Effects of carbon to nitrogen ratios on amounts and composition of *Bacillus subtilis* biofilms – Work in progress

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Introduction

In natural environments, bacteria can be found as multicellular communities exhibiting a high degree of structure, denominated biofilms. Biofilms are composed of microbial cells, often of multiple species, embedded within a matrix of extracellular polymeric substances (EPS). The exact composition, physical and chemical properties, and amounts of these components varies depending on their growth conditions. However, it remains unclear how nutrient availability drives the allocation into cell growth and/or EPS production, especially under conditions found in soils**. Here we aimed to evaluate the effect of various C/N ratios on *Bacillus subtilis* biofilm growth (spatial expansion and structure) and their EPS composition.** Hypothesis: increasing EPS production with a nutrient imbalance in C/N ratios, especially high C availability compared to N limitations.

Methods

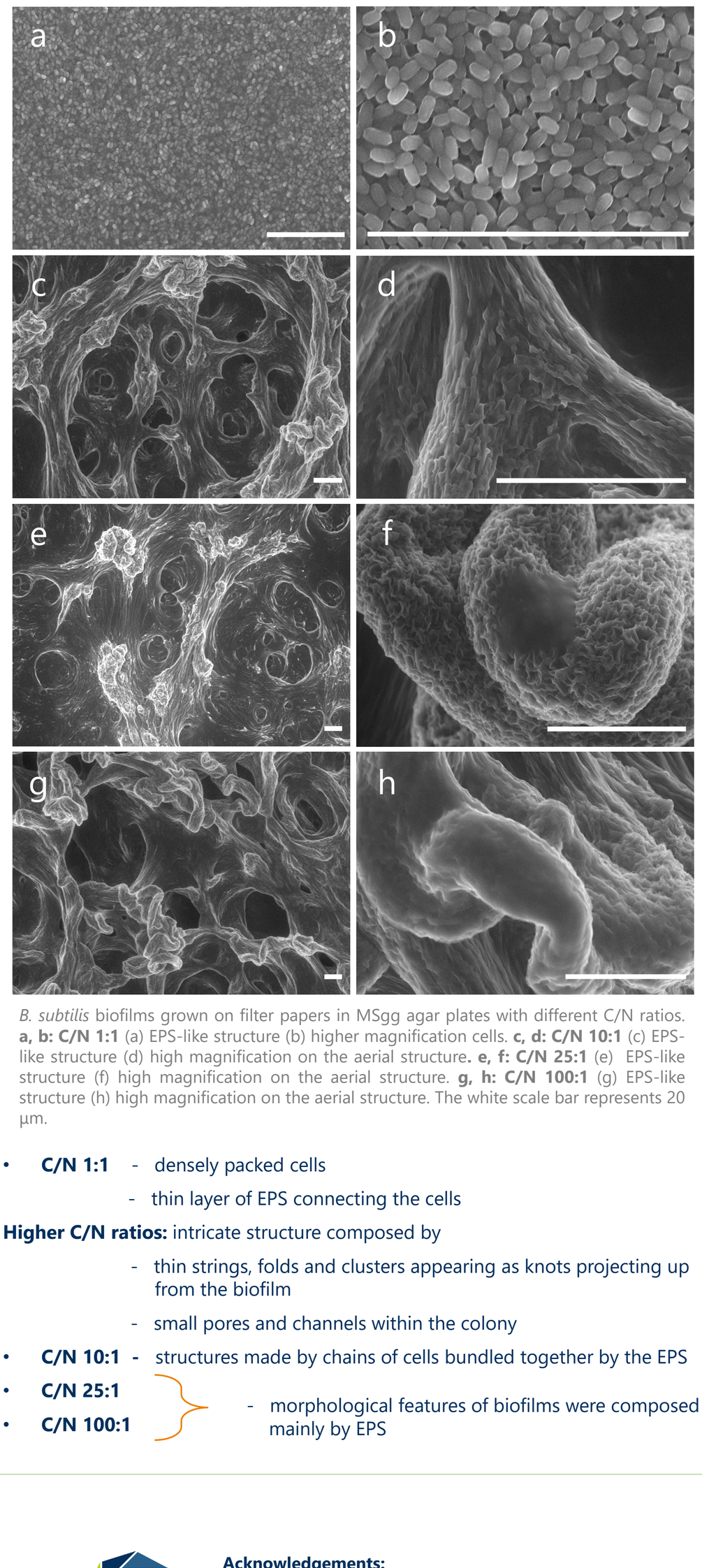
Biofilms of *B. subtilis* NCIB 3610 were grown on filter papers on MSgg agar plates with C/N ratios of 1:1, 10:1, 25:1 and 100:1

1) Radial expansion of the biofilm: measured via macroscopic imaging
2) EPS extraction to quantify:
   - biomass and EPS weight
   - proteins (Frolund et al., 1995) and polysaccharides (Dubois et. al. 1956)

Work in progress

1) Biofilm structures of hydrated biofilm samples: scanning electron microscopy (SEM) within the environmental mode (ESEM) at approximately 97% humidity
2) Fixed, dehydrated and embedded samples: used to evaluate the biofilm height and internal structure with SEM in high vacuum mode

(3) ESEM



B. subtilis biofilms grown on filter papers in MSgg agar plates with different C/N ratios. **a, b: C/N 1:1** (a) EPS-like structure (b) higher magnification cells. **c, d: C/N 10:1** (c) EPS-like structure (d) high magnification on the aerial structure**. e, f: C/N 25:1** (e) EPS-like structure (f) high magnification on the aerial structure. **g, h: C/N 100:1** (g) EPS-like structure (h) high magnification on the aerial structure. The white scale bar represents 20 µm.

- **C/N 1:1** - densely packed cells
  - thin layer of EPS connecting the cells

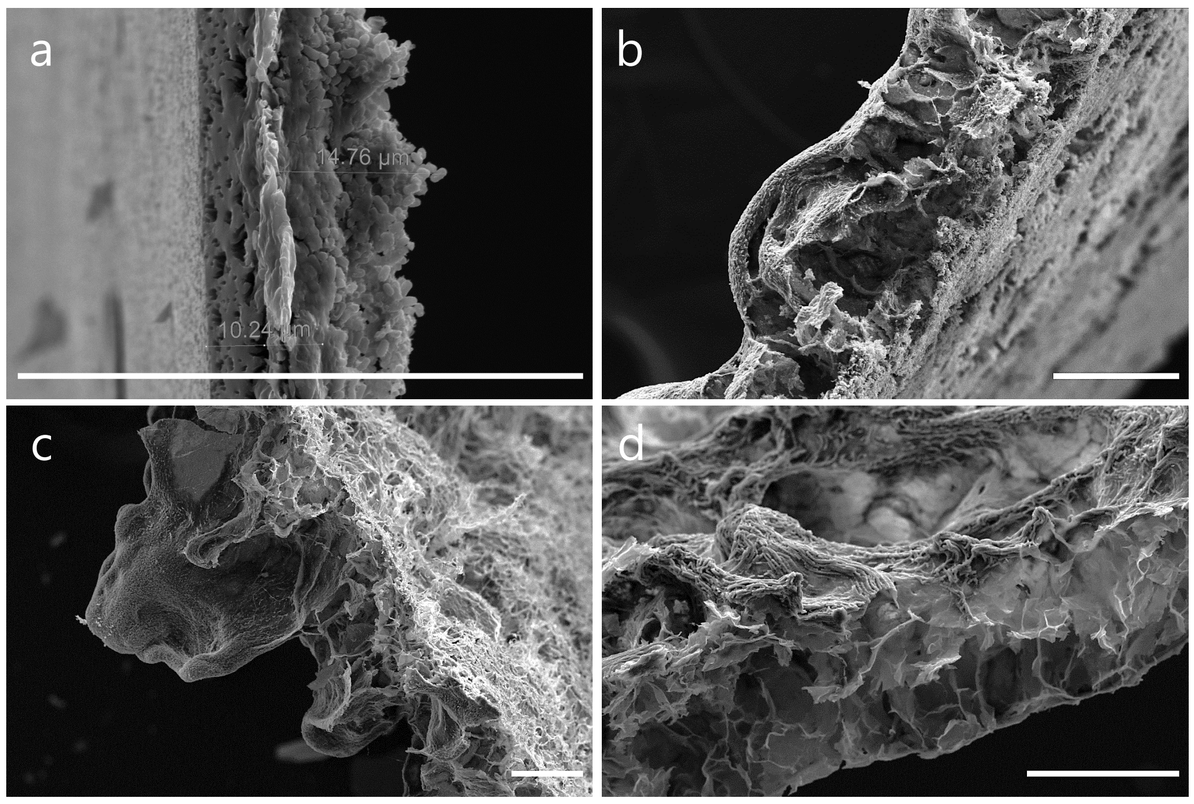
Higher C/N ratios: intricate structure composed by

- thin strings, folds and clusters appearing as knots projecting up from the biofilm
- small pores and channels within the colony

- **C/N 10:1** - structures made by chains of cells bundled together by the EPS
- **C/N 25:1**
- **C/N 100:1**

  - morphological features of biofilms were composed mainly by EPS

(4) SEM



Cross-sectional area of B. subtilis biofilms grown on membranes in MSgg agar plates with different C/N ratios: (a) 1:1 (b) 10:1 (c) 25:1 and (d) 100:1. The scale bar represents 50µm

- **C/N 1:1** - densely packed cell and a thin layer of EPS connecting the cells
- **C/N 10:1** - higher amount of EPS forming a complex internal structure
- **C/N 25:1** - high presence of cells as part of these structures
- **C/N 100:1** - visible cells mainly on the biofilm surface
  - mainly EPS in the internal structural features
- average biofilm height*:

C/N 1:1	C/N 10:1	C/N 25:1	C/N 100:1
10.3 µm	61.0 µm	57.3 µm – 123.8 µm	65.8 µm

* Preliminary results

Conclusions

- first results indicate: large impact of C/N ratio on the biofilm development and structure, with different allocations into microbial cells and EPS
- results obtained until now indicate that higher C/N ratios induce a higher EPS production, probably because energy from the carbon excess is used for polysaccharide biosynthesis. This suggests that environments containing a high ratio between carbon and the limiting nutrient, often nitrogen, may favor EPS production
- a combination of microscopy methods is necessary to gain more detailed information on the biofilm structure and composition

Next steps

- This study: quantitative analysis by differentiating EPS from cells
- Future: - testing the effects of other C/N/P ratios on other soil biofilm forming bacteria
  - testing other boundary conditions for the EPS production

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References

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