Endophytic microbial diversity in seagrass beds of *Thalassia testudinum* and *Syringodium filiforme* from Cabo Rojo, Lajas and Vieques, Puerto Rico

Sandra L. Maldonado Ramírez
Rafael Montalvo Rodríguez
Department of Biology
University of Puerto Rico- Mayagüez

Microbial endophytes

- Invade plant tissues causing asymptomatic infections.
  - endophytic fungi in terrestrial plants contribute to the growth and protection of the host plant.

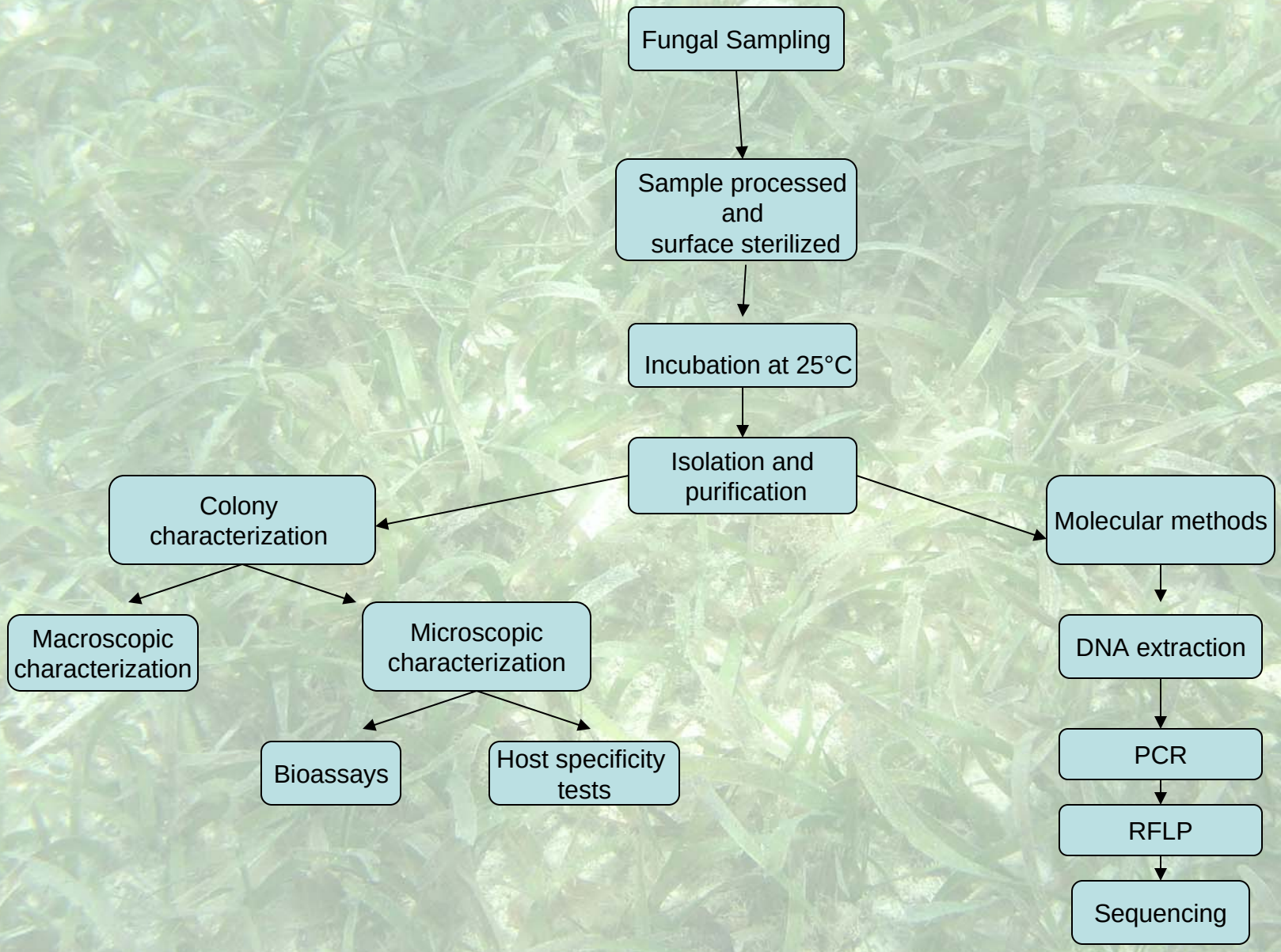
T. testudinum and *S. filiforme*

- Angiosperms abundant from the north of the Gulf of Mexico to the north coast of South America.
  - contribute to sustain phytoplankton, epiphytic flora, invertebrates, and algae.
  - helps to stabilize the substrates where they grow.
  - provide an essential habitat for species of economic importance and in danger of extinction.

Research Objectives

- Determine endophytic microbial diversity associated to well establish sea grass beds of *T. testudinum* and *S. filiforme* from Las Salinas, Buyé, La Parguera and Vieques island by culturing techniques and molecular characterization.
  - **S**ea **G**rass **E**ndophytic **C**ulture **C**ollection (SGECC).
    - Data on SGECC will be available on line.
- Determine temporal and spatial distribution, host specificity and seasonality of the endophytic flora.
- Bioprospecting extracellular secondary metabolites produced by the endophytic flora recovered.

Processing of foliar samples for fungi



Surface sterilization

Sodium hypochlorite (0.50%)

1 min

Distilled water

1 min

Distilled water

Blot dry

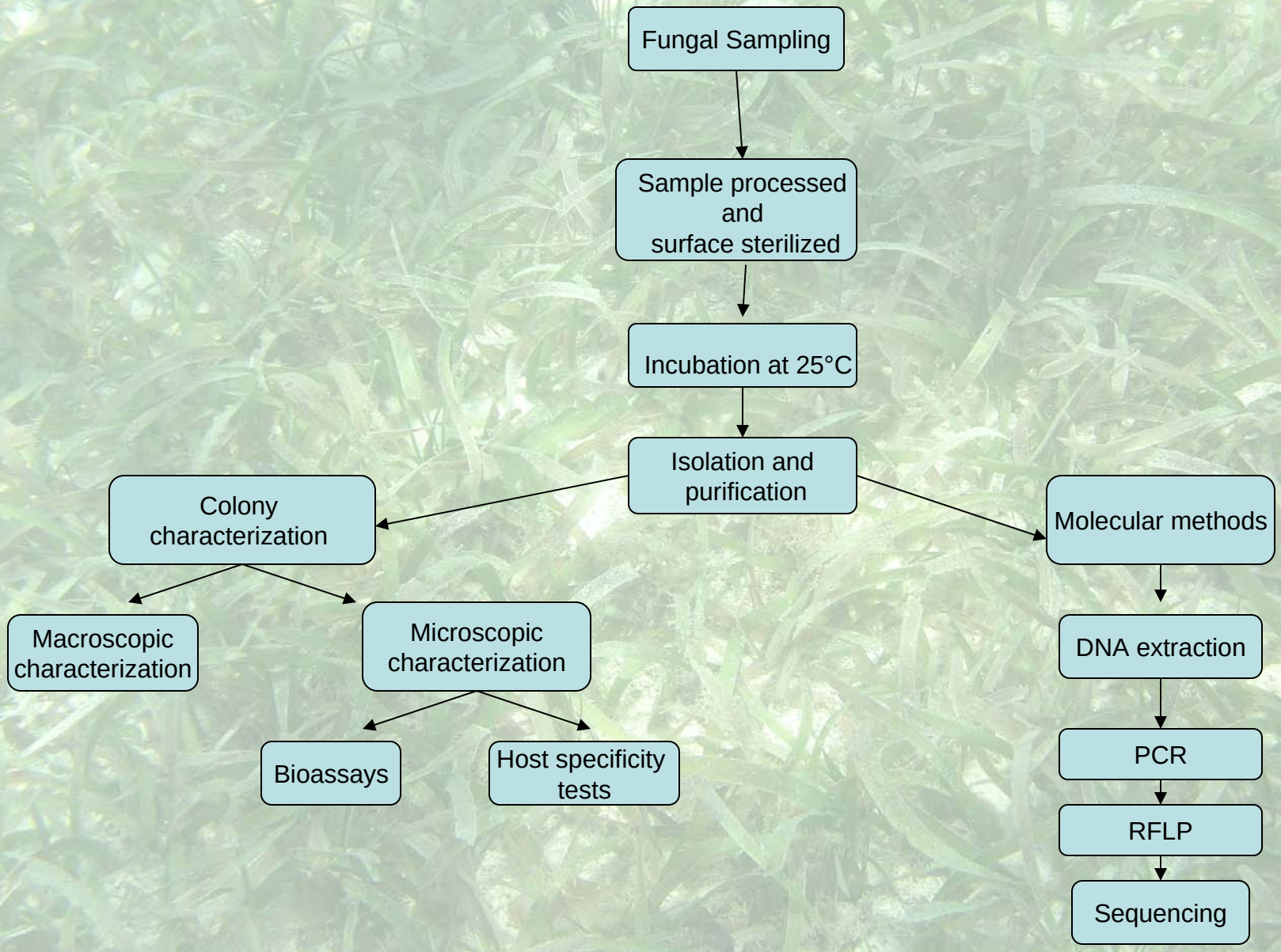
Cut in sections

9 fragments per section

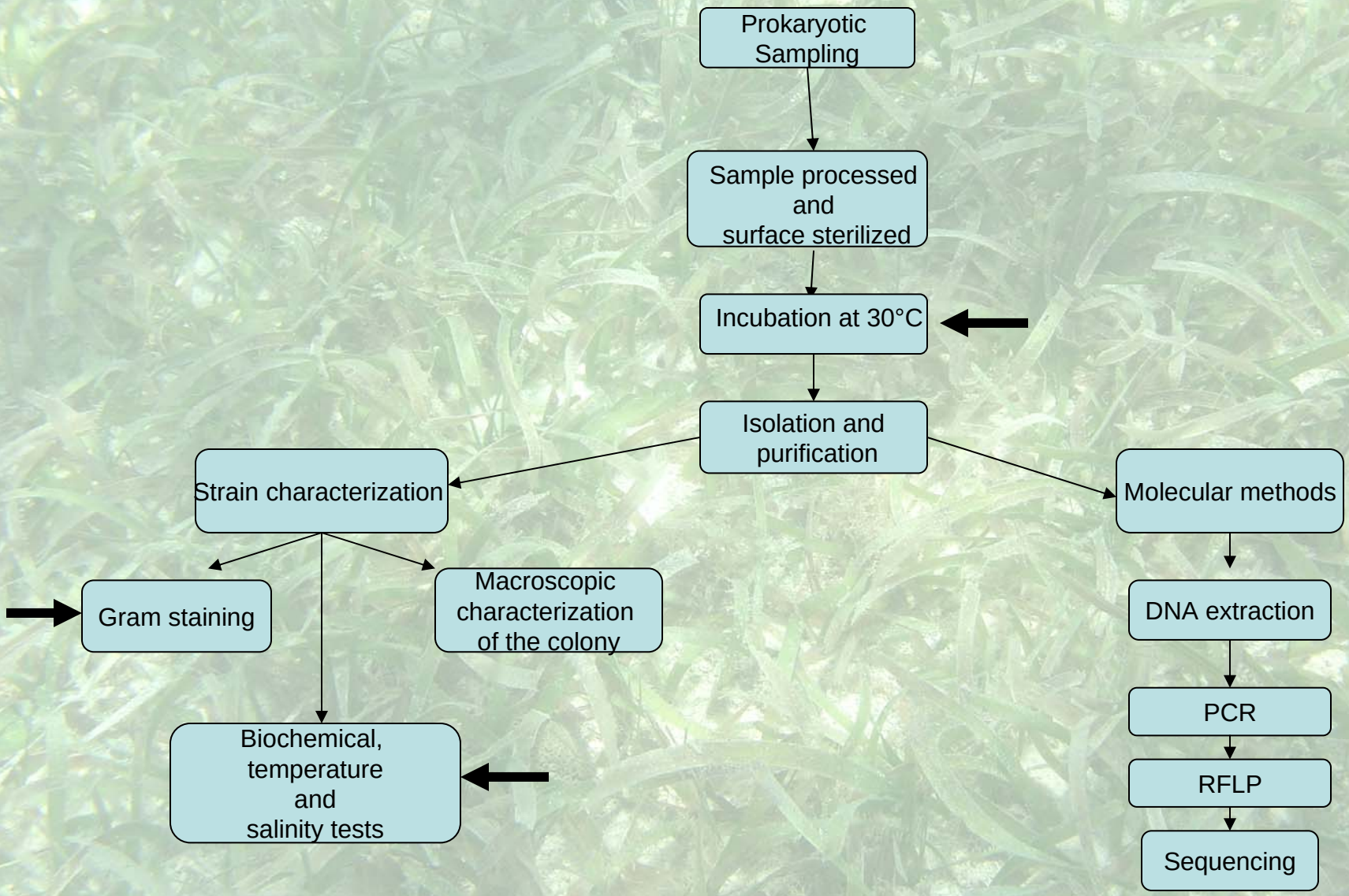
Fragment inoculation in MA

Incubate at 25° C for 7 days

Processing of foliar samples for fungi



Processing of foliar tissue for prokaryotes



First sampling at Buyé Beach, Cabo Rojo Fungi

- 270 plates were incubated.
- We have 35 different morphotypes purified.
  - Sub-cultivated on Marine Agar, Oatmeal Agar, Potato Dextrose Agar y Malt Extract Agar to stimulate sporulation.
- Interestingly, thirteen morphotypes (37%) are exudate- producing fungi.

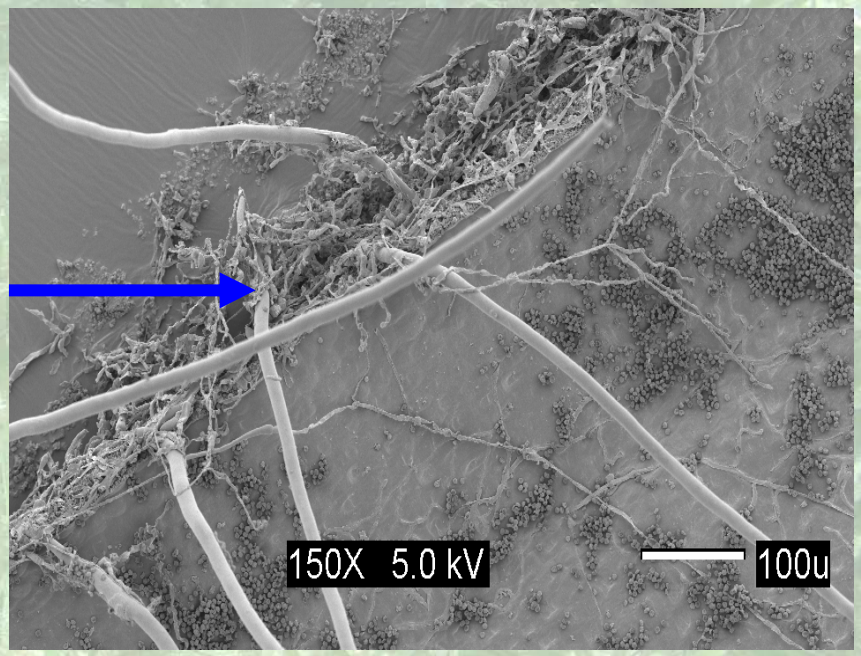


Figure 1. Mycelium emerging through surface sterilized tissue.

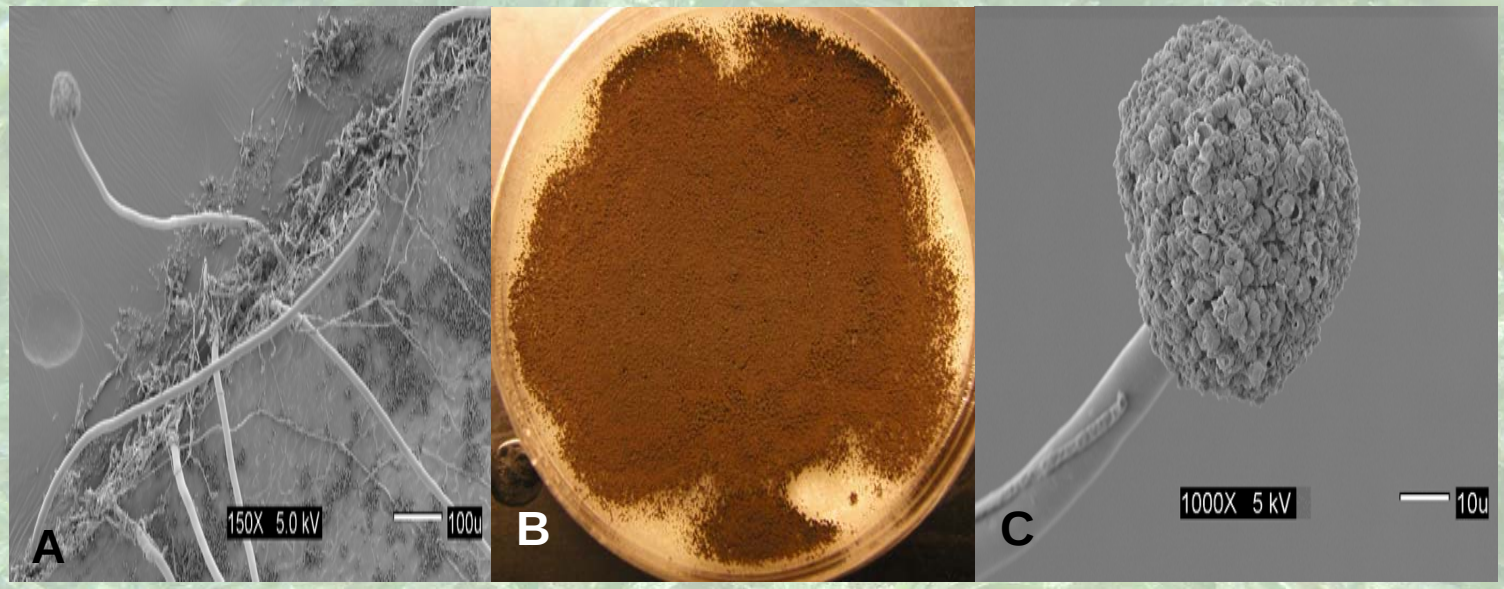


Figure 2. *Aspergillus niger* emerging from surface sterilized tissue (A), colony growing on MA (B), and details of microscopic head on SEM (C).

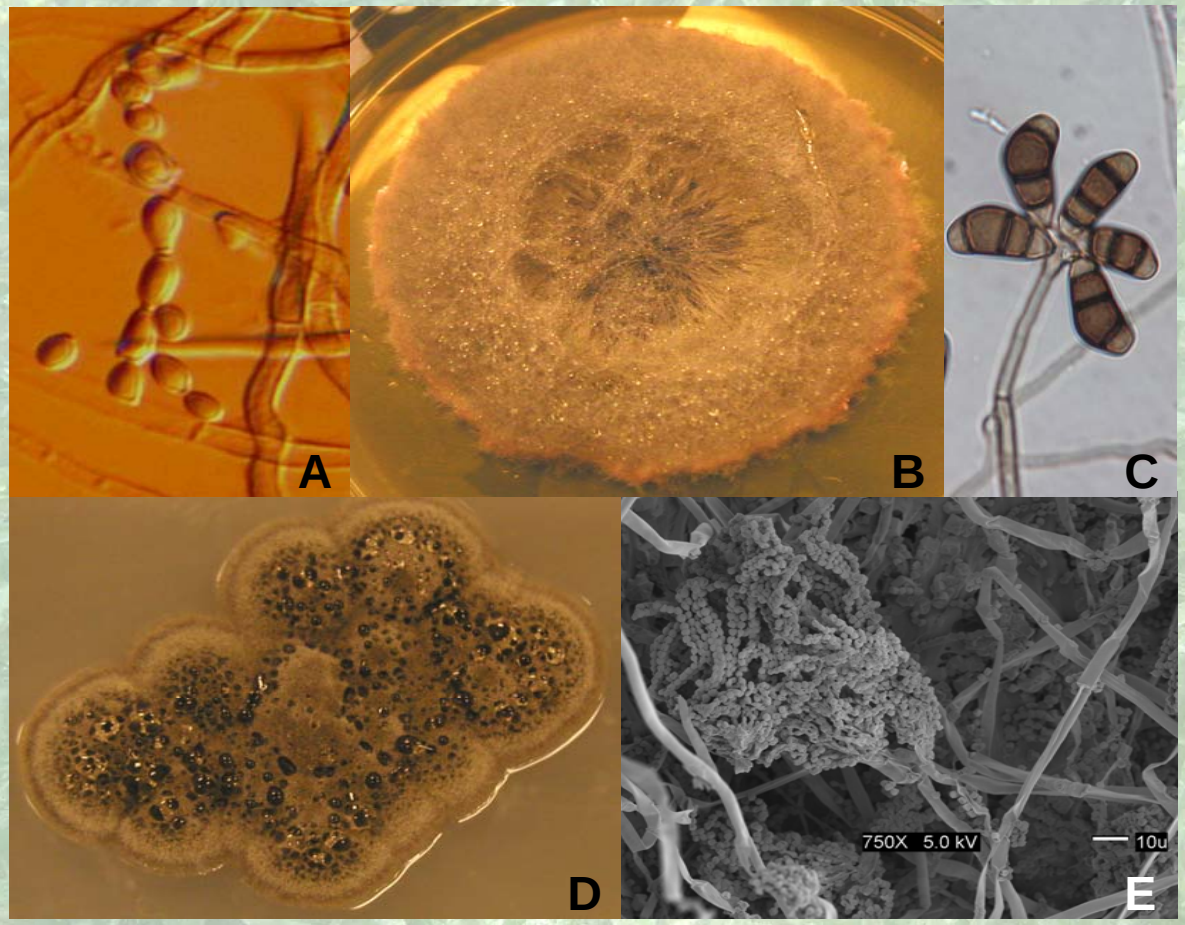


Figure 3. Exudate-producing fungi: *Cladosporium* (A), *Curvularia lunata* (B-C), and *Penicillium janthinellum* (D-E).

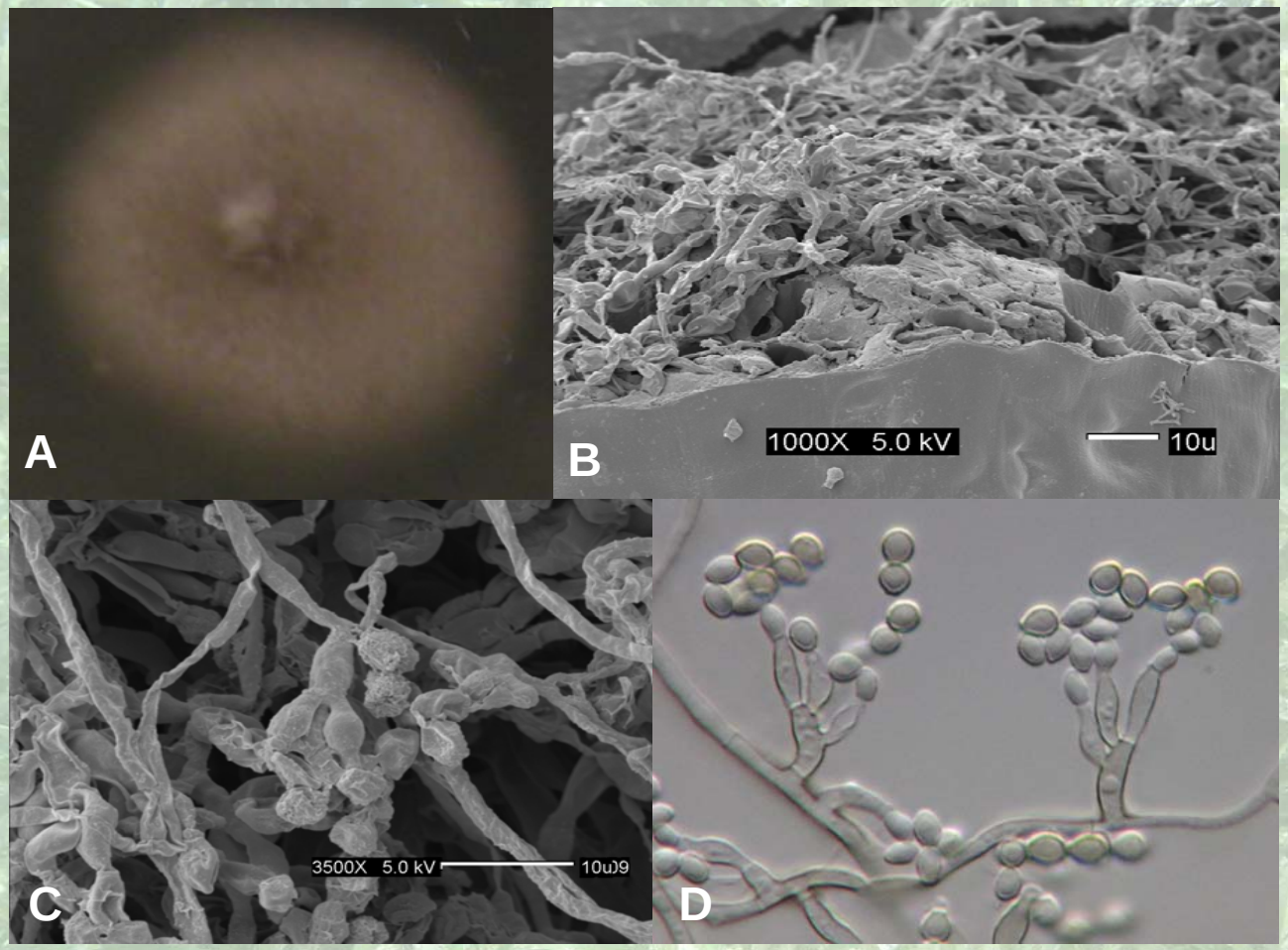
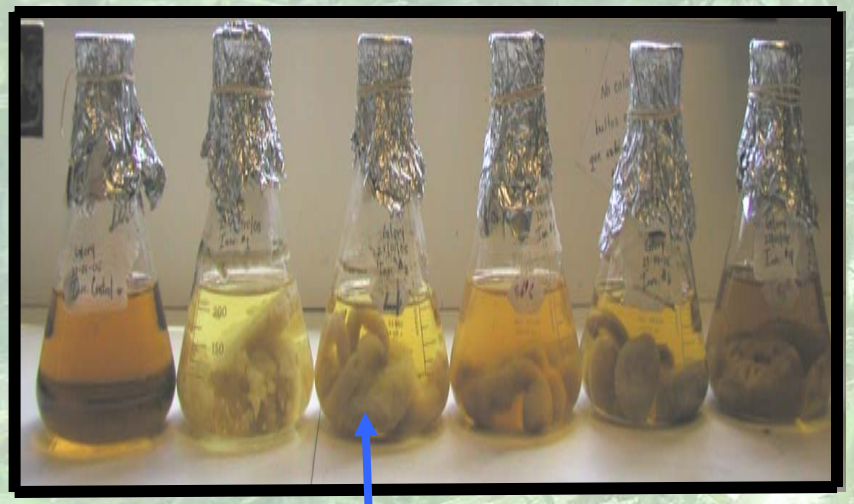


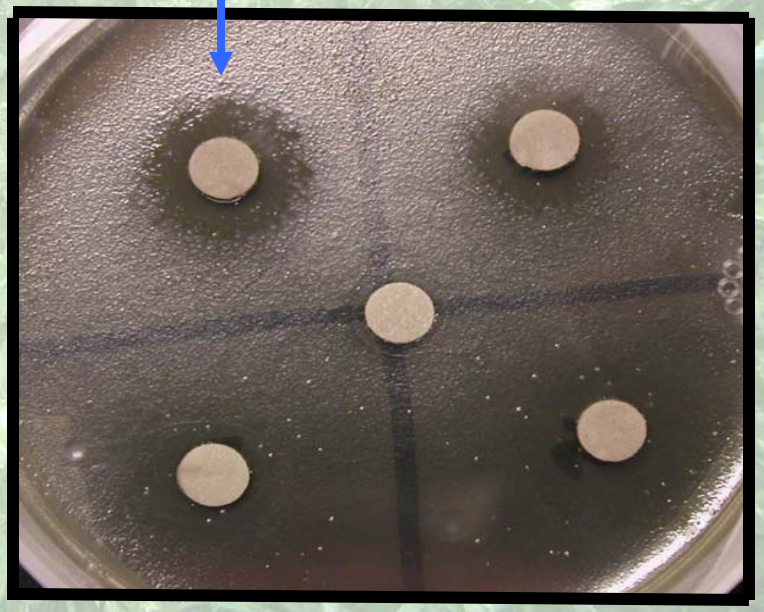
Figure 4. Exudate-producing fungi: *Scopolariopsis sp* on MA (A), mycelia emerging from surface sterilized tissue (B), microscopic details under SEM (C), and morpholology under light microscopy (D).

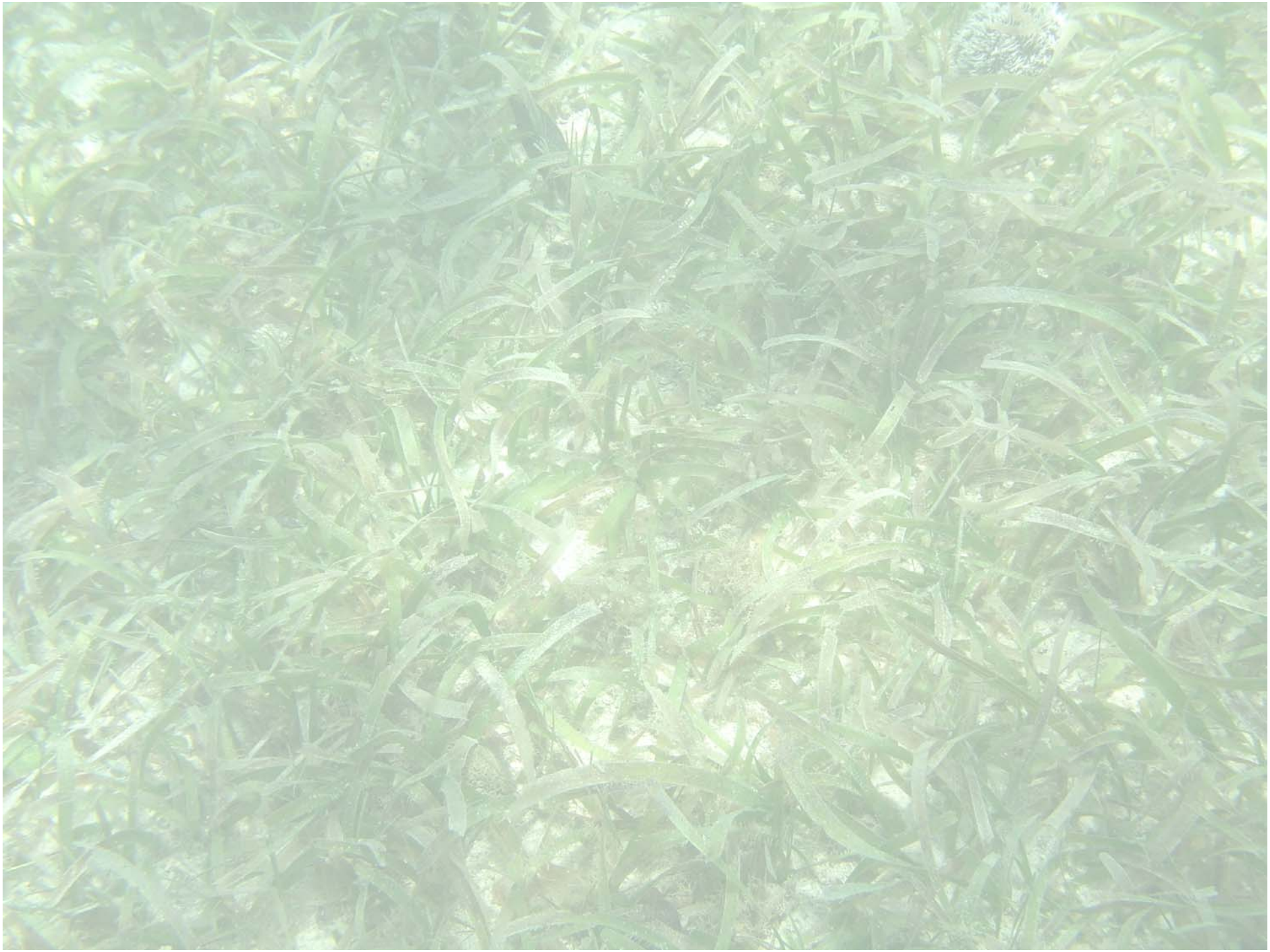
Bioassays



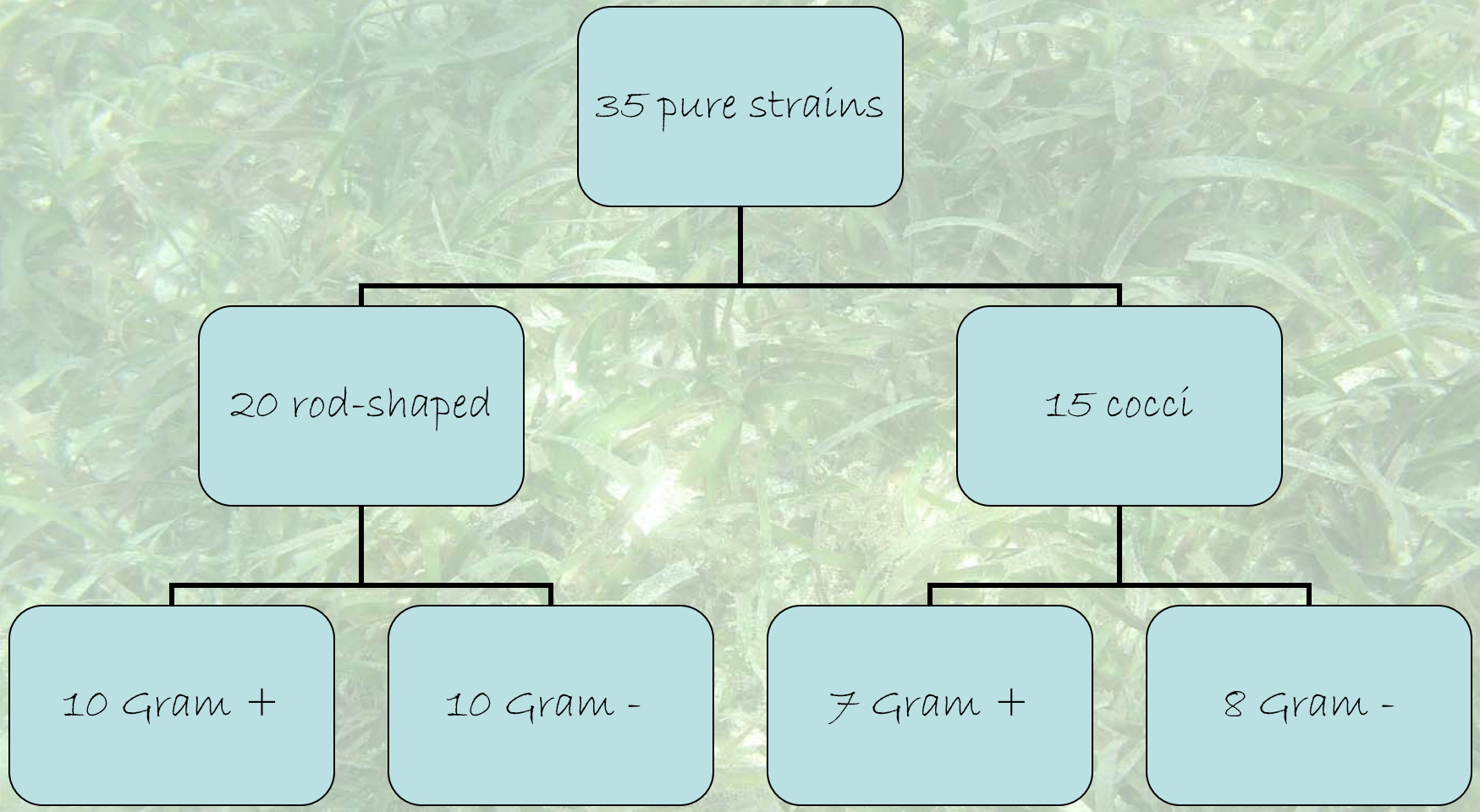
Growth of the endophytic fungi
on liquid malt extract

Inhibition zone around
Pseudomonas aeruginosa

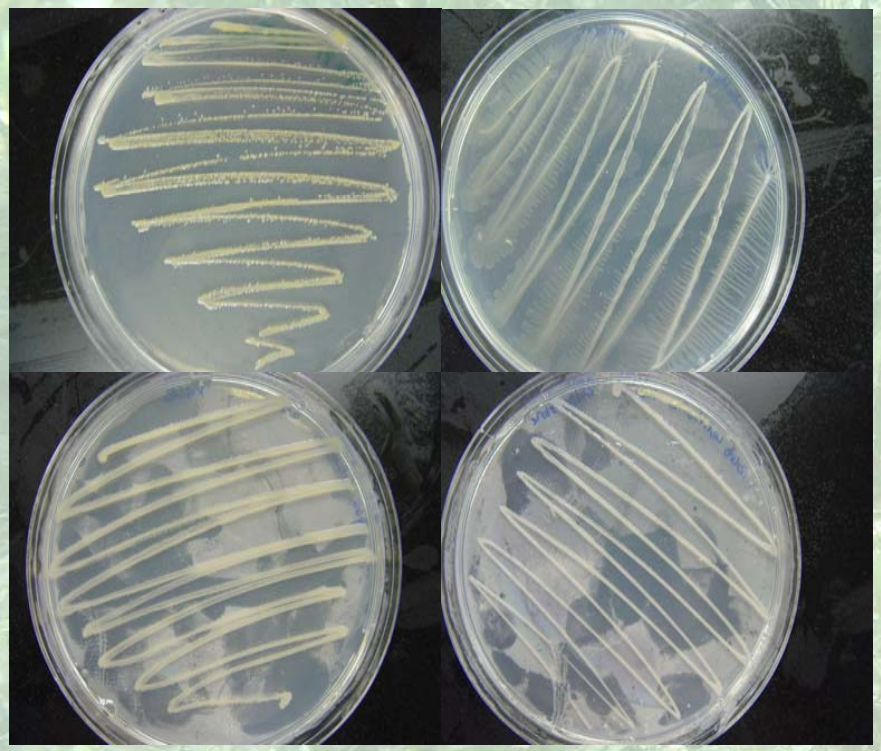


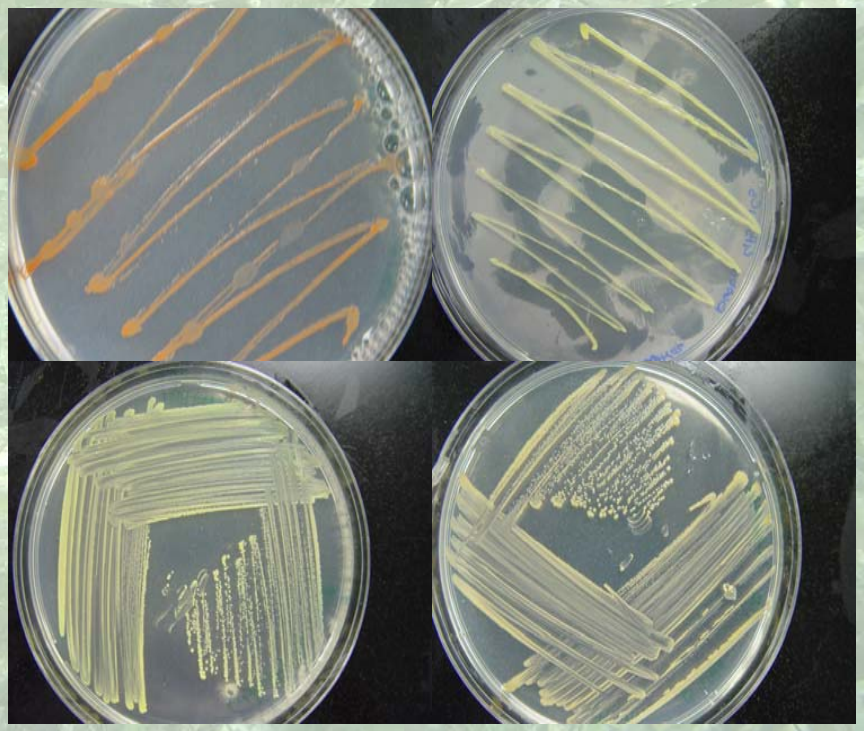


Prokaryotic diversity from preliminary sampling



Purified strains



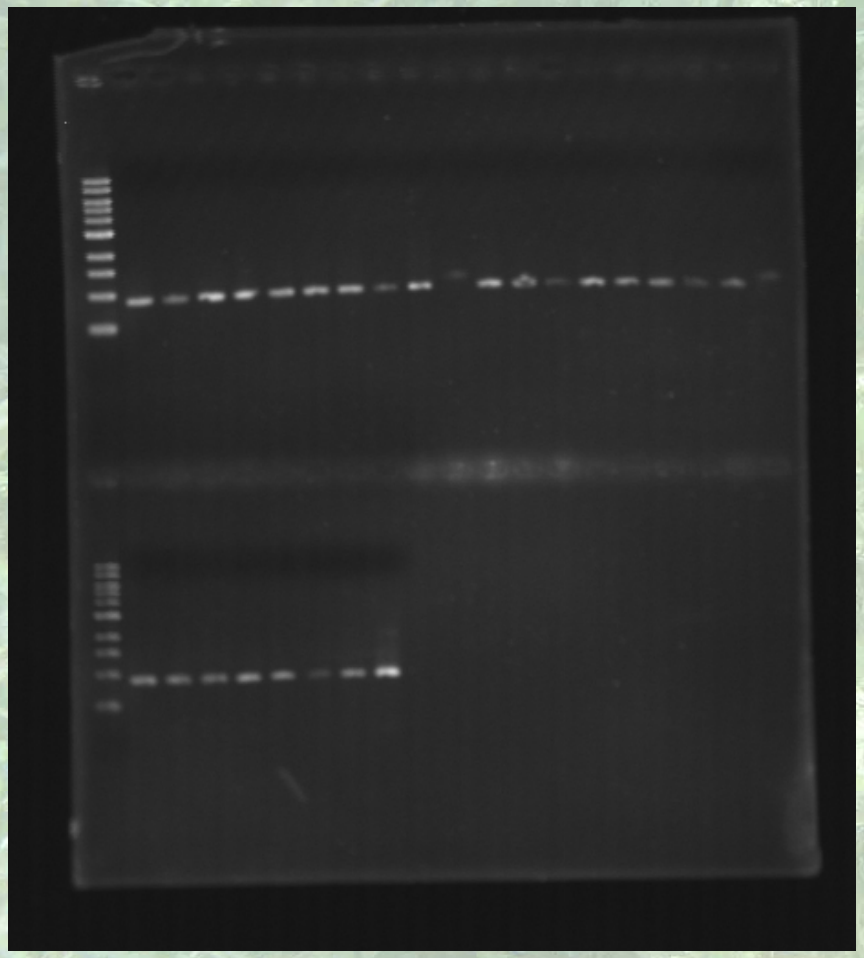


Prokaryotic diversity from preliminary sampling

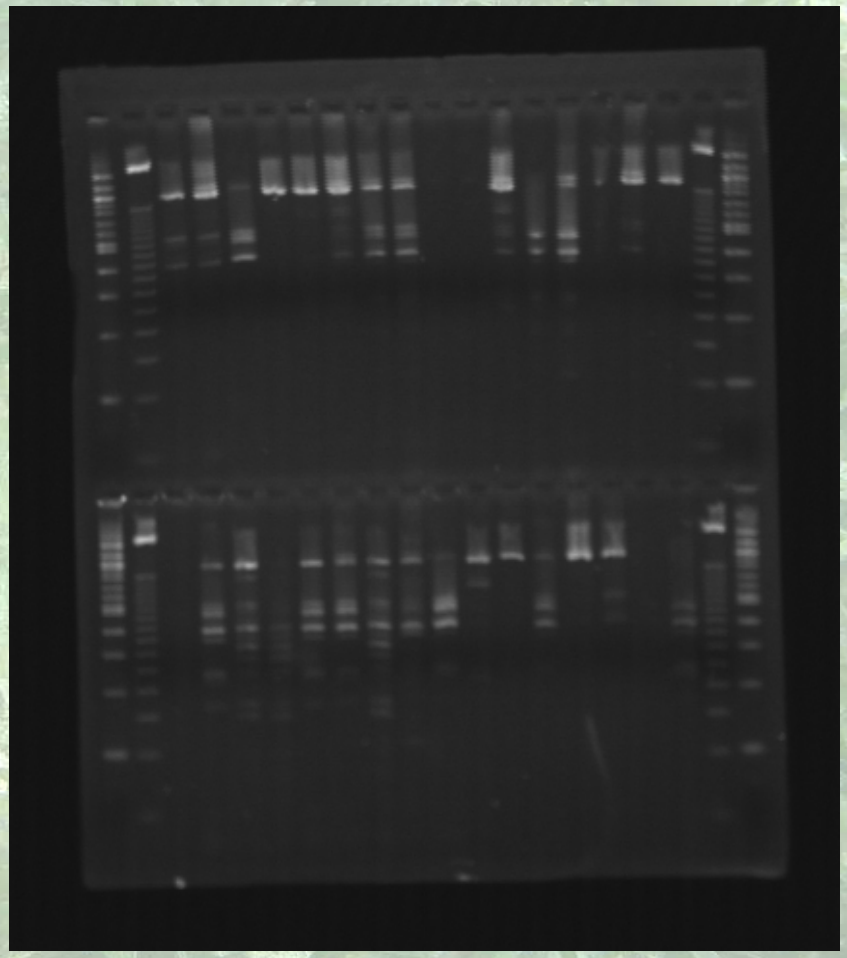
- DNA extraction and PCR from all 35 pure strains.
- RFLP reactions were performed.
  - 8 different patterns in 16S rRNA gene.
- Sequencing of these 16S rRNA genes will be performed to know the identity of these 8 different microorganisms.

PCR and RFLP: gel electrophoresis

PCR



RFLP



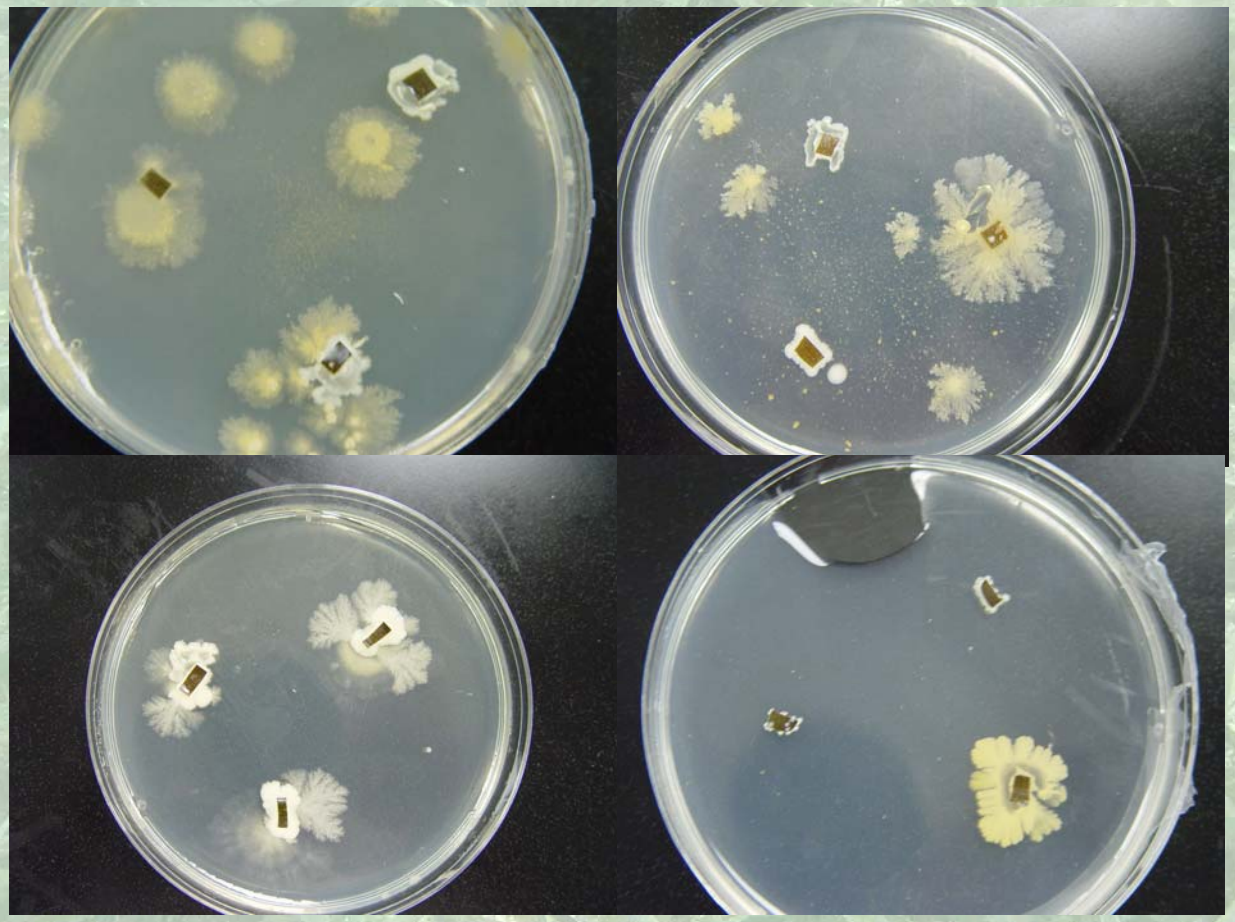
First sampling at Buyé Beach, Cabo Rojo

- 3 different quadrants varying in depth; 2 healthy leaves were taken from 5 random plants of each quadrant.
- 270 plates were incubated for isolating endophytic prokaryotic strains.
- Until now we have 450 strains purified.
- There are predominantly 15 phenotypes of strains:

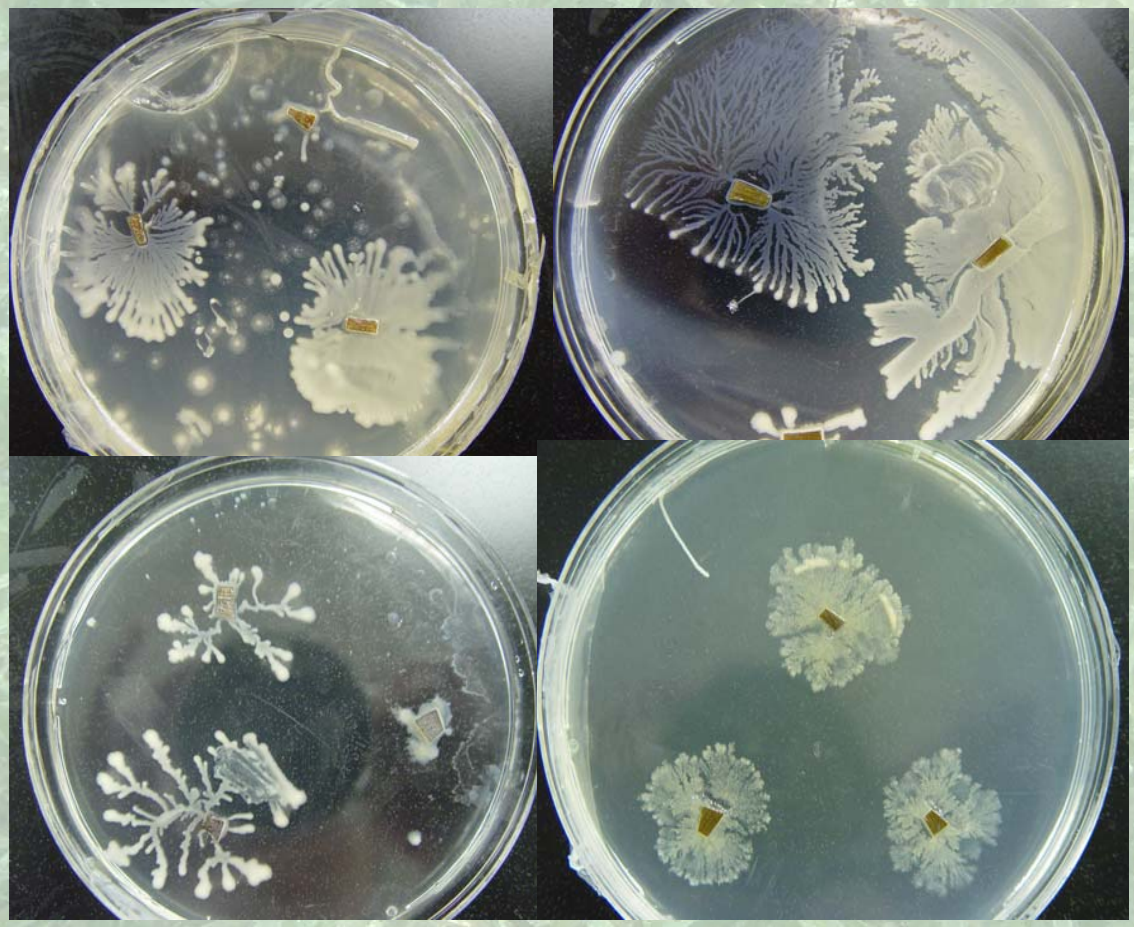
rhizoid white colonies	orange colonies
yellow colonies	bright pink
transparent white colonies	cream rhizoid colonies
dark cream colonies	others.

- Interestingly, different colony phenotypes are predominant depending on the depth from which each plant was isolated.

Endophytes isolated



Endophytes isolated



Acknowledgements

- Sea Grant at UPR-M for the financial support.
- Magaly Zapata, microbiology technician for providing the bacterial cultures used for the bioassays.
- Carolyn Rivera from the Laboratory of Tropical Mycology.
- For their help during the samplings:
  - Hernán Torres -Agnes Martínez
  - Carlos Virella -Tavania Espinosa
  - Angel A. Cruz - Miguel Ortíz
  - Roberto Román
- Gualberto Rosado and José Almódovar for their collaboration with the photographic material.
- Gloriner Morell for providing the photographic material on the presentation.

Questions?