# Mycoviruses: are they an important issue for the quality control of a fungal collection?

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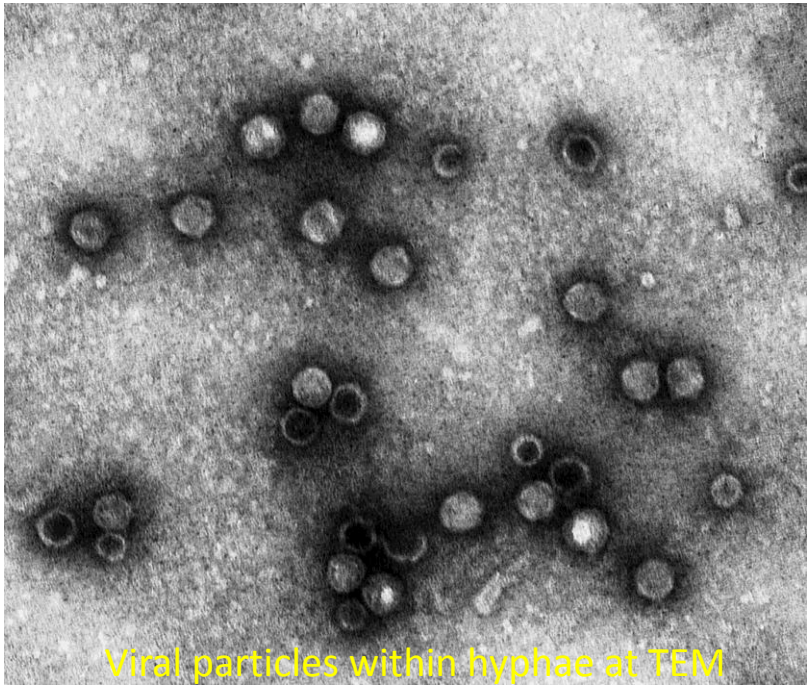
# Why Mycoviruses (MVs) are so intriguing?

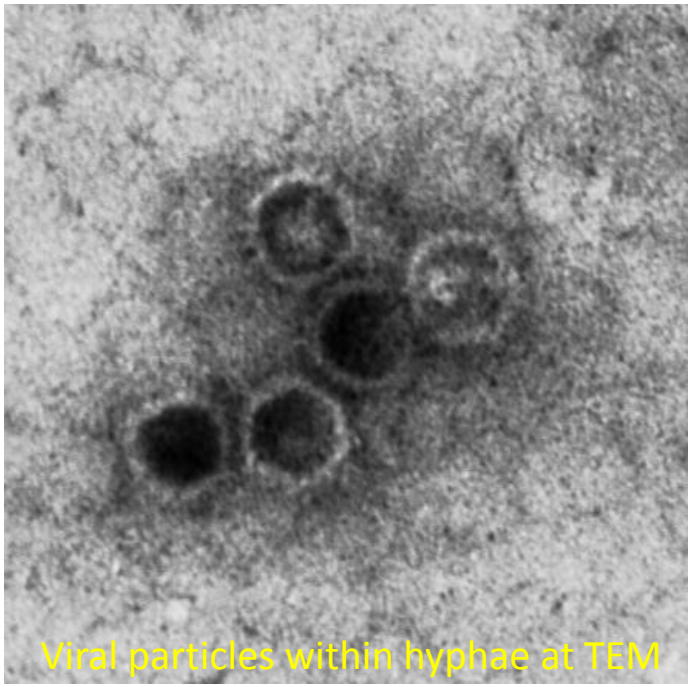
1. They are widespread in all major fungal groups.

2. Fungi can be infected with two or more viruses.

3. They have different morphologies (encapsidated or capsidless) and genomes: **dsRNA** (about 70%), ssRNA (+/-) and circular ssDNA.

4. They are transmitted among different individuals through cellular fusion (anastomosis).

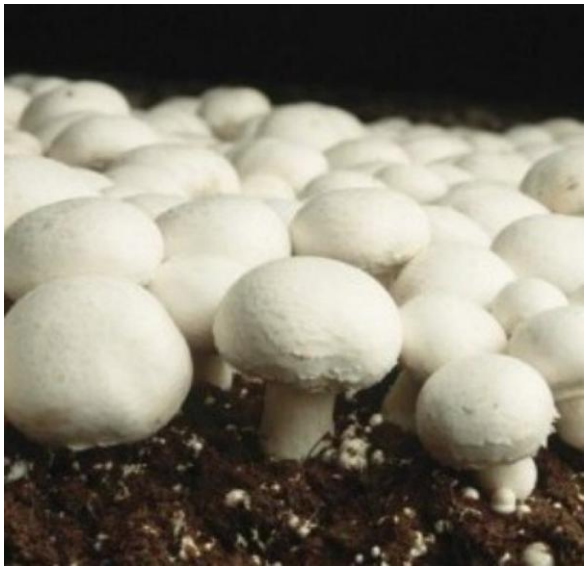

Viral particles within hyphae at TEM


Viral particles within hyphae at TEM

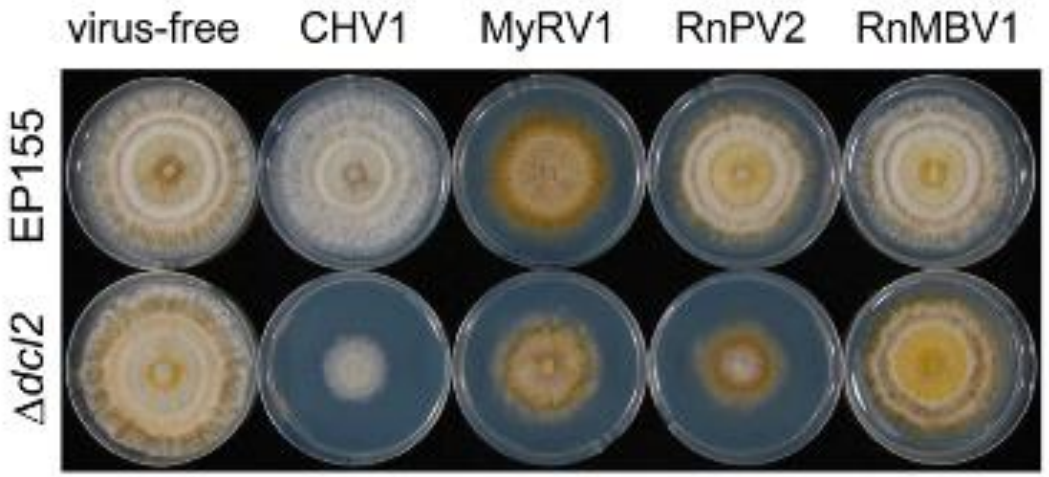
# Why Mycoviruses are so intriguing?

3. Phenotypic effects of MVs can vary from **advantageous** to **deleterious**, but most of them are **asymptomatic or cryptic**.
However**, attention was mainly focused on negative effects**: decreased growth rate, lack of sporulation, **attenuation of virulence**, reduced germination of basidiospores, reduced yield.

*La France* disease *in A.*



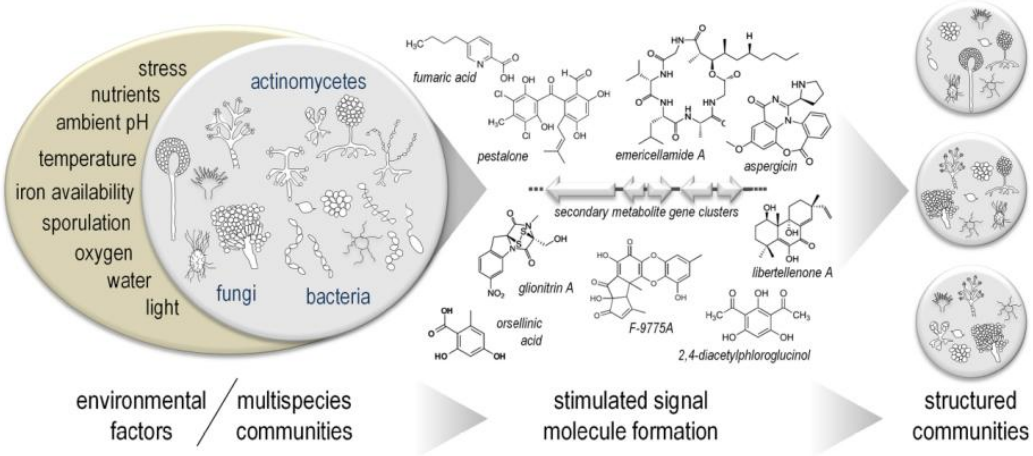
Virus-mediated hypovirulence of the chestnut blight fungus *C. parasitica*

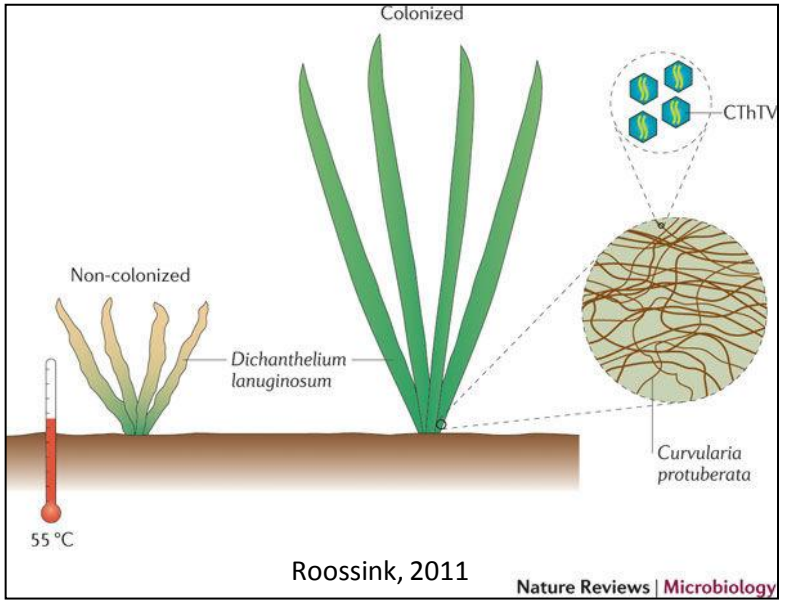


# Why Mycoviruses are so intriguing?

4. **MVs can promote adaptive changes** of their hosts in specific ecological niches, favouring the fitness of fungi and of other organisms (i.e. tripartite symbiosis virus – fungal endophyte – plants).

Many putative ecological roles.




**Many biotecnological implications: the presence/absence of MVs can impact the chemical fingerprint of fungi**

(i.e. change of production of useful metabolites or of dangerous ones i.e. mycotoxins)

# Searching Mycoviruses in a collection (91 isolates) of marine fungi using all the available tecniques to search for any kind of virus

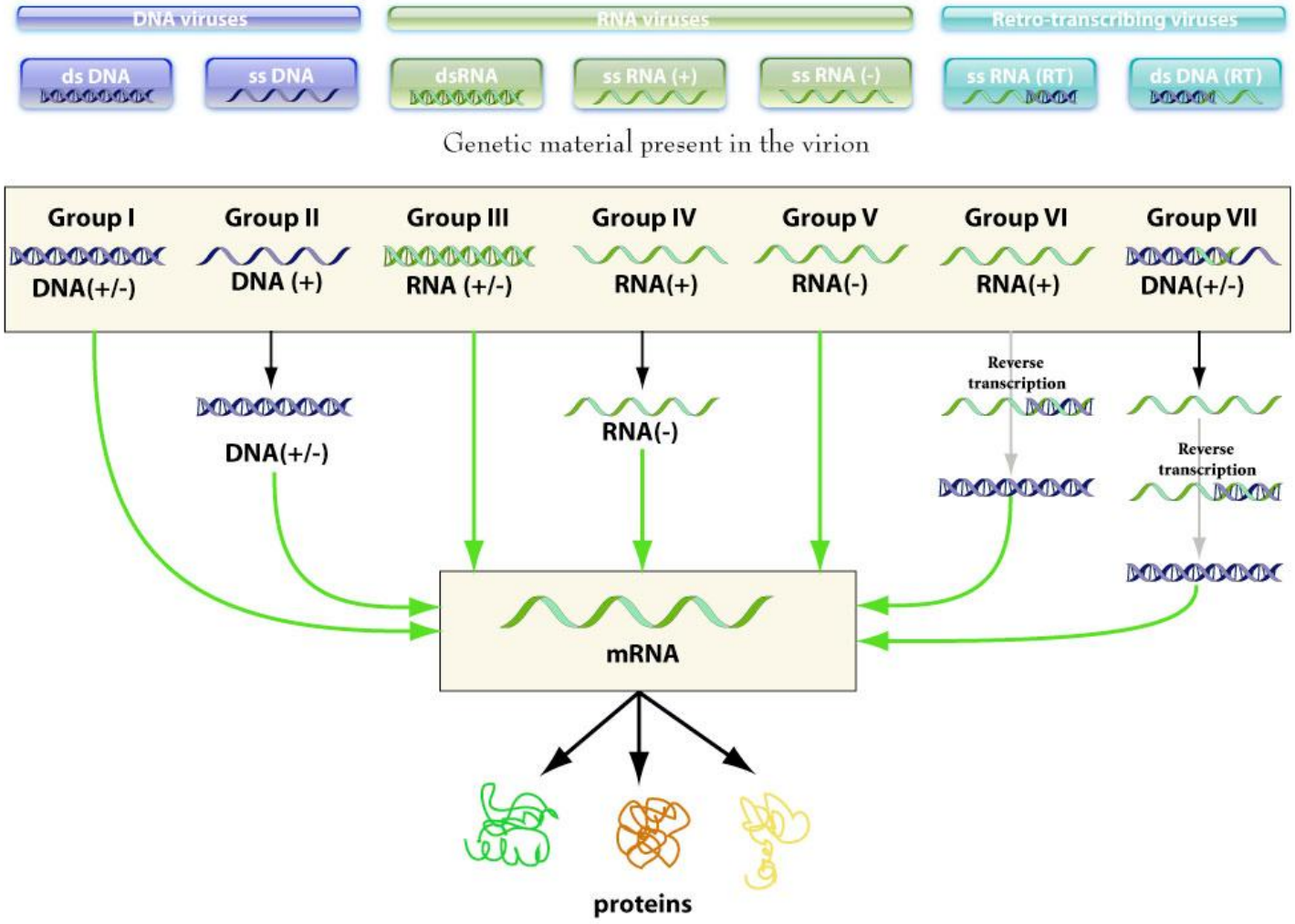
Biomass in SF

Different techniques to detect any kind of virus

1. dsRNA purification + cDNA libraries and Northern blot analysis
2. Total DNA extraction (endogenized viral genomes)
3. Rolling cicle amplification (circular ssDNAvirus)
4. **Total RNA extraction and RNAseq**
5. Assembly of mycovirus sequences from small RNA (sRNA) libraries

+ Viral particle purification and TEM

# Why RNAseq?

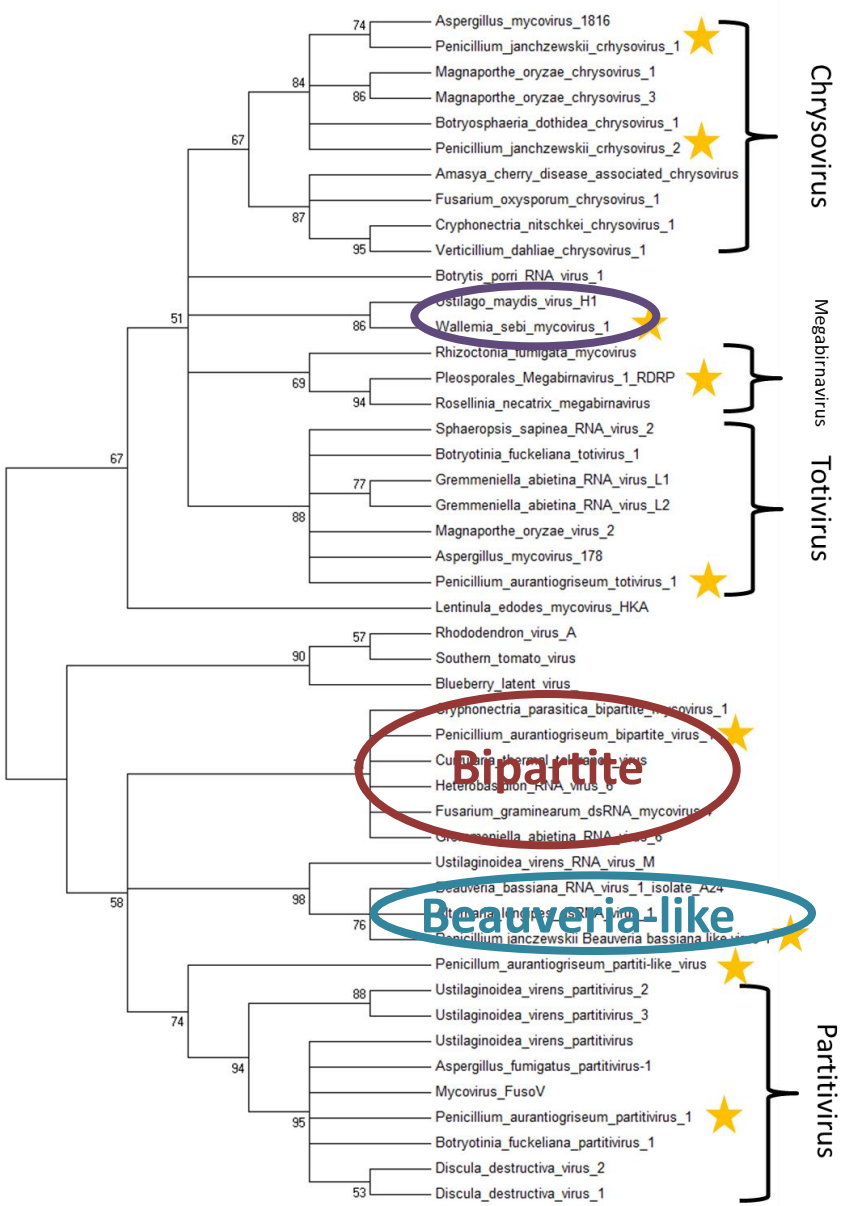



**Theoretically, all types of viruses can be detect**

Nerva et al., 2017. Different approaches to discover mycovirus associated to marine organisms. **Springer Book, Viral Metagenomics: Methods and Protocols**

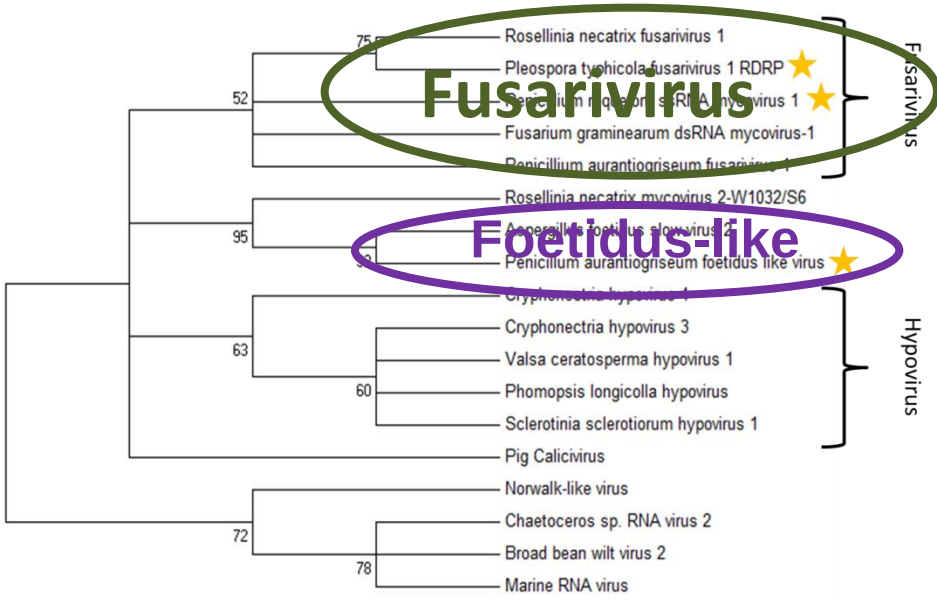
# New viral groups in fungi from *Posidonia oceanica*

## dsRNA




**15% of fungi were infected by viruses**

## ssRNA




*Nerva et al., Virus Research 2016*

# *Pencillium aurantiogriseum* MUT 4330

**hosts 6 mycoviruses:**

Bipartitivirus (PaBV1)

Aspergillus foetidus slow virus 2 (PaFLV1)

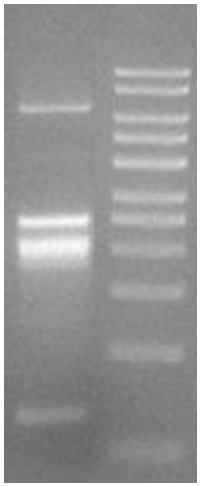
Fusarivirus (PaFV1),

Unknown virus (εPartiti-virus? - PaPLV1)

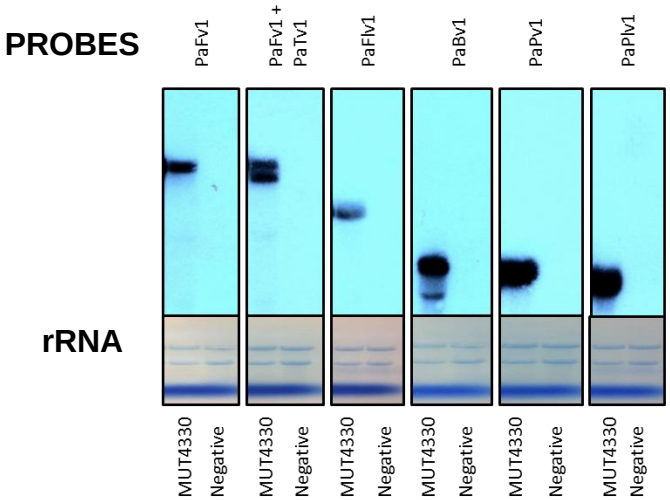
γPartitivirus (PaPV1)

Totivirus (PaTV1)

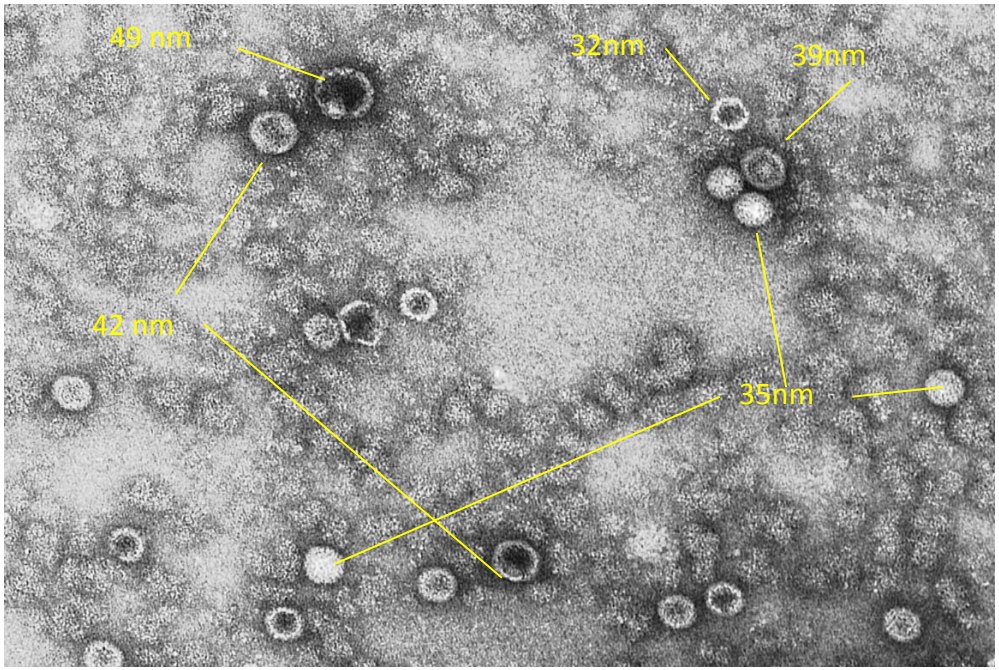
## Molecular characterization



## Northern blot analysis

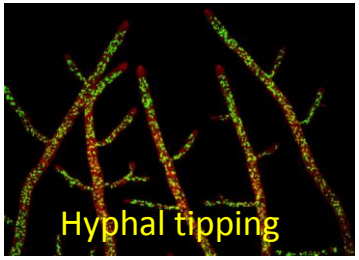



## Morphological characterization (TEM)



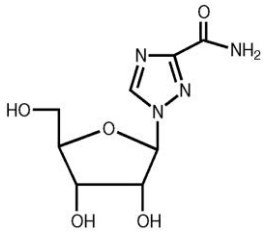
# Searching for mycoviruses effects on fungal phenotype

**Curing methods:** 2 techniques combined with 2 antiviral drugs

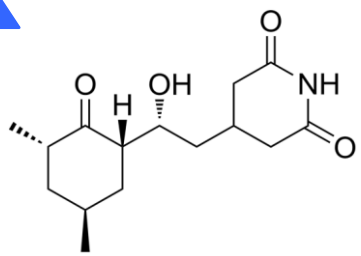




X




Ribavirin




Cycloheximide



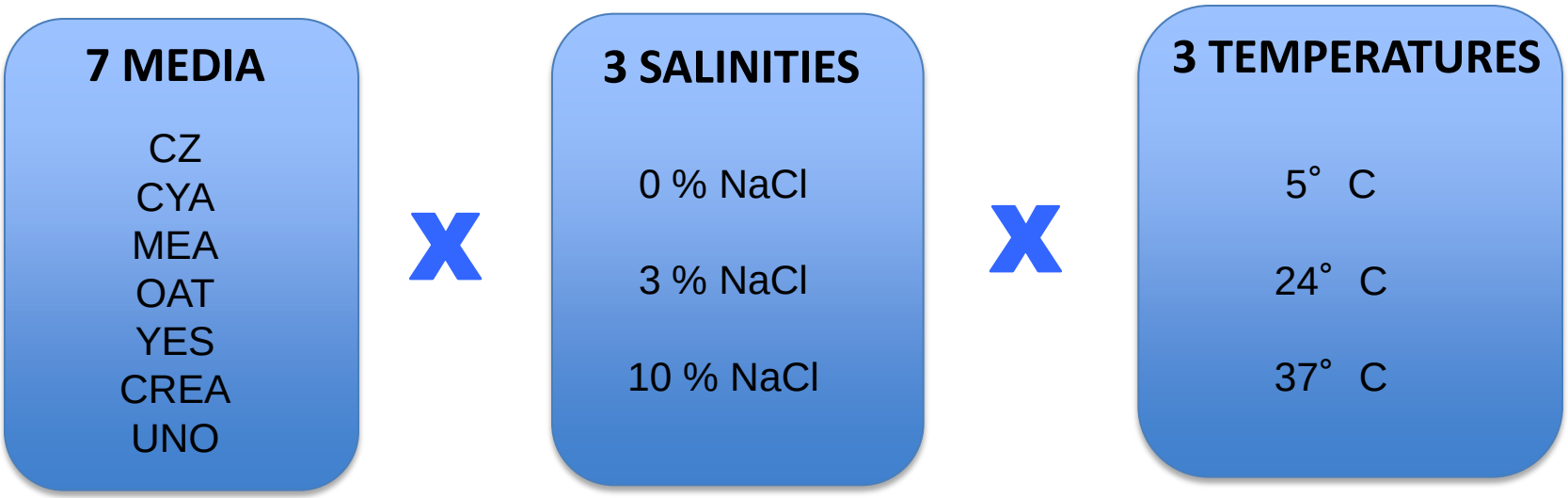
**We got *14 P. aurantiogriseum* MUT 4330 virotypes: isogenetic fungal isolates with different MVs combinations**

**The 14 *P.* aurantiogriseum MUT 4330 virotypes:**
**1 wild-type (6 MVs)**
***12* semi-cured isolates with different MVs combination**
**1 completely cured isolate (VF)**




| Virotypes | | | | | | |
|---|---|---|---|---|---|---|
| | PaFV1 | PaTV1 | PaFLV1 | PaPV1 | PaPLV1 | PaBV1 |
| WT | + | + | + | + | + | + |
| V5-A | - | + | + | + | + | + |
| V5-B | + | + | + | + | + | - |
| V5-C | + | + | + | + | - | + |
| V5-D | + | + | + | - | + | + |
| V4-A | - | + | + | + | + | - |
| V4-B | - | + | + | + | - | + |
| V4-C | + | + | + | + | - | - |
| V4-D | + | + | + | - | + | - |
| V3-A | - | + | + | + | - | - |
| V3-B | + | - | + | + | - | - |
| V2-A | + | - | - | - | - | + |
| V1-A | - | - | - | - | - | + |
| VF | - | - | - | - | - | - |

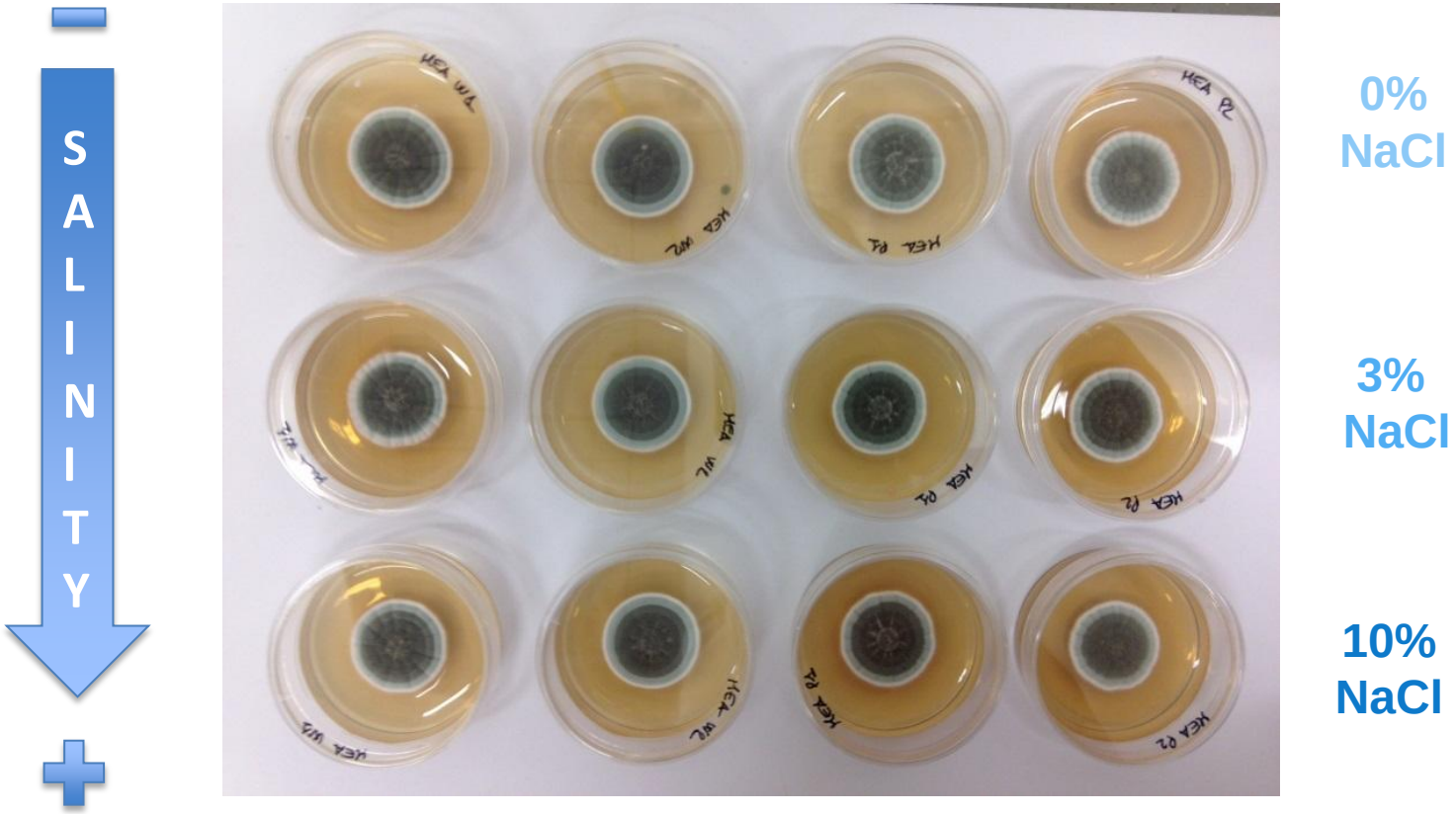
# Phenotypical characterization of the different virotypes of *P. aurantiogriseum* MUT 4330




Evaluation of growth parameters at 7 and 14 days

# wild type VS semi-cured

WT1 WT2 SC1 SC2

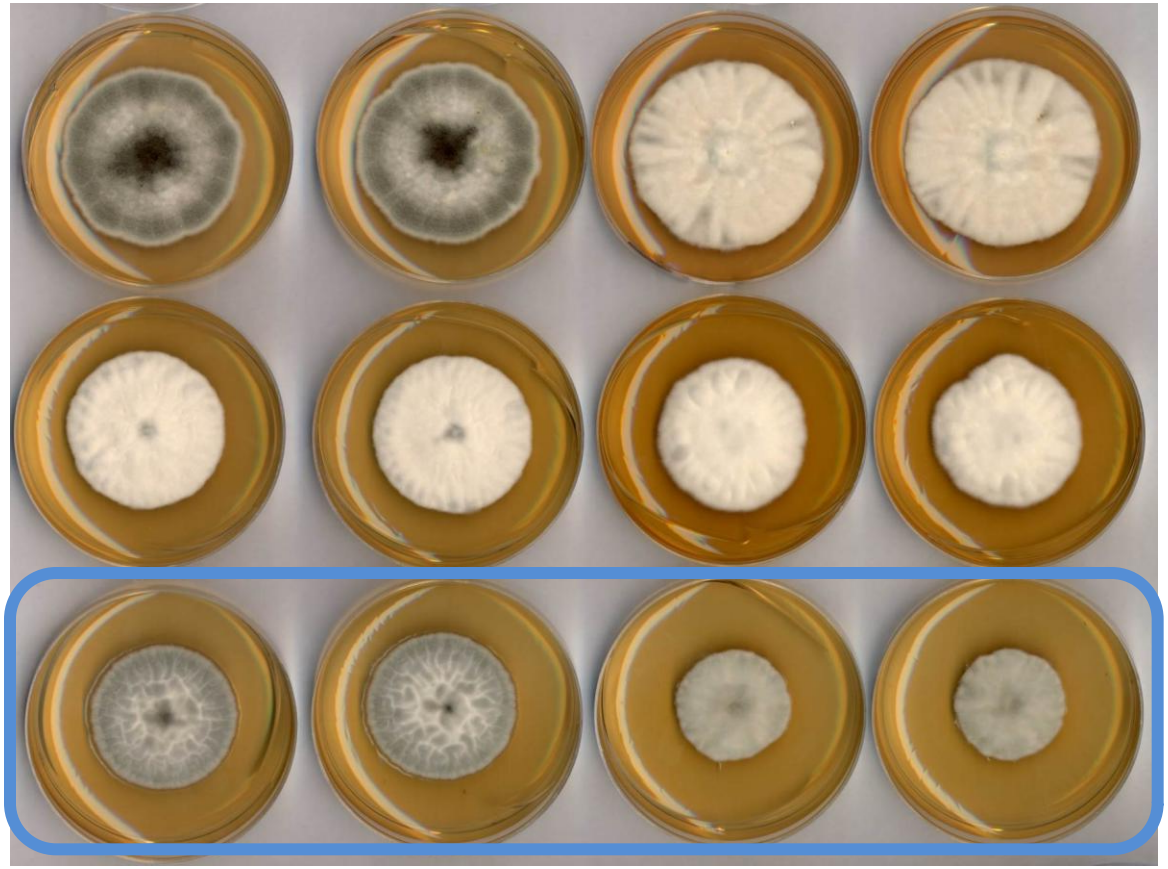



MEA medium – 14 days – 24° C – 2 biological replicas – 3 replicas per each condition

**No significant effects on growth rate and the morphology of semi-cured strains**

# wild type VS semi-cured

WT1 WT2 SC1 SC2



SALINITY

0% NaCl

3% NaCl

10% NaCl

**The presence of MVs increase the salt tolerance of this marine fungus!**

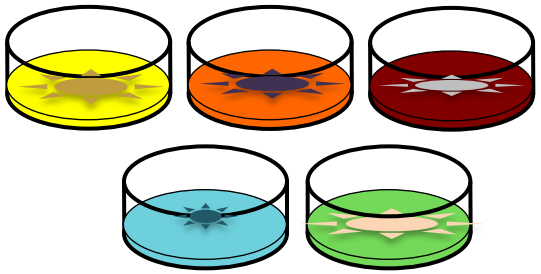
MEA medium – 14 days – 24° C – 2 biological replicas – 3 replicas per each condition

**The lost of MVs can strongly affects the growth rate and the morphology (i.e. pigmentation) of semi-cured strains !!!**

# Does the presence/absence of MVs impact also the chemical fingherprint of the fungus?

**WT vs Semicured**

5 media



different in:
- C:N ratio
- C and N sources
- salt concentration

3 Weeks at 24° C

SSF (biomasses + media)

Solvent Extraction

1:1 MeOH-$CH_2Cl_2$
ca 1:10 p/v

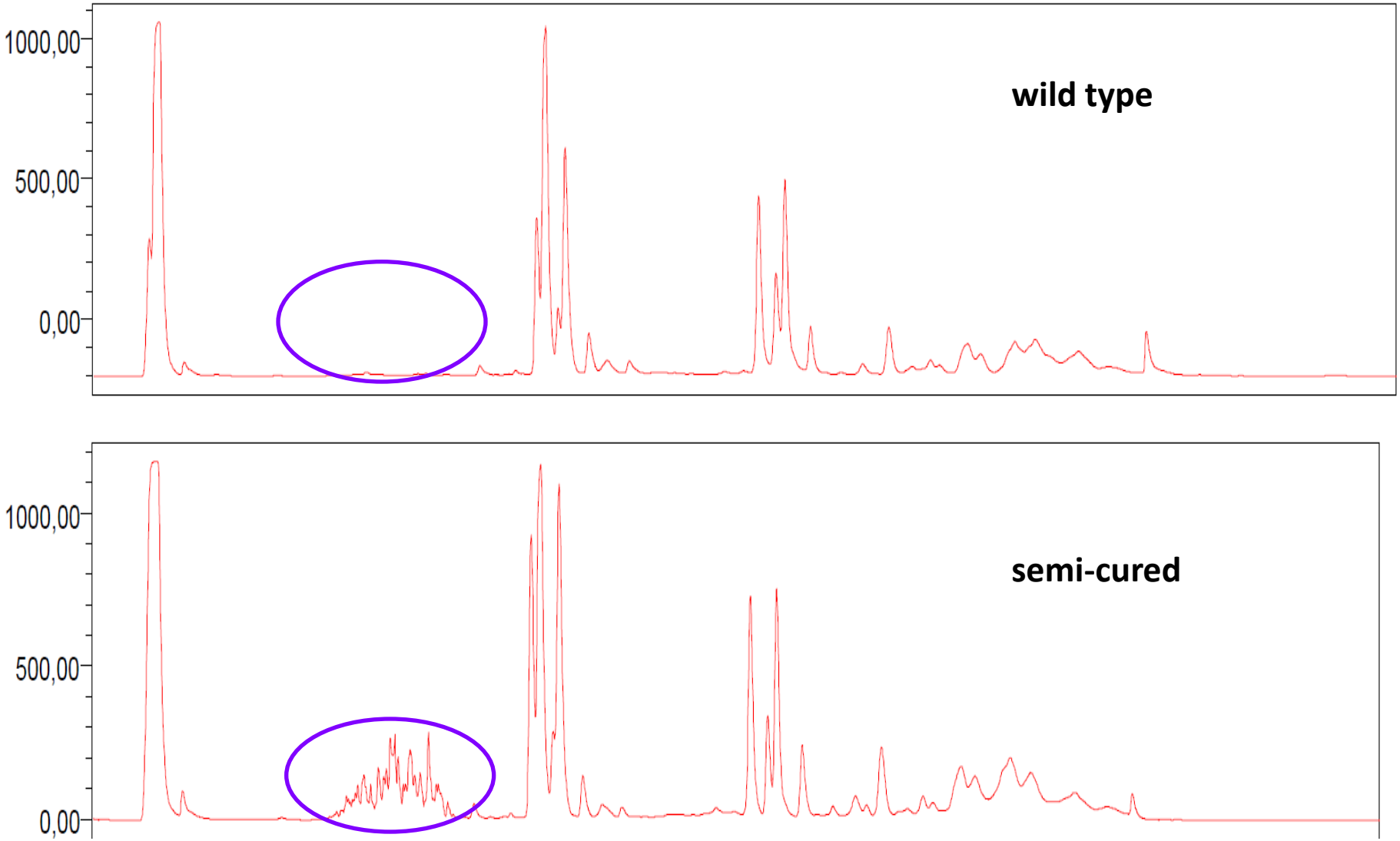
Crude Extracts

Chemical Analysis

HPLC-DAD-ESD

# HPLC-DAD-ESD WT vs SC

Raw extract MEA medium 14 days 24° C



# WT vs SC

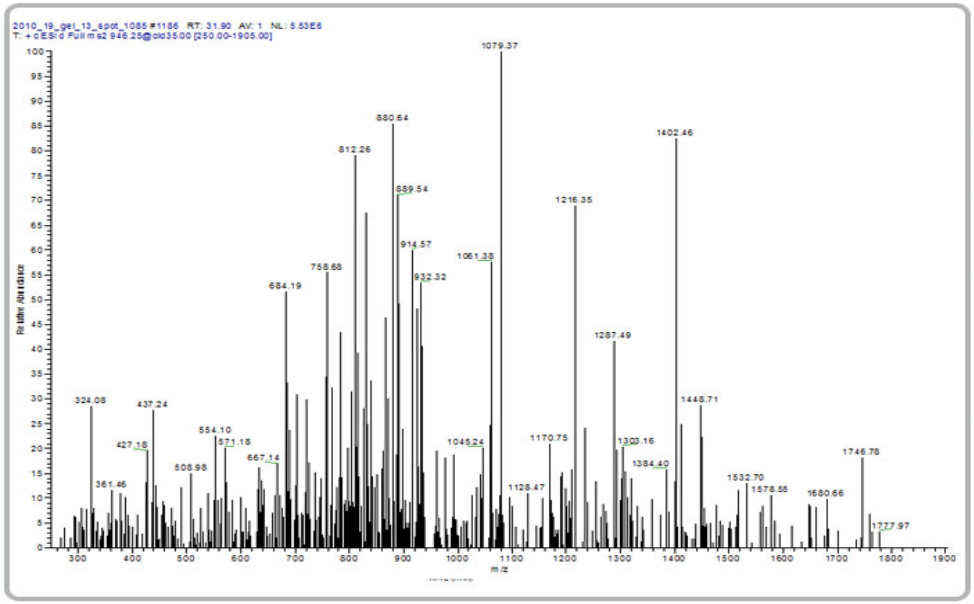
## Chemical fingerprint

Biomasses on SSF

Solvent Extraction

Crude Extracts

Chemical Analysis

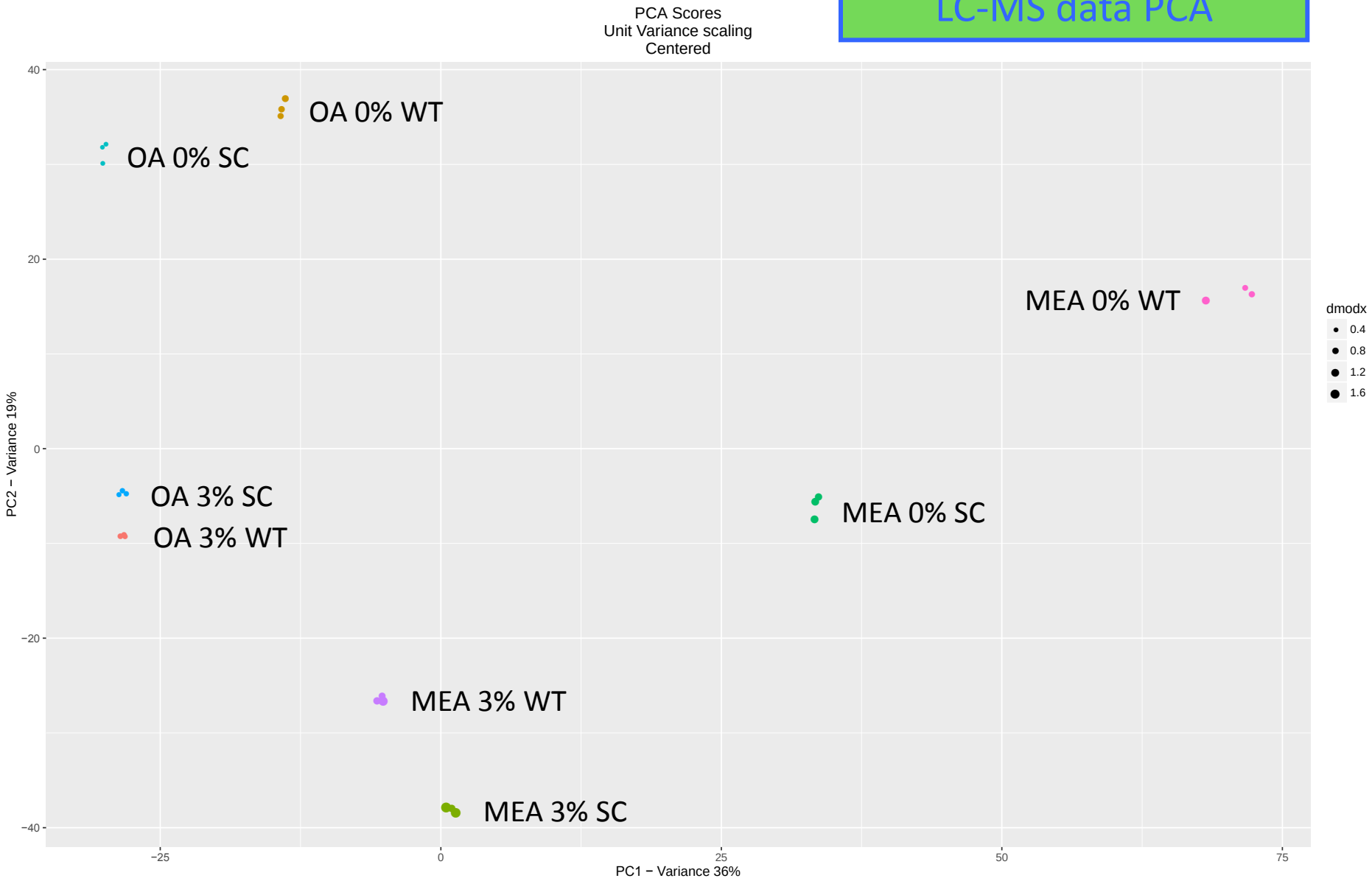



HPLC-DAD-ESD

LC-MS

PCA

# LC-MS data PCA

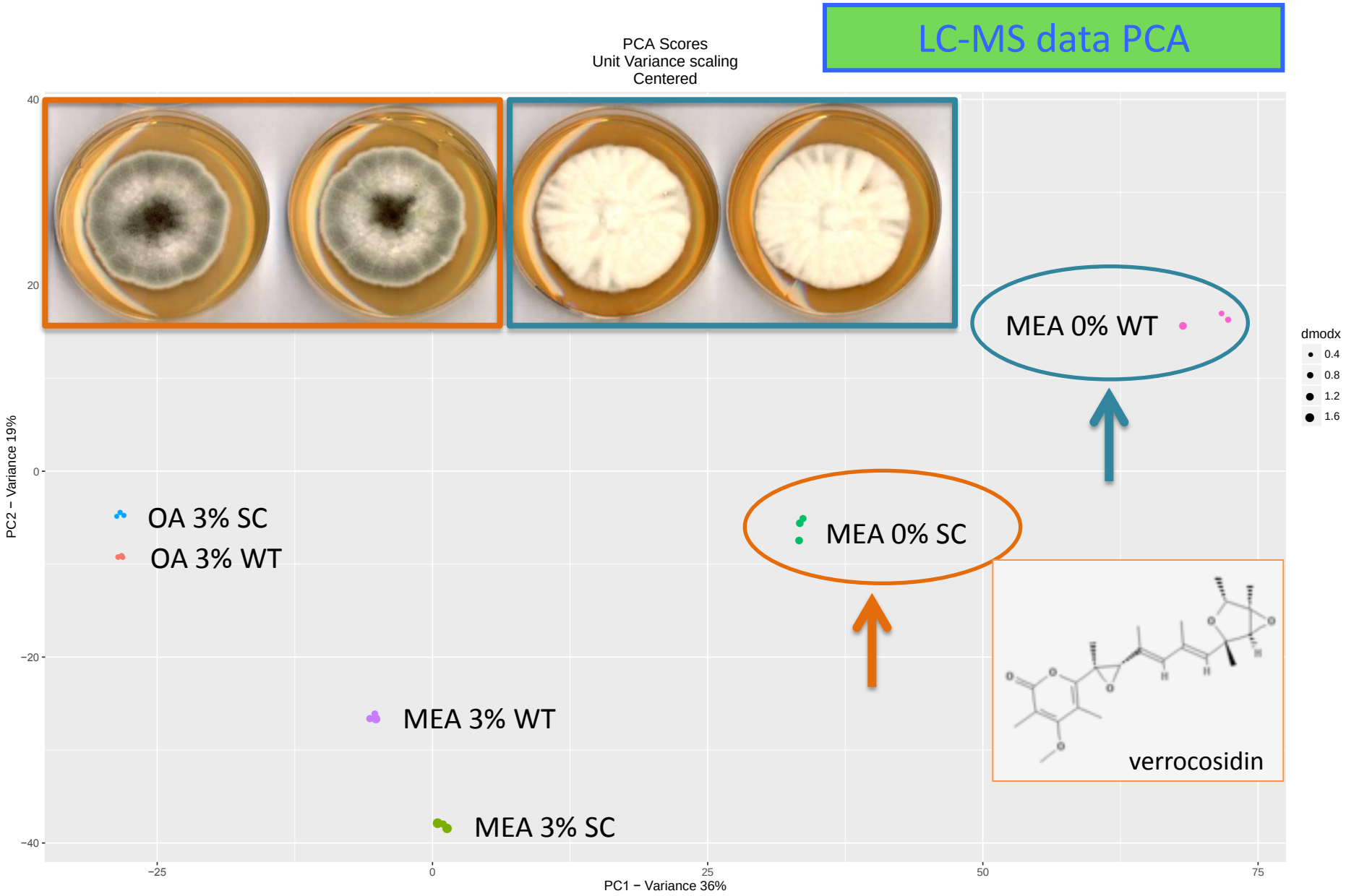



MEA 0% = Malt Extract Agar 0% NaCl
MEA 3% = Malt Extract Agar 3% NaCl

OA 0% = Oatmeal Agar 0% NaCl
OA 3% = Oatmeal Agar 3%

WT = wild type strain
SC = semi-cured strain

# LC-MS data PCA




MEA 0% = Malt Extract Agar 0% NaCl
MEA 3% = Malt Extract Agar 3% NaCl

OA 0% = Oatmeal Agar 0% NaCl
OA 3% = Oatmeal Agar 3%

WT = wild type strain
SC = semi-cured strain

# Pathogenicity test MUT4330



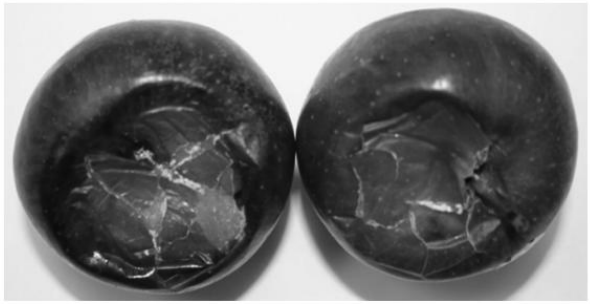


**FIG. 1.** Apple fruits with blue mold symptoms.

borate, 0.002 M ethylenediaminetetraacetic acid, pH 8) and visualized on a UV transilluminator after ethidium bromide staining. Gel images were acquired with a Gel Documentation System (Uvitec, Cambridge, United Kingdom).

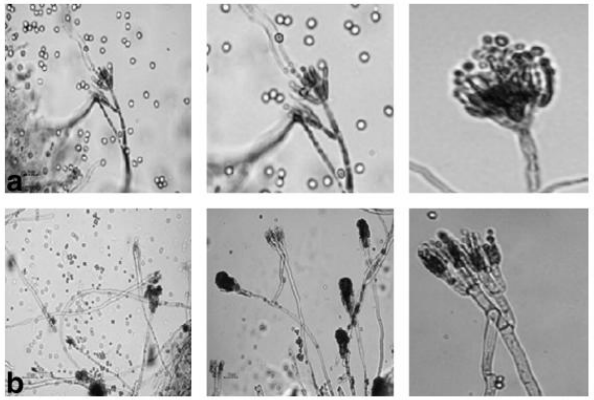
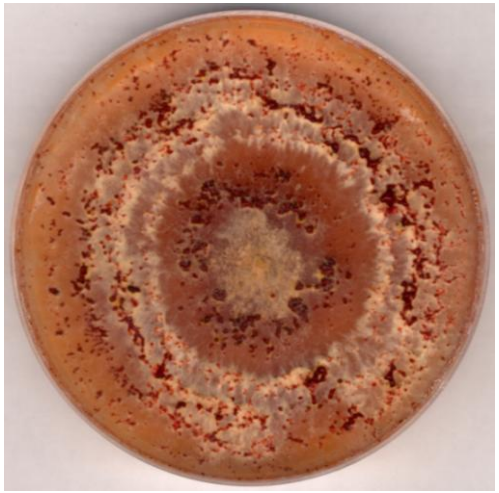


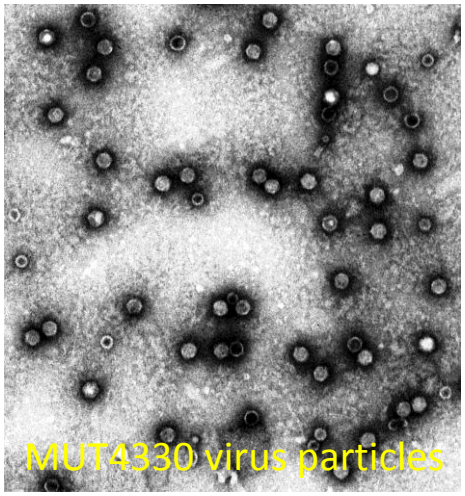

**FIG. 3.** Micromorphology of *P. griseofulvum* (**a**) and *P. aurantiogriseum* (**b**).

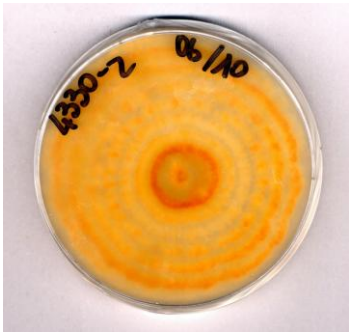
Nor WT nor the other virotypes showed patogenicity

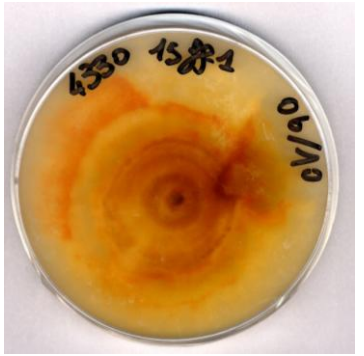
# Transmission to a model host

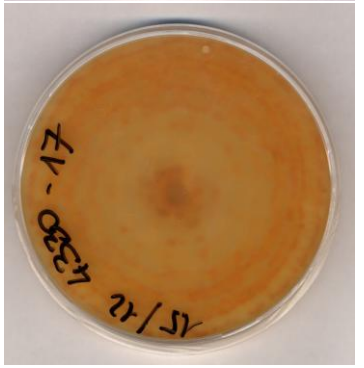
**Cryphonectria parasitica: a model host for virus-fungus interaction**



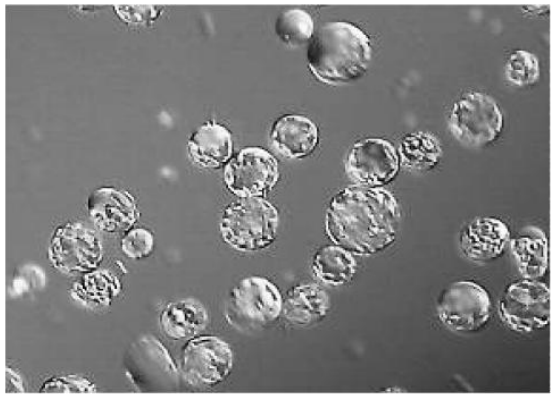


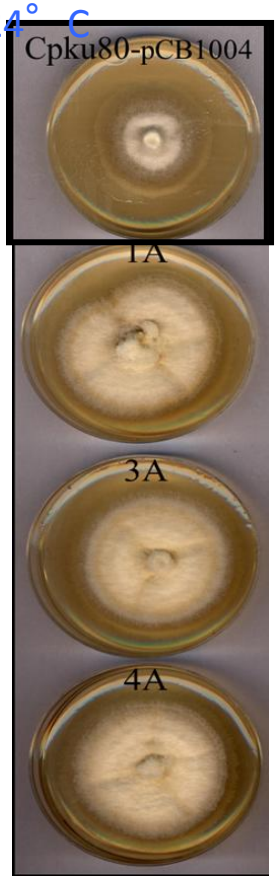

We got protoplasts from *C. parasitica* KU80 isolate



From 3 different transfection experiments we obtained 3 isolates stably infected by PaPLV1 (different titer)

YES - 3%NaCl – 24° C

# Transmission to a model host




4 virotypes (WT and 3 transfected isolated with different PaPLV1 titer were screened for growth experiments and pathogenicity test.

**Growth test**: **5 media** (PDA – PDYA – MEA, YES, YPY) at **3 salinities** (0 - 1.5 - 3% NaCl) and **3 temperatures** (10, 24, 30° C)

**The infected isolates grow more in present of salt.**

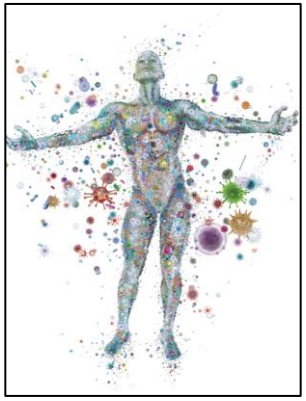
**The presence of virus did not interfere with *C. parasitica* virulence**

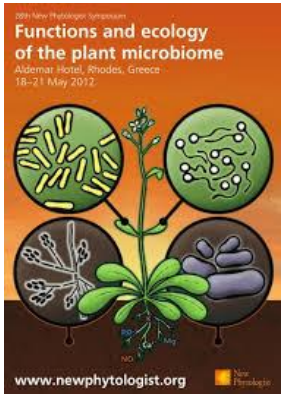

**RNAseq analyses showed showed that 2 isolates accumulates non-synonimous mutations suggesting adaptation to the new host**

*Nerva et al., 2017. Transmission of Penicillium aurantiogriseum partiti-like virus 1 to a new fungal host (Cryphonectria parasitica) confers higher resistance to salinity and reveals adaptive genomic changes. Environmental Microbiology, In press.*

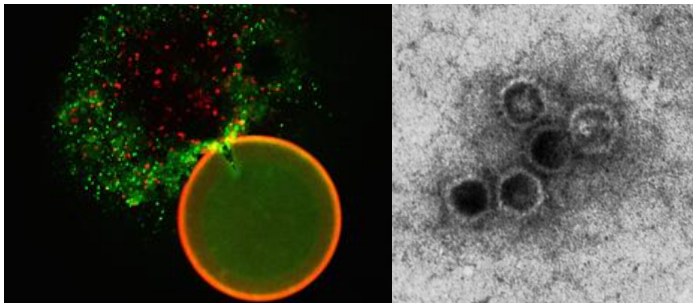
# Conclusions

1) Fungi are widely (15%) colonized by MVs,  but their associated virome is almost always unkown.






FUNGAL MICROBIOME



"Extended genome of fungi"

2) First record of MVs in marine fungi:  12 MVs belonging to different viral families of dsRNA, (+) ssRNA and (-) ssRNA lineages have been described.

3) MVs are able to impact the phenotype of fungi and can play important roles in adapting to extreme environments (i.e. saline tolerance).

**MVs presence should be investigated in fungi of possible biotechnological and/or ecological relevance.**
**Virome analisis as Quality Control pipeline for mBRC.**

4) For some of the marine fungi already studied we clearly showed MVs affect the morphology and the physiology of the host.


**Can some MVs inhibit the production of mycotoxins? Case study of *A. ochraceus* of marine origin.**

5) We showed the possibility of MVs cross-species transfection in *C. parasitica* to artificially extend MVs host range. In out case the virus transfection confers higher resistance to salinity.

**MVs transfections can became an important tool to change the phenotype of fungi and also of other organisms (i.e plants) bypassing natural barriers.**

**MVs are a driving force at evolutionary level.**

# Acknowledgements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