Protein content of the *Oenococcus oeni* extracellular vesicles-enriched fraction
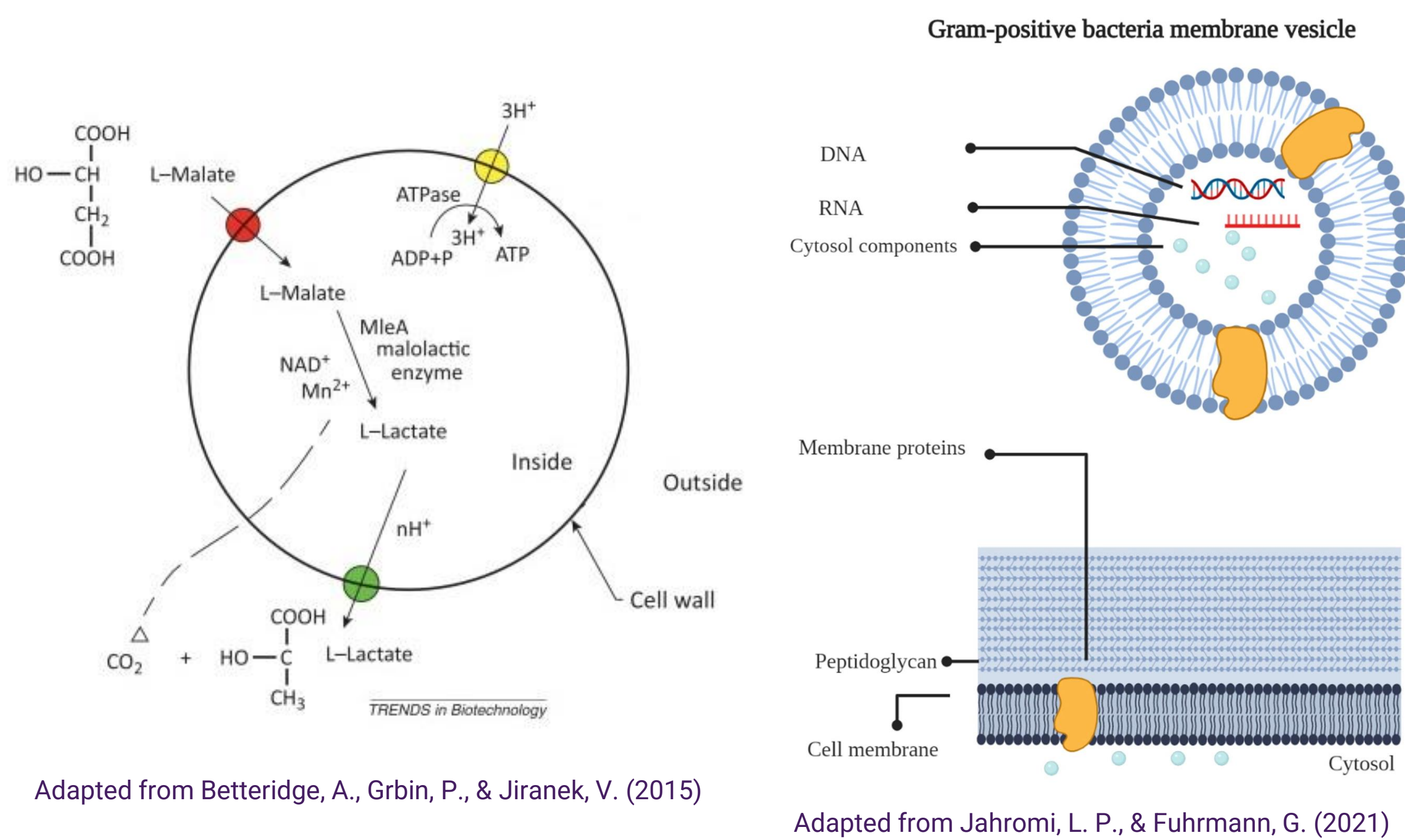
Miguel Mejías Ortiz, Ana Mencher, Pilar Morales, Jordi Tronchoni, Ramon Gonzalez

Instituto de Ciencias de la Vid y el Vino (ICVV-CSIC-UR-GR)

miguel.mejias@icvv.es

Instituto de Ciencias de la Vid y del Vino

Gobierno de La Rioja

CSIC

UNIVERSIDAD DE LA RIOJA

MALOLACTIC FERMENTATION AND EV'S

Adapted from Betteridge, A., Grbin, P., & Jiranek, V. (2015)

Gram-positive bacteria membrane vesicle

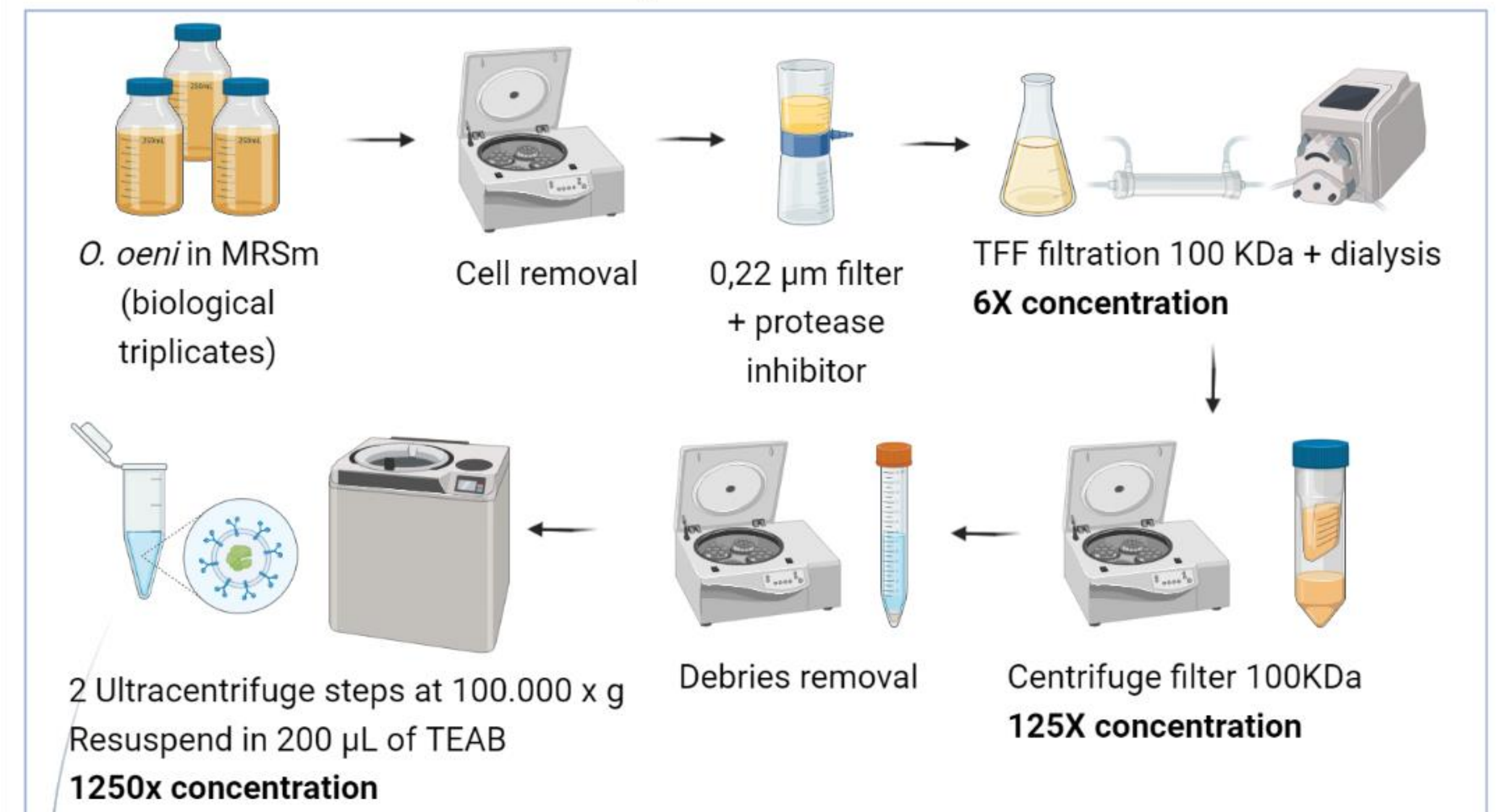Adapted from Jahromi, L. P., & Fuhrmann, G. (2021)

- Malolactic fermentation (MLF) is almost essential for most red wines and some white wines.
- MLF is performed by lactic acid bacteria. *Oenococcus oeni* is the main responsible of spontaneous MLF. For this reason, commercially available starters for MLF usually contain *O. oeni* strains.
- EV's are non-replicative particles delimited by a lipid bilayer and released by cells. They are involved in intra- and interspecific interactions and can carry diverse components inside them.
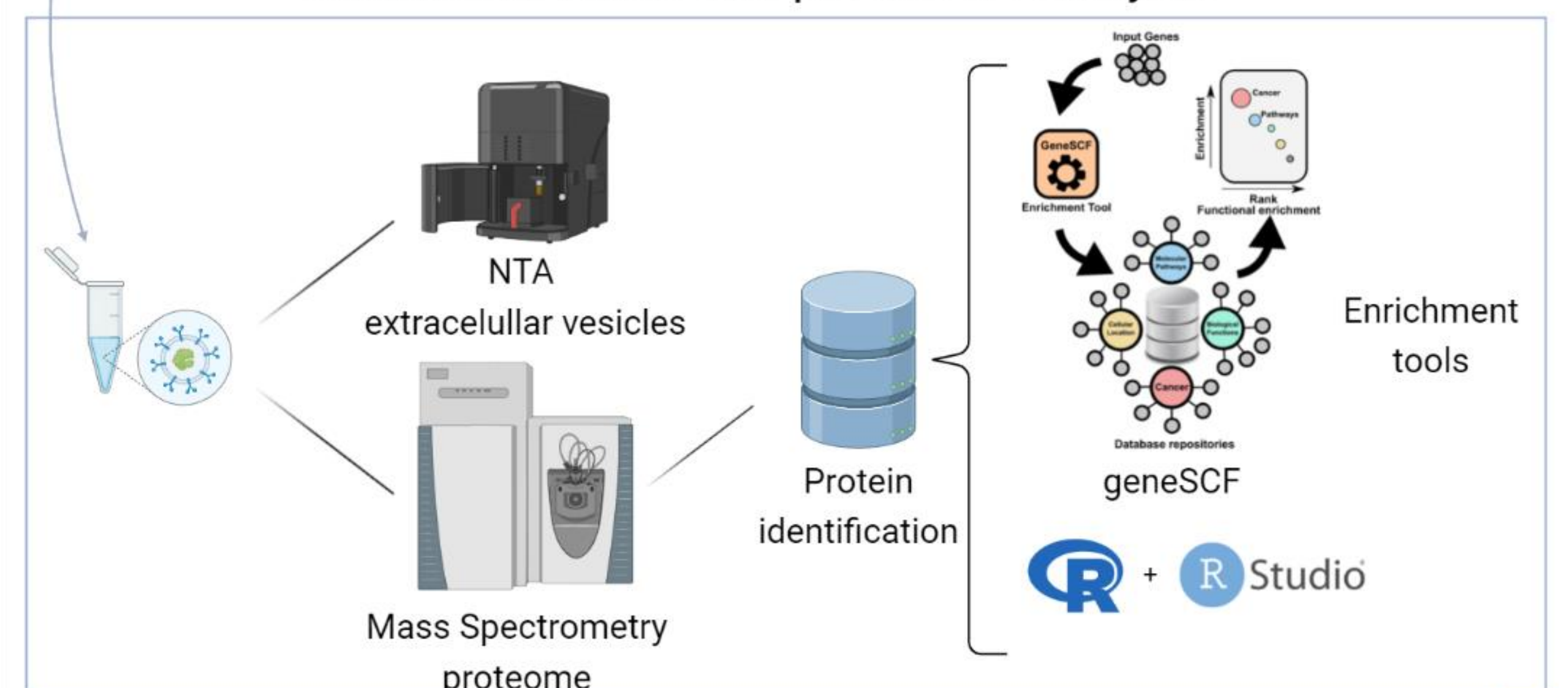
To understand the interactions between microbial starters, extracellular vesicles (EV) have been hypothesized as part of the interaction mechanisms.

METHODOLOGY
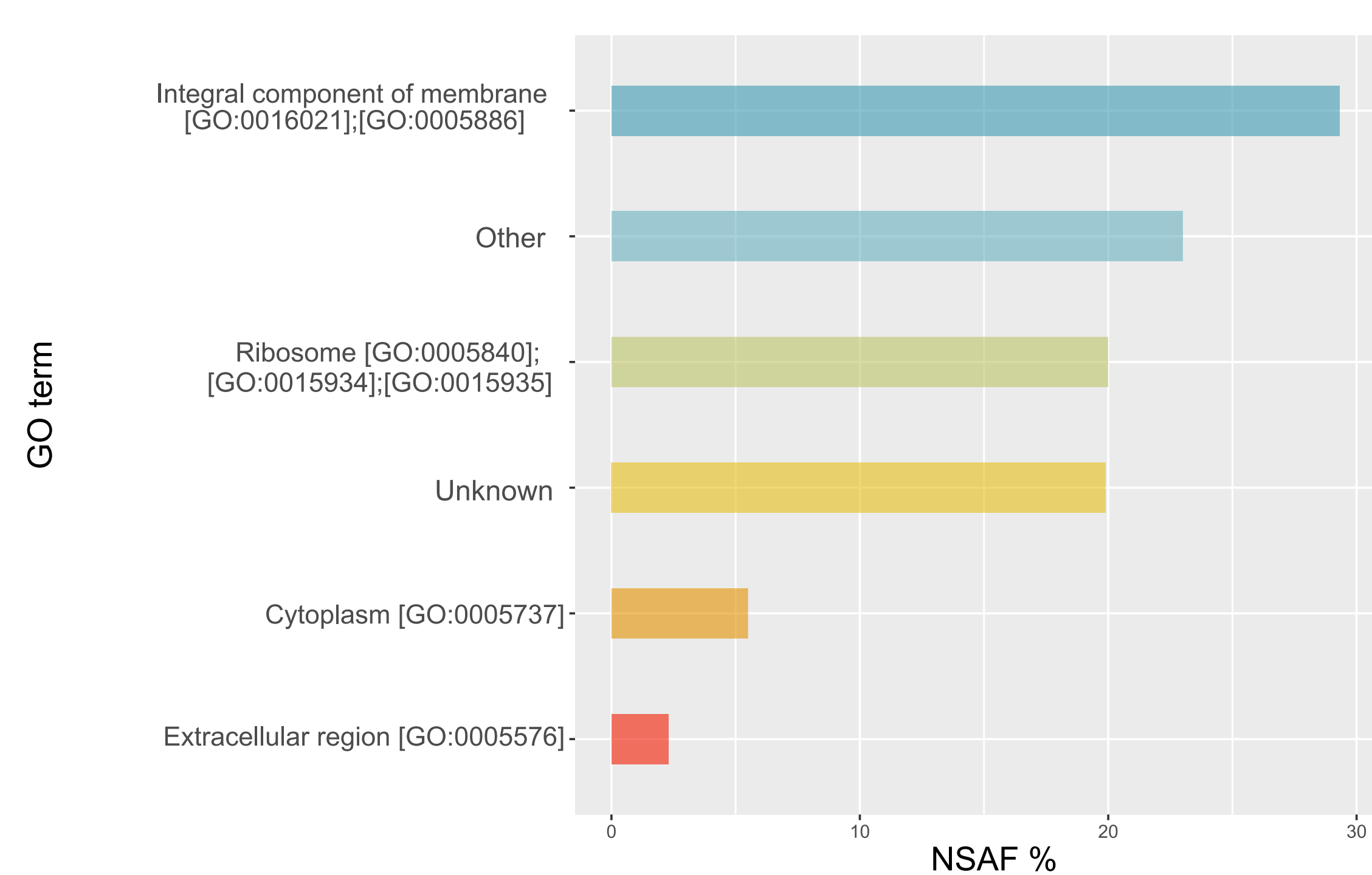
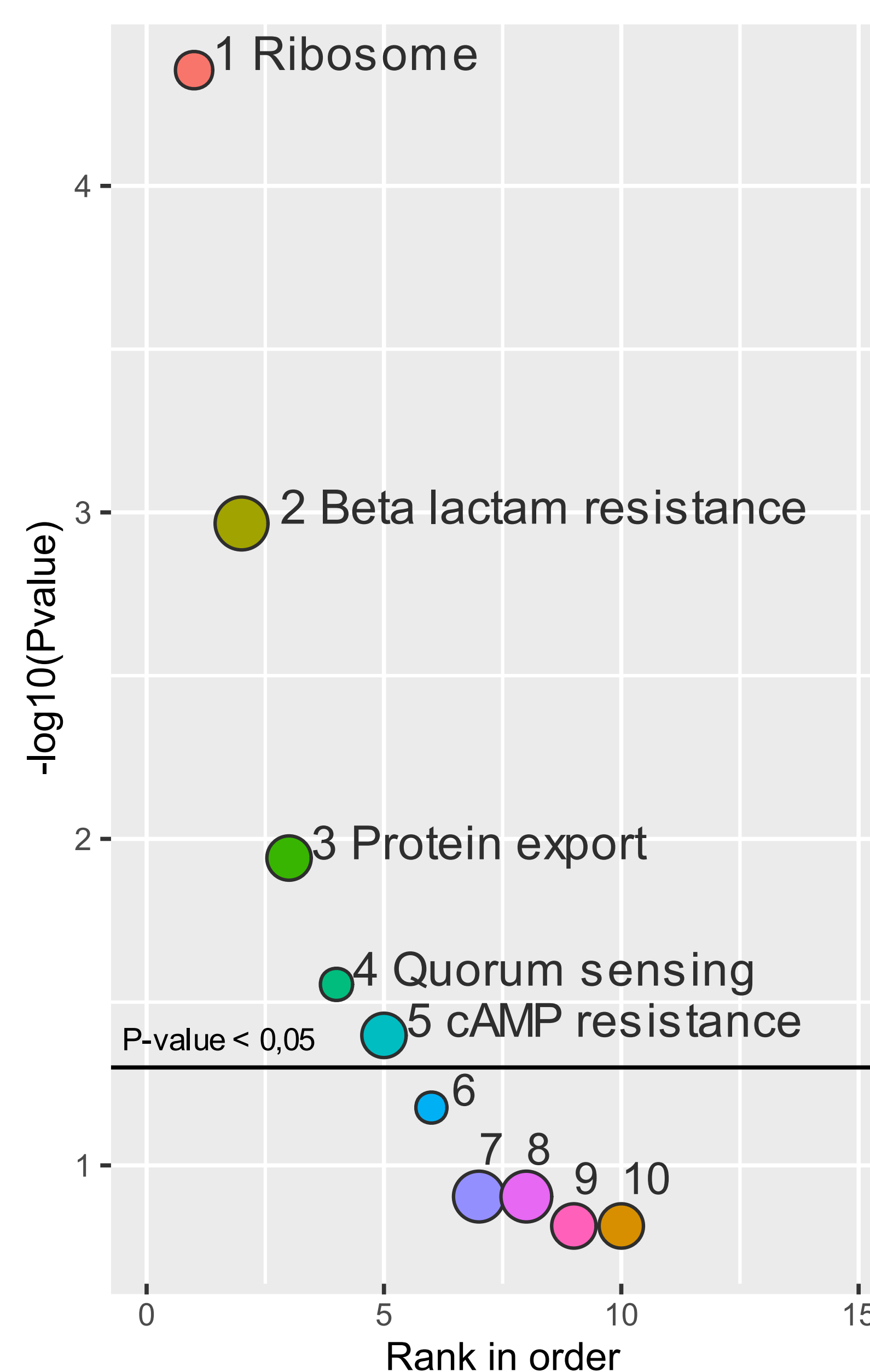### Extracellular vesicles purification and concentration

O. oeni in MRSm (biological triplicates) → Cell removal → 0,22 μm filter + protease inhibitor → TFF filtration 100 KDa + dialysis **6X concentration**

2 Ultracentrifuge steps at 100.000 x g Resuspend in 200 μL of TEAB **1250x concentration** ← Debries removal ← Centrifuge filter 100KDa **125X concentration**

Characterization and proteome analysis

NTA extracellular vesicles

Mass Spectrometry proteome

Protein identification

geneSCF

Enrichment tools

Created in **BioRender.com**

RESULTS

NSAF %

GO term:
Integral component of membrane [GO:0016021];[GO:0005886]
Other
Ribosome [GO:0005840]; [GO:0015934];[GO:0015935]
Unknown
Cytoplasm [GO:0005737]
Extracellular region [GO:0005576]

Cellular component GO term of the most abundant proteins present in EV-enriched fraction.

-log10(Pvalue) vs Rank in order

1 Ribosome
2 Beta lactam resistance
3 Protein export
4 Quorum sensing
5 cAMP resistance
6
7 8 9 10
P-value < 0,05

MP share
○ 15 %
○ 20 %
○ 25 %
○ 30 %
○ 35 %

GeneSCF graph representing significant KEGG categories from the *O. oeni* EV-enriched proteome. "MP share" stands for the percentage of the corresponding metabolic pathway covered by the proteins included in each category.

L. plantarum   *O. oeni*

261   32   31
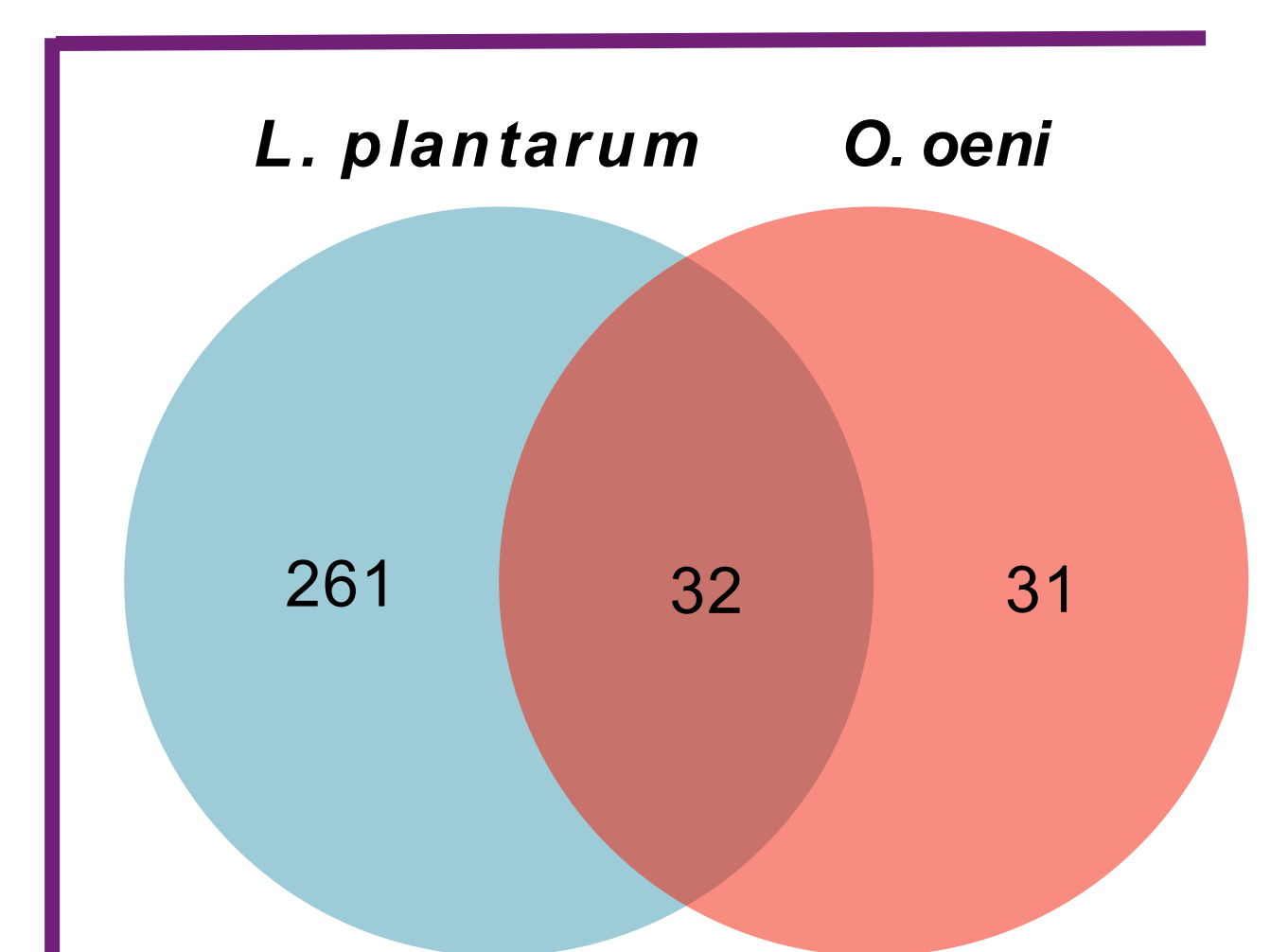
Number of proteins shared by *O. oeni* and *L. plantarum* EV-enriched fractions.

CONCLUSIONS

- *O. oeni* EV's are similar to other bacterial EV's described before.
- Ribosomal and integral membrane proteins are the most abundant protein categories in the EV-enriched fraction.
- These features are also found in other bacterial and eukariotic species.

REFERENCES

Betteridge, A., Grbin, P., & Jiranek, V. (2015). *Trends in Biotechnology*, *33* (9).

Jahromi, L. P., & Fuhrmann, G. (2021). *Advanced Drug Delivery Reviews*, *173*.

FUNDING