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Effects of carbon to nitrogen ratios on amounts and composition of Bacillus subtilis biofilms

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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 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. We hypothesized that the largest biofilm development and highest EPS production by *Bacillus subtilis* would be caused by a nutrient imbalance reflected in C/N ratios, especially high C availability. Biofilms were grown on membranes on MSgg agar plates with C/N ratios of 1:1, 10:1, 25:1 and 100:1. Several methods from macroscopic observations over EPS extraction and determination up to various microscopic visualisation techniques were used. The radial expansion of the biofilm was measured, followed by EPS extraction to quantify EPS-proteins and EPS-polysaccharides. Hydrated biofilm samples were studied regarding their biofilm structures by scanning electron microscopy (SEM) within the environmental mode at approximately 97% humidity. Fixed, dehydrated and embedded samples were used to evaluate the biofilm height and internal structure with SEM in high vacuum mode. Low C/N ratio (1:1) resulted in the smallest biofilms in terms of radial expansion and biofilm height, with densely packed layers of cells and low amounts of EPS. Our first results revealed that the highest biofilm productions were observed at C/N ratio of 10:1 and 25:1. The microscopic approaches indicated that biofilms growing at C/N ratios of 100:1 produced the highest amount of EPS. Furthermore, changes in the microscopical features of the biofilms were detected with different structures along the biofilm regions affected by the nutrient conditions. These results suggest that the C/N ratio has a large impact on the biofilm development and structure, with different allocations into microbial cells and EPS. Overall, the results obtained until now allowed us to accept the initial hypothesis, indicating that higher C/N ratios induce a higher EPS production. This suggests that environments containing a high ratio between carbon and the limiting nutrient, often nitrogen, may favour polysaccharide production, probably because energy from the carbon excess is used for polysaccharide biosynthesis.