

**MELISSA**

**TN 5**

TN5 Detailed experimental plan

General comment : standardization of the following methods applicable for the 4 compartments is required : centrifugation, sterilization processes (outputs), inoculation processes (inputs), storage, ... .

WP6100 : Thermophilic Clostridia :

Pure cultures of Clostridium thermocellum and C. thermosaccharolyticum.

- a. Definition of synthetic media : range of substrates.
- b. Choice of appropriate strains from various culture collections and batch cultures : control for axeny.
- c. Definition of a chemical formula with emphasis of N, C and possibly S.
- d. Definition of physiological characteristics : log phase, growth rate, methods of conservation, conditions of sporulation, autolysis, temperature range : growth, temperature range : survival, definition of practical diagnostic tests.
- e. Substrate metabolization : yields, detection of major endproducts, substrate conversion versus biomass.
- f. Processing of supernatant fluid : 1) identification of methods : filtration, sterilization ; 2) choice of diagnostic assays : H<sub>2</sub>S, fatty acids, ethanol, NH<sub>4</sub><sup>-</sup> and assays before and after sterilization (at least in a few key experiments) ; 3) identification of methods to introduce supernatant (peristaltic pumping).

WP6200 : Rhodobacter capsulata and Rhodospirillum rubrum

- a. Definition of synthetic media for photo-organotrophic growth and photo-autotrophic growth. The organotrophic growth is foreseen to convert fatty acids, etc... into biomass, the photo-autotrophic growth is purposed to recycle gases (H<sub>2</sub>, ...) evolved during the growth of Clostridia.
- b. Choice of appropriate strains from culture collections, batch cultures and axeny control.

- c. Definition of a chemical formula in both conditions with emphasis on carbon and nitrogen. Specific attention has to be given to production of  $\text{NH}_4^+$ .
- d. Physiological characteristics : lag phase, growth rate, methods of conservation, range of carbon sources, stability of photo-autotrophic growth, temperature range : survival, definition of practical diagnostic tests.
- e. Substrate metabolization : low chain fatty acids, ethanol, aminoacids : yields, detection of major endproducts, substrate conversion versus biomass in both conditions : photoheterotrophic and photo-autotrophic.
- f. Production of  $\text{NH}_4^+$  during growth on amino acids.
- g. Processing of supernatant : centrifugation, filtration, sterilization, assays before and after sterilization.

6.300 : Nitrobacteria

- a. Definition of synthetic media and choice of appropriate strains from culture collections.
- b. Growth of Nitrosomonas sp. at expenses of  $\text{NH}_4^+$ ,  $\text{CO}_2$ ,  $\text{O}_2$  : assay of nitrite. Control of axeny.
- c. Growth of Nitrobacter sp. at expenses of  $\text{NO}_2^-$ ,  $\text{CO}_2$  and  $\text{O}_2$  : assay of  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{CO}_2$  and  $\text{O}_2$ . Control of axeny.
- d. Definition of chemical brutto formula and of stoichiometric equations.
- e. Cocultures of Nitrosomonas and Nitrobacter at expenses of  $\text{NH}_4^+$  : assays of  $\text{NH}_4^+$ ,  $\text{CO}_2$ ,  $\text{O}_2$  and  $\text{NO}_3^-$ .
- f. Physiological characteristics : log phase, growth rate, conservation and storage of strains, diagnostic tests, control of axeny.
- g. Processing of supernatant : centrifugation, filtration, sterilization, assays ...

WP7100 : Growth of Clostridia at expenses of a waste source : rat faeces

- a. Production of faeces from an animal husbandry : sterilization by autoclaving.

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- b. Growth of Clostridia (C. thermocellum, C. thermosaccharolyticum, possibly C. thermohydrosulfuricum) on faeces and cellobiose in synthetic media. In some experiments, faeces have to be added as sole nitrogen and sulphur source. Various ratios of faeces/cellulose would be assayed.
  - c. Axeny controls.
  - d. Assay of residual products : fatty acids, alcohols,  $\text{NH}_4^+$ .
  - e. Correlation with WP6100 with emphasis on growth rate, substrate conversion, lag phase, etc.
  - f. Processing of supernatant fluid : 1) identification of methods : decantation, filtration, sterilization ; 2) choice of diagnostic assays :  $\text{H}_2\text{S}$ , fatty acids, ethanol,  $\text{NH}_4^-$  and assays before and after sterilization (at least in a few key experiments).

WP7200 : Pure cultures of Rhodospirillum rubrum and Rhodobacter capsulata on supernatant fluids coming from WP6100

- a. Supernatant as described in WP6100 (NB : not 7100) - \$f\$ : choice of a dilution process (facultative).
- b. Batch cultures : definition of physiological characteristics of R. rubrum and Rh. capsulata.
- c. Cocultures : definition of physiological characteristics, use of selective plates to count both coculture partners.
- d. Assay of  $\text{NH}_4^+$  : the careful assay nitrogen balance is essential at this stage : biomass nitrogen versus free inorganic nitrogen.
- e. Correlation with 6.200.

WP7300 : Pure cultures of nitrifying bacteria on supernatant fluids coming from WP6200

- a. Batch cocultures of Nitrosomonas sp. and Nitrobacter with supernatant fluids.
- b. Correlation with 6.300.

WP7400 : Pure cultures of Spirulina on supernatant fluids coming from WP6.300

- a. Batch culture with supernatant fluid from coculture nitrosomas/nitrobacter.
- b. Comparison with results of WP3000.

This experiment is one of the most critical experiments of the project.

WP8100 : Spirulina toxicity tests

These tests have to be performed out with supernatants from 7200 and 7300. These supernatants have to be sterilized and should come from cultures at their final phases with extensive substrate metabolization and, if possible, with very low residual concentrations of substrates : this can be achieved in test cultures grown on limiting amounts of substrate.

WP8200 : Sulphur cycle

- a. Theoretical considerations

Working hypothesis

- H<sub>2</sub>S production in compartment 1 : Clostridium thermohydrosulfuricum.
- H<sub>2</sub>S recycling in phototrophic conditions : Thiocapsa roseopersicina.
- Sulphur recycling in aerobic conditions : choice of a Thiobacillus (various strains possible).
- The final choice will depend on the tolerance to H<sub>2</sub>S in the various compartments.

- b. Choice of a Thiocapsa roseopersicina strain :

The chosen strain will be originated from a waste processing system (thus not from a marine environment). The strain has probably to be reisolated and reassessed with a specific attention for taxonomy and axeny.

c. Batch culture in synthetic media.

d. Physiological characteristics : growth rate, stability, storage ...

e. Choice of a Thiobacillus strain.

WP8300 : Selection of a proteolytic thermophilic bacterium.

Definition of a selection strategy from the DRANCO plant managed by RUG.  
(dry anaerobic composting system).