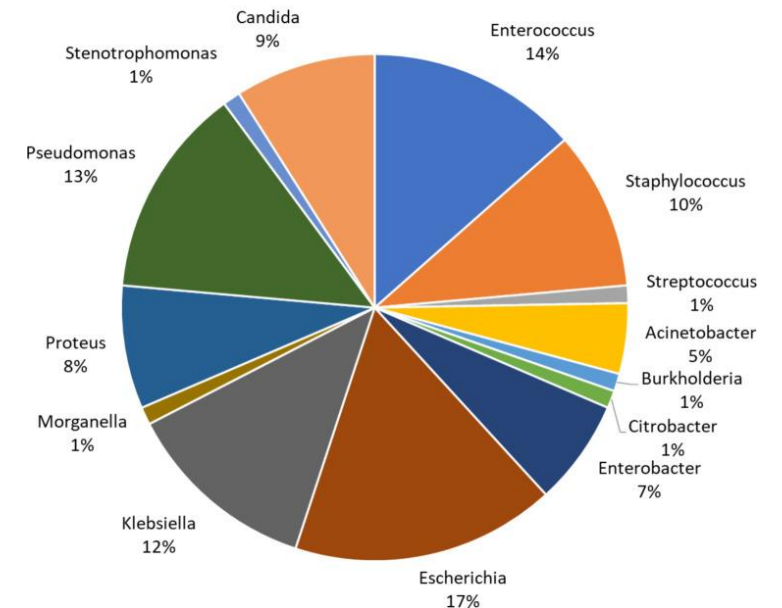
<http://www.genome.gov/dmd/img.cfm?node=Photos/Microorganisms&id=79092>

- *E. faecalis* :
  - Gram positive bacterium
  - Part of the intestinal microbiota of mammals
  - Pathogenic especially in immunocompromised / hospitalized patients

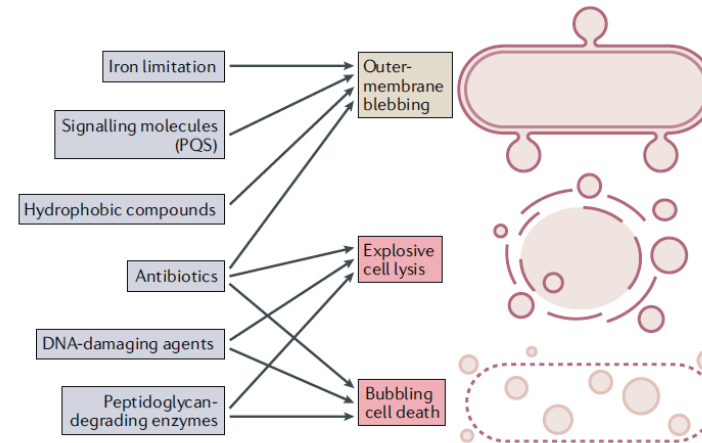
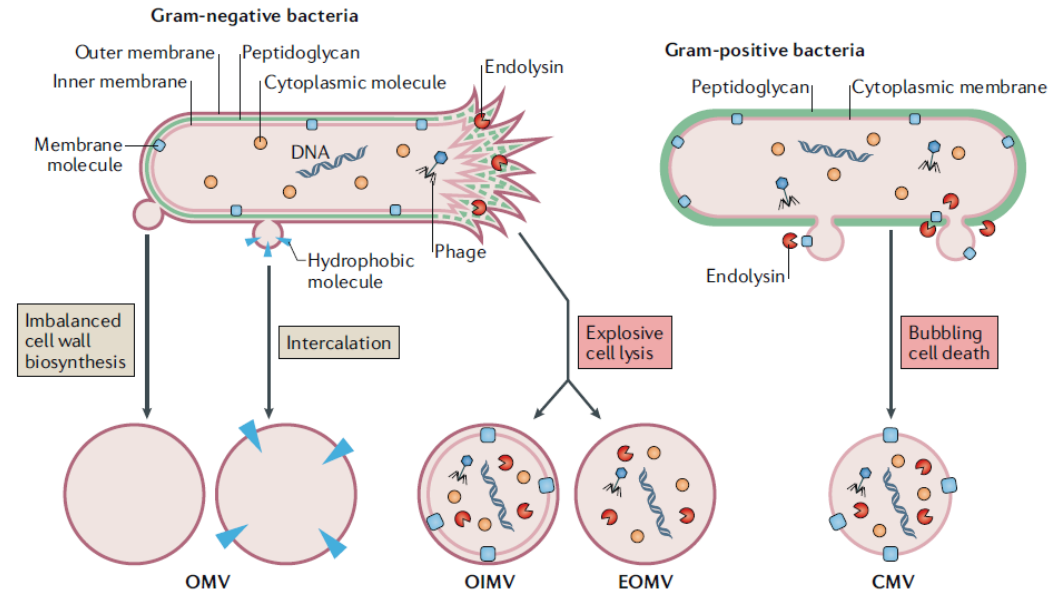
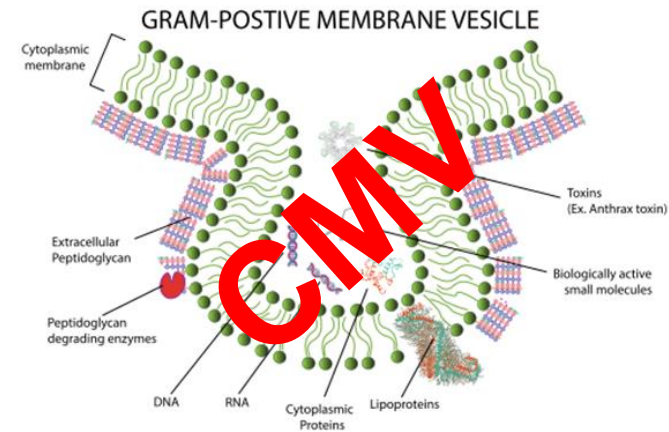
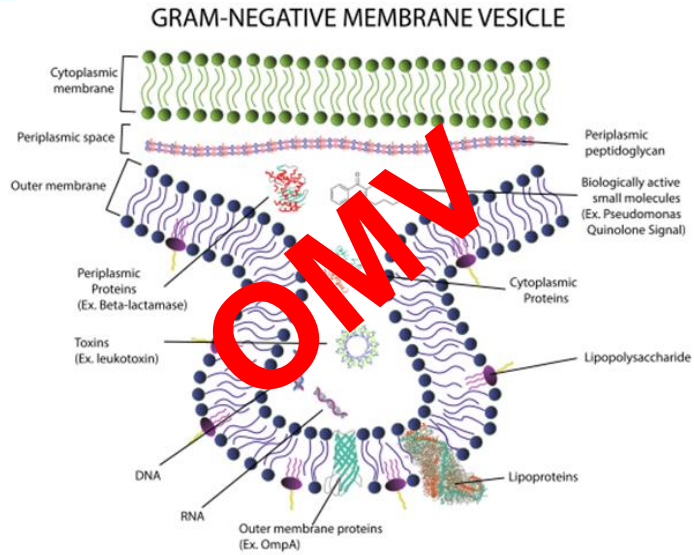
Incidence of urinary tract pathogens (%)



Micro-organism genus identification



# What do we already know about bacterial MVs ?



Villageliu DN and Samuelson DR (2022) The Role of Bacterial Membrane Vesicles in Human Health and Disease. *Front. Microbiol.* 13:828704.

# What do we already know about *Enterococcus faecalis* MVs ?

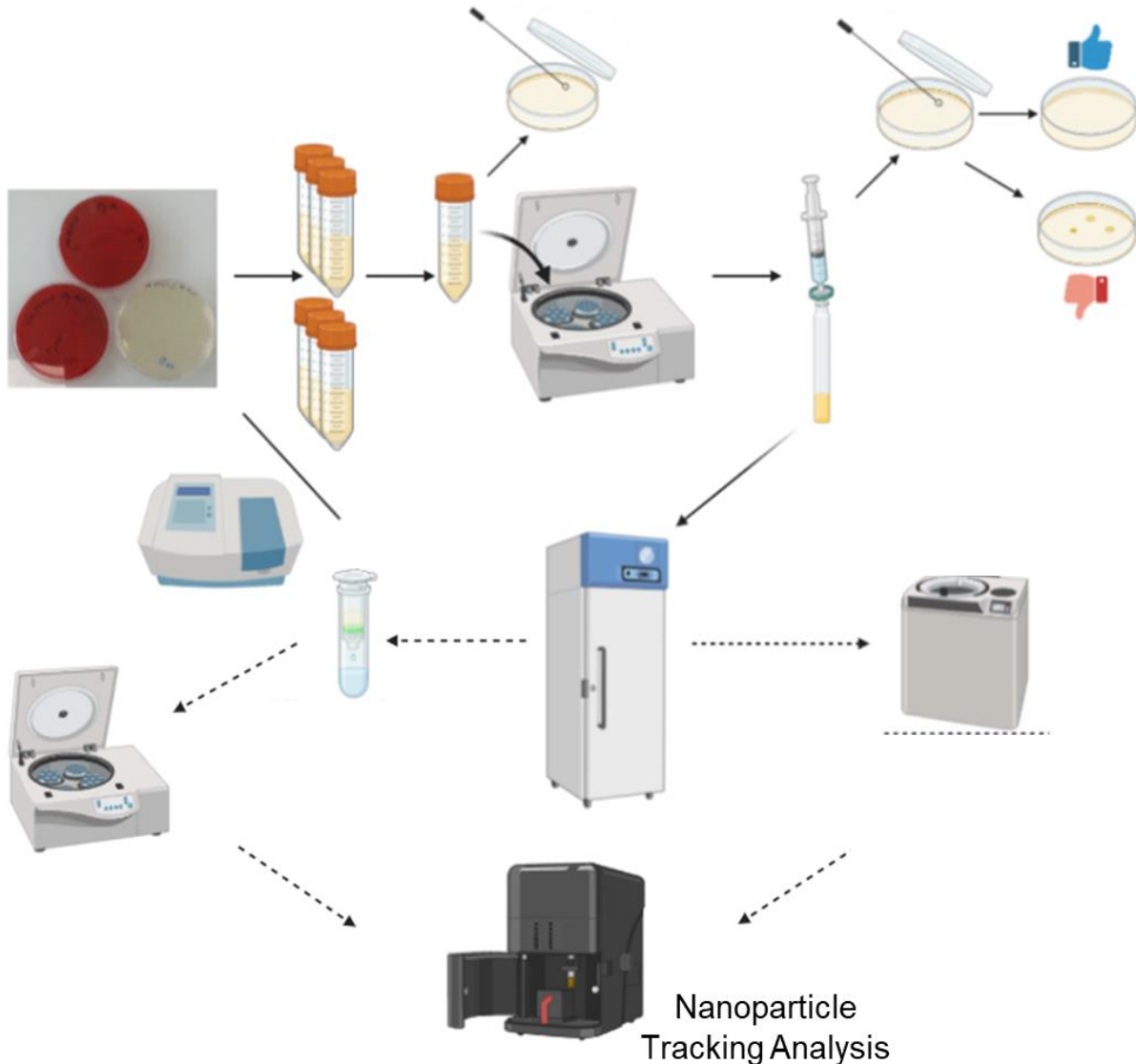
- EFS produces MVs with a size range of 50-400 nm
  - EFS MVs are enriched in unsaturated lipids
  - EFS MVs contained DNA
  - EFS MVs contained proteins :
- **What about « real life » ?**
    - **Clinical strains vs lab strains**
    - **Different clinical site of isolation**
    - **Different matrix (Urine / Broth)**

Protein	Position in MVs (of 225)	Position in WCL (of 397)	Function
Aad	1	8	Aldehyde-alcohol dehydrogenase
GroL	2	15	Chaperonin
OG1RF_12 384 IreK	3	168	Non-specific serine/threonine protein kinase
OG1RF_10 124	6	151	ABC superfamily ATP binding cassette transporter, binding protein
OG1RF_12 203	8	155	ABC superfamily ATP binding cassette transporter, substrate-binding protein
OG1RF_10 125	9	67	ABC superfamily ATP binding cassette transporter, binding protein
OppA	11	33	Oligopeptide ABC superfamily ATP binding cassette transporter, binding protein
TraC2	12	93	Peptide ABC superfamily ATP binding cassette transporter, binding protein
PrsA	15	118	Peptidyl-prolyl cis-trans isomerase
RplB	16	44	50S ribosomal protein L2
Pbp1A	4	n/p	Penicillin binding proteins 1A
CnaB	5	n/p	Cna protein B-type domain protein
OG1RF_12 508	7	n/p	Thiamine biosynthesis lipoprotein
PenA	10	n/p	Penicillin-binding protein 2 gene
OG1RF_12 235	13	n/p	S1 family extracellular protease
Pbp1B	14	n/p	Penicillin-binding protein 1B
MreC	19	n/p	Rod shape-determining protein MreC
MetQ	20	n/p	ABC superfamily ATP binding cassette transporter, binding protein
OG1RF_11 061	26	n/p	Phage tail protein
LytR	32	n/p	Response regulator



AI generated picture

# The ExCellEnt project : EXtra CELLular vesicles in ENTerococcus



## • 3 EFS strains

- VACA → Infective endocarditis strain
- VB → Urinary Tractus Infection strain
- V583 → Lab strain Vancomycin resistant

## • 2 culture media

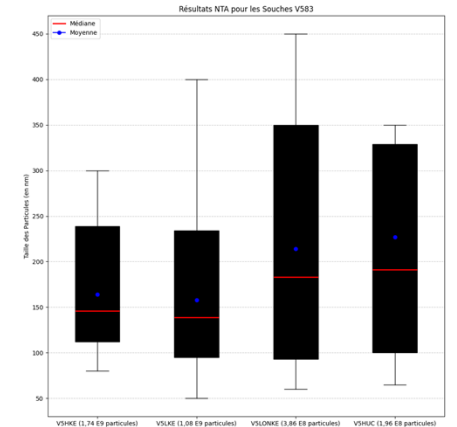
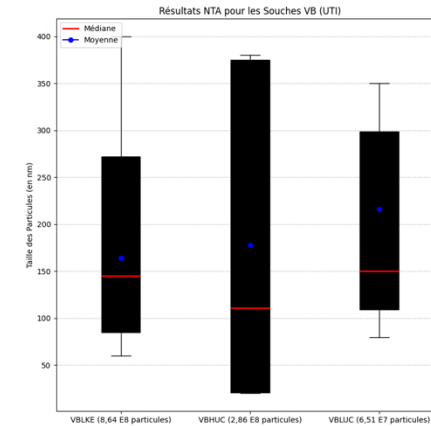
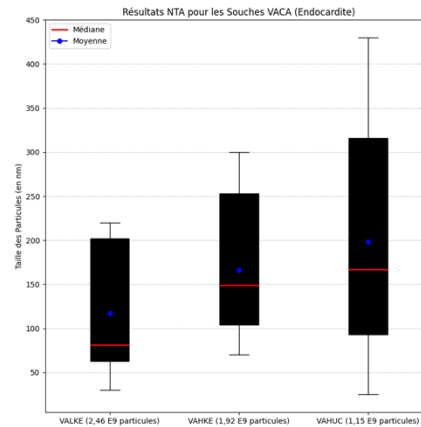
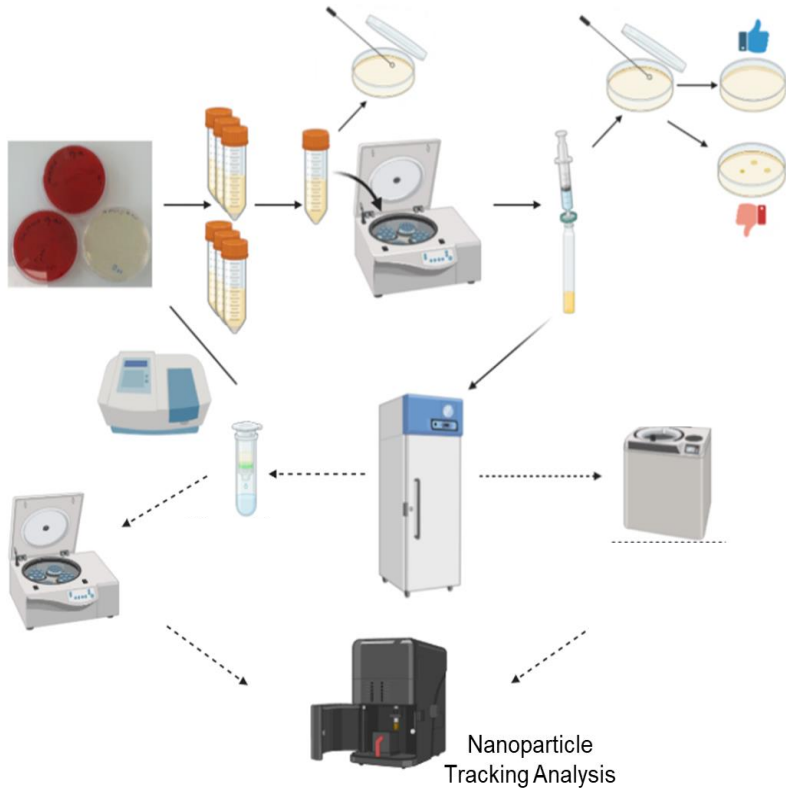
- Luria-Bertoni broth
- Human urinary

## • 2 MVs extraction methods

- Ultracentrifugation
- ExoEasy (Qiagen®)

➔ Differences in MVs production / MVs physiological impact / MVs components ?

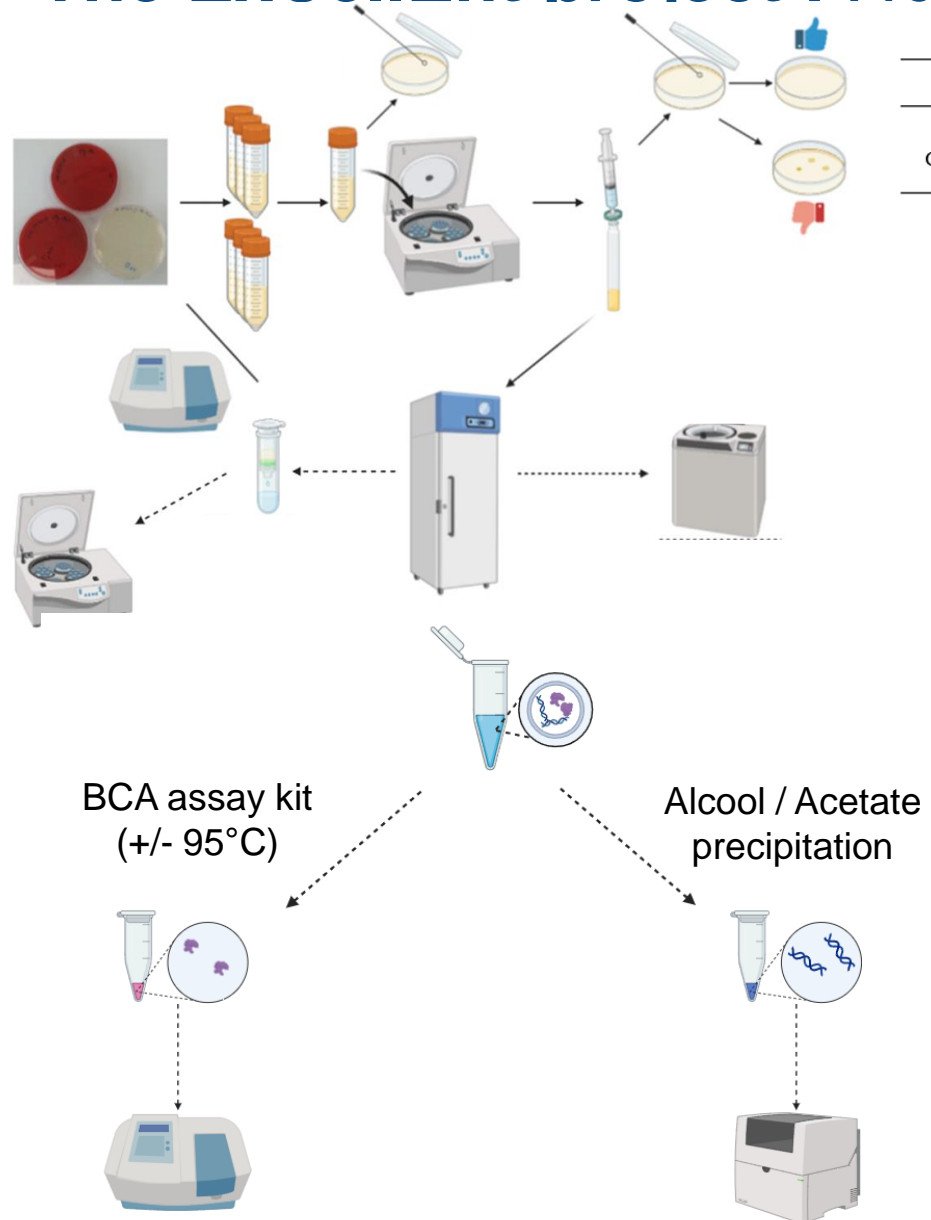
# The ExCellEnt project : Nanoparticle Tracking Analysis results



Taille moyenne	204,75 nm
Taille minimale	~30 nm
Taille maximale	430 nm

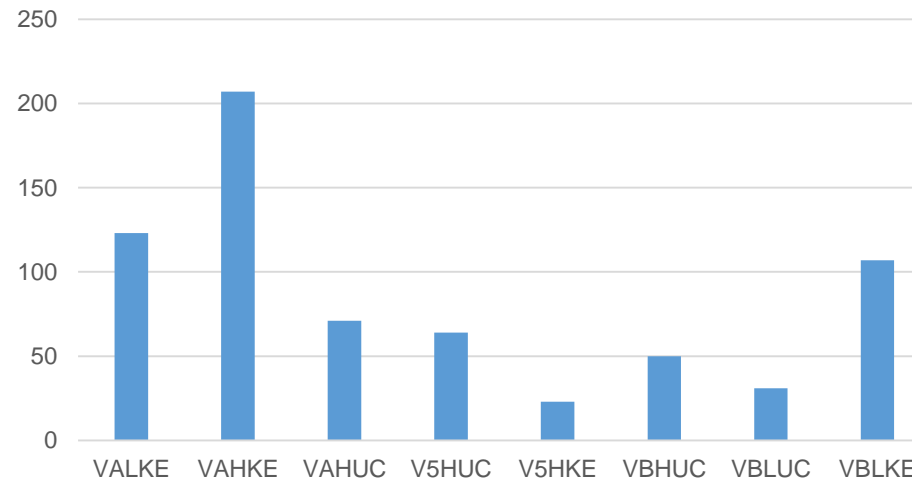


# The ExCellEnt project : Proteins / DNA quantification



Echantillons	VALKE	VAHKE	VAHUC 95°	VAHUC	V5HUC 95°	V5HUC	V5HKE	VBHUC 95°	VBHUC	VBLUC 95°	VBLUC	VBLKE
DO 562 (nm)	0,016	0,029	0,006	0,01	0,009	0	0,003	0,014	0,007	0,005	0,004	0,014
Concentration Protéines (µg/mL)	123,08	207,142857	42,8571429	71,4285714	64,2857143	0	23,08	100	50	35,7142857	30,8	107,7

Protein concentration

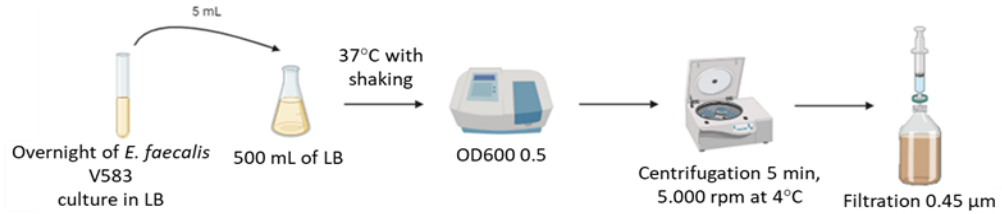


**Need for DNA extraction protocol improvement**

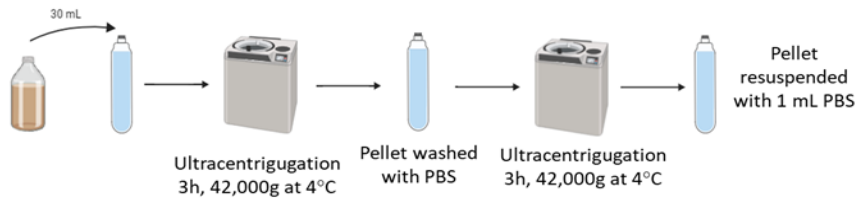


# The ExCellEnt project : Proteins composition

## Sample preparation



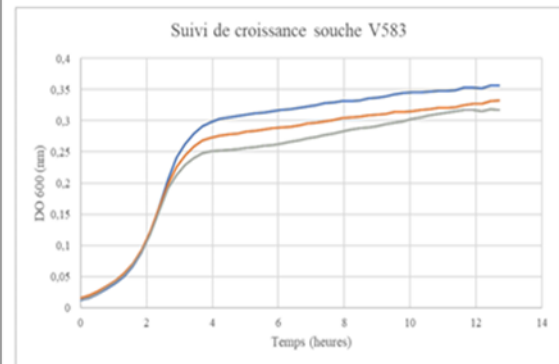
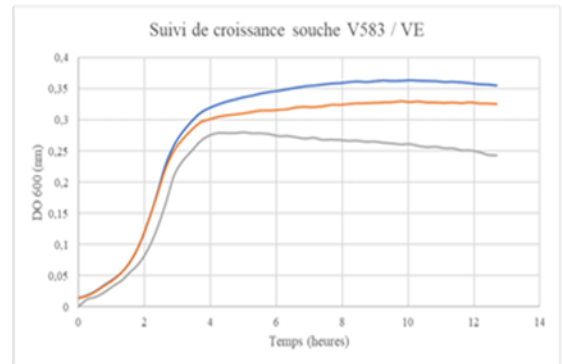
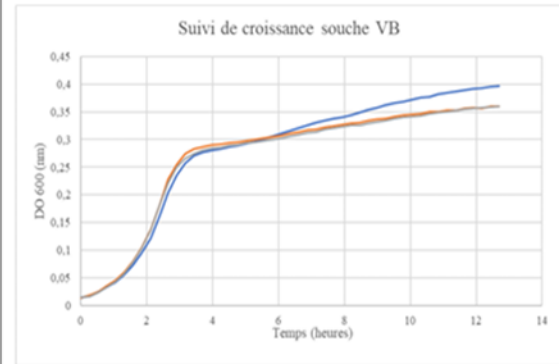
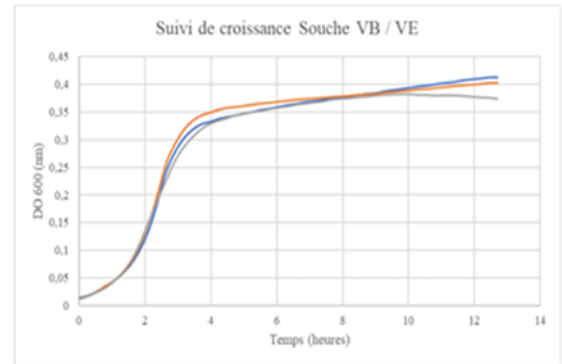
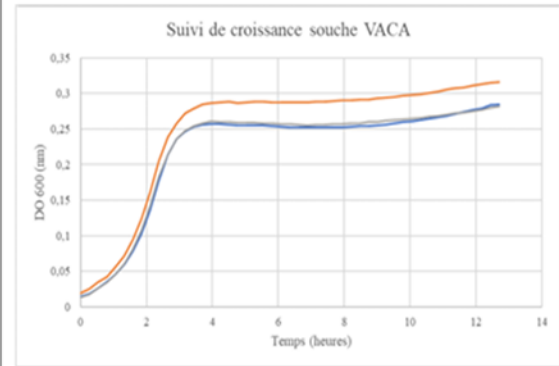
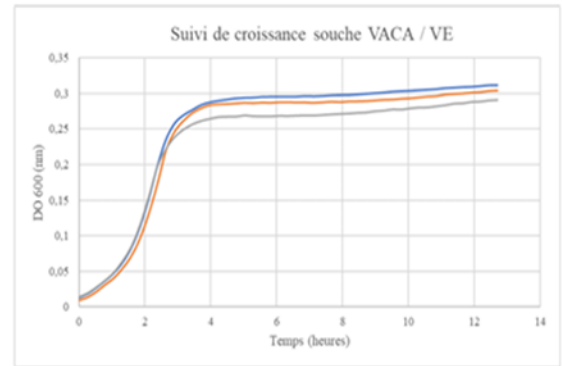
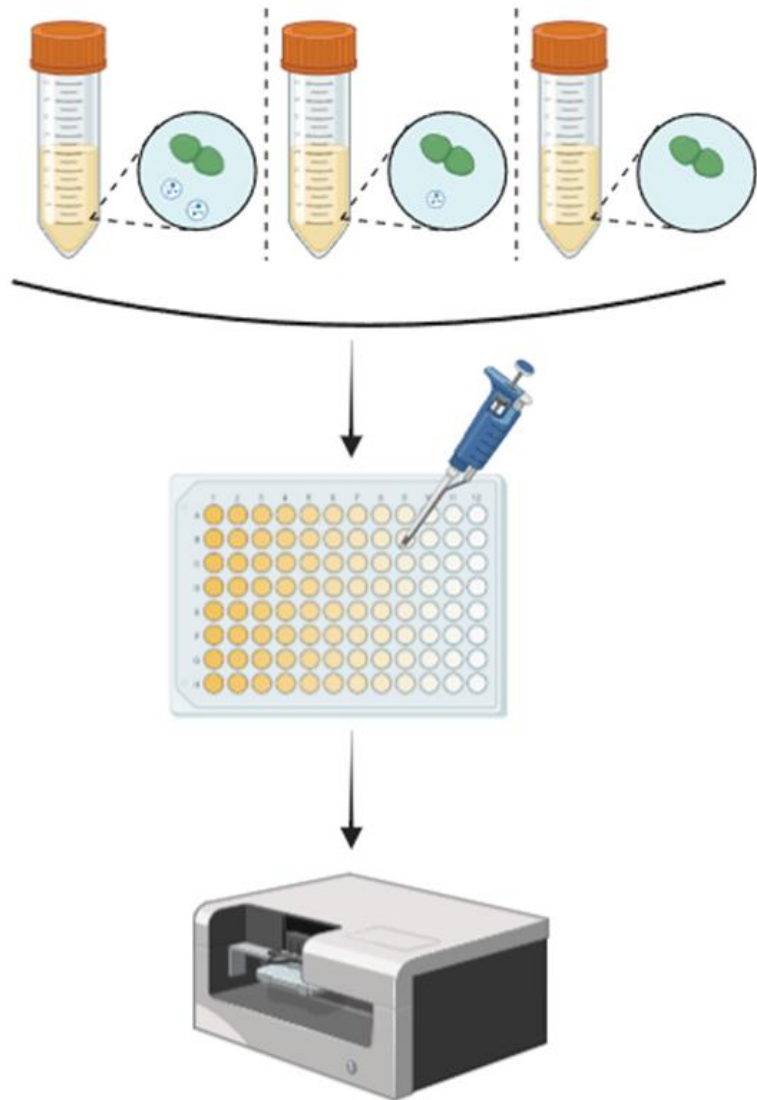
## Ultracentrifugation steps



**Beads and Trypsin lysis + MS analysis**

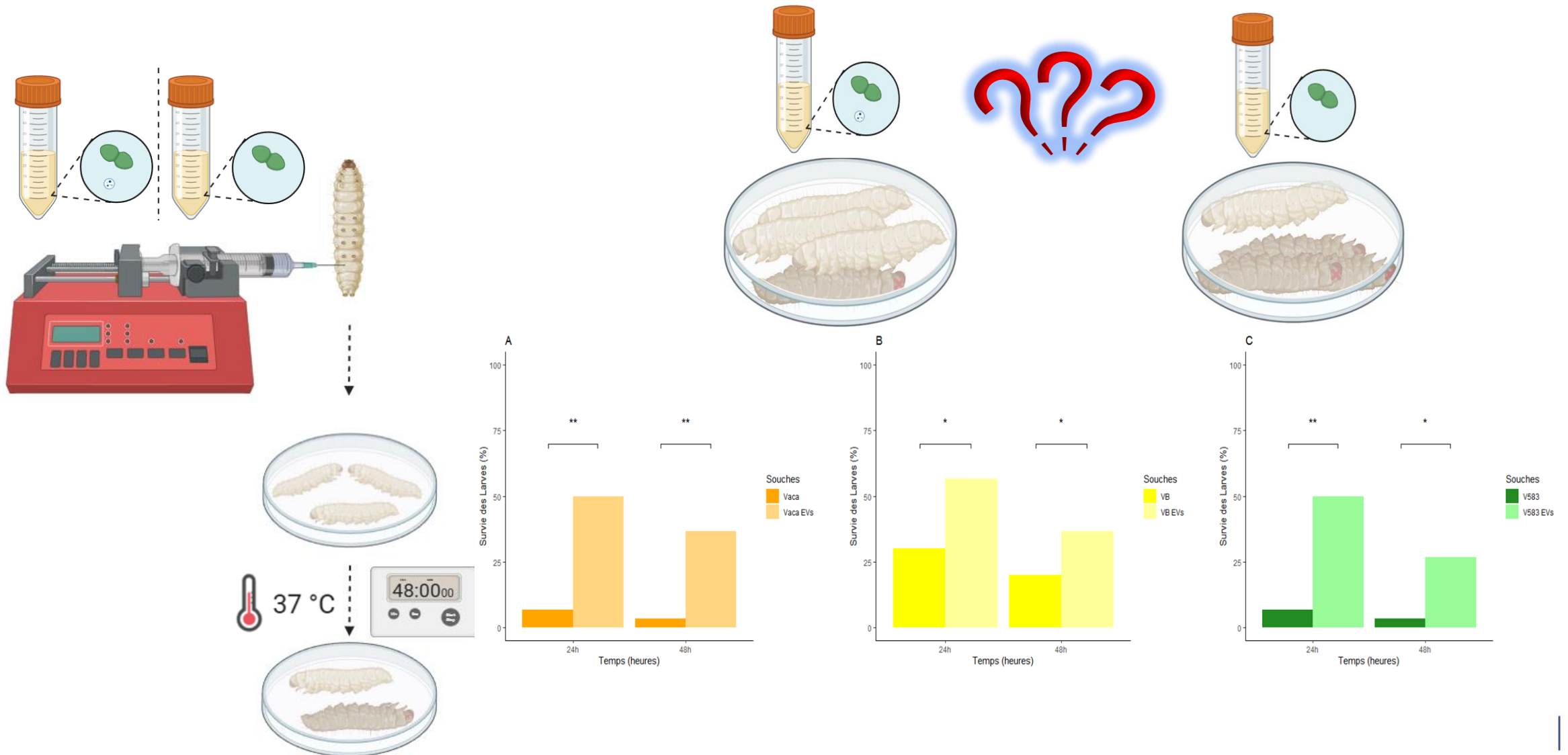
Proteins	Functions
Fumarate reductase flavoprotein subunit putative	Pyruvate metabolism
Phosphoglycerate kinase	Pyruvate metabolism
Pyruvate dehydrogenase E1 component subunit alpha	Pyruvate metabolism
Pyruvate dehydrogenase E1 component subunit beta	Pyruvate metabolism
Chaperonin GroEL	Stress protein Adhesion, virulence
Glyceraldehyde-3-phosphate dehydrogenase	Energy metabolism Virulence
Aldehyde-alcohol dehydrogenase	Energy metabolism
Enolase	Energy metabolism Adhesion, biofilm formation
Basic membrane protein family	Membrane protein
LysM domain protein	Anchored protein
Secreted antigen putative	Putative antigen
Pheromone cAD1 lipoprotein	Quorum sensing
Pheromone cAM373 lipoprotein	Quorum sensing
Small ribosomal subunit protein uS3	Traduction
Sulfatase domain protein	Lipoteichoic acid synthesis
Portal protein	Phage protein

# The ExCellEnt project : Impact of MVs on growth kinetic



— 1 µL — 10 µL — 20 µL

# The ExCellEnt project : Impact of MVs on EFS virulence



# TAKE HOME MESSAGE

- *E. faecalis* clinical strains produced Membrane Vesicles



- EFS MVs are compounded by intracellular proteins

  - Presence of « essential » vesicular proteins (e.g. *GroEL*, *Aad*) ??



- EFS MVs decreased EFS virulence in *Galleria mellonella* model



- EFS MVs do not interfered with bacteria kinetic growth



## NEXT STEPS

- MultiOMICS analysis
  - Proteins composition in different media
  - DNA sequencing
  - RNA sequencing
- Strains recruitment (non human strains / different clinical isolates)
- Are MVs can be used as **diagnostic biomarkers** or **antibiotherapy adjuvants** ?

# Acknowledgements



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& RESPIRATOIRES

Pr. A Rincé  
Dr. M Rami



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