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Supplemental Information

**Bacterial growth in multicellular aggregates
leads to the emergence of complex life cycles**

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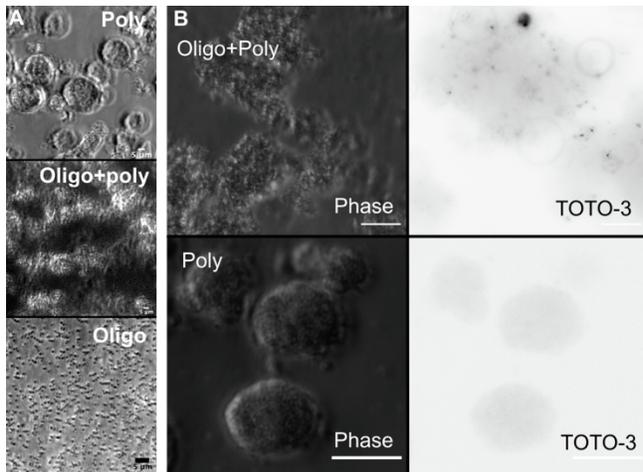


Figure S1. Structural properties 12B01 clonal groups formed during growth on alginate polysaccharide, in comparison to growth on oligosaccharide, related to Figure 1. A) Morphology of 12B01 grown on alginate oligosaccharides (Oligo), a combination of alginate oligosaccharides and low viscosity polysaccharide (Oligo+Poly), or polysaccharide only (Poly). Scale bar = 5 μm . **B)** Staining of 12B01 during growth on alginate polysaccharide or oligosaccharide with TOTO-3 iodide, a cell-impermeant fluorophore that detects extracellular DNA and dead cells. Left-hand companion images were acquired in phase-contrast and show the area occupied by 12B01. For TOTO-3 staining, darker signal indicates increased staining of DNA. Scale bar= 20 μm .

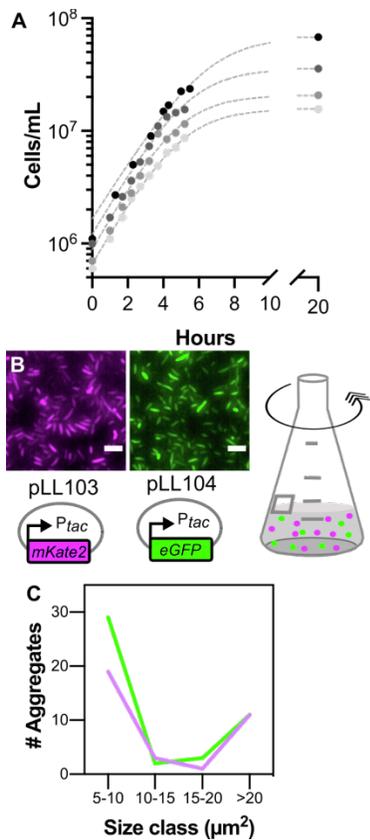


Figure S2. Growth of 12B01 on alginate oligosaccharides and in clusters, related to Figure 2. **A)** Growth of 12B01 on varying concentrations of alginate oligosaccharides. Lines indicate a fit of a logistic growth equation to the data. Points indicate individual measurements. All replicates are shown. **B)** Construction of fluorescent-protein expressing 12B01. Plasmids pLL103 and pLL104 express mKate2 or eGFP from the synthetic P_{tac} promoter. The plasmid backbone encodes a chloramphenicol acetyltransferase, and a *Vibrio*-specific origin of replication. Scale bar= 2 μm . Populations of the two plasmid-expressing isogenic strains were mixed to initiate the experiment. **C)** Distribution of group sizes for each fluorescent marker, measured for clusters collected from a single time-point during stage ii self-organization. Raw dataset analyzed is the same as shown in Figure 2D.

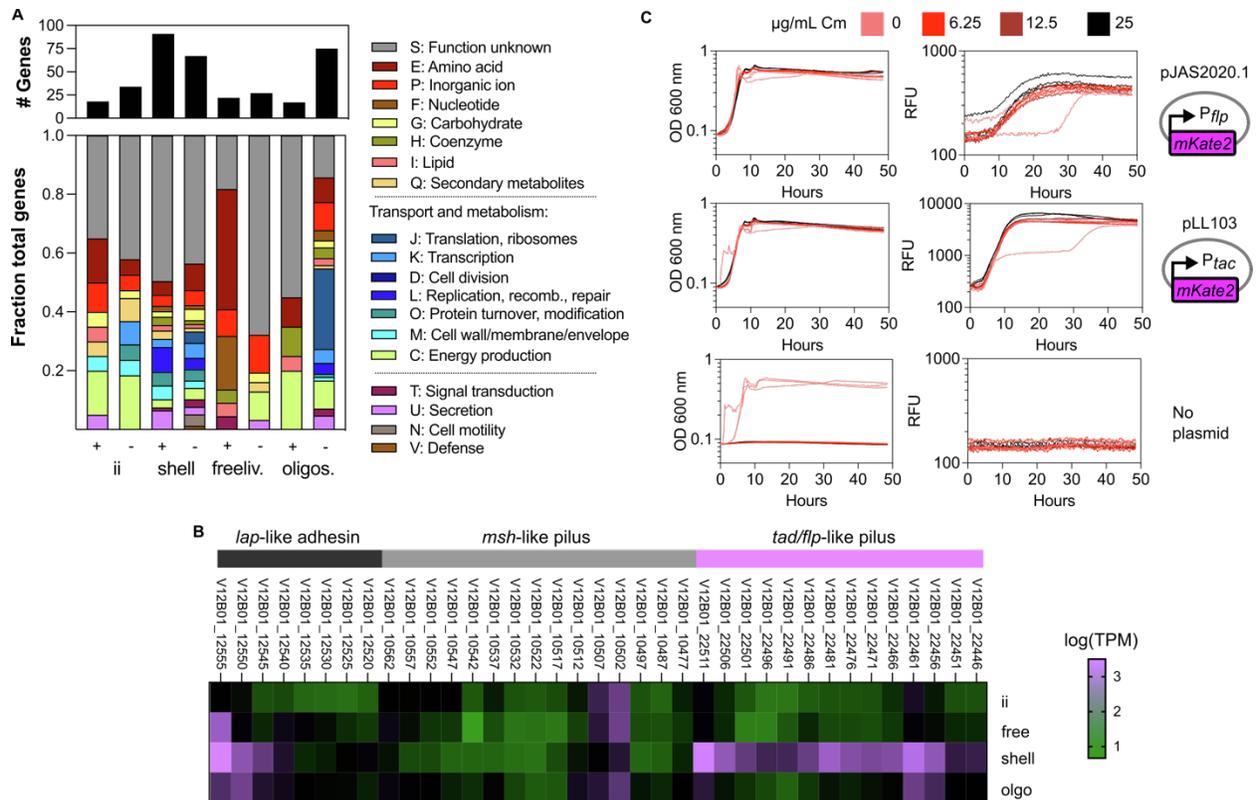


Figure S3. Characterization of 12B01 transcription, related to Figure 3 and Tables S1 and S2. **A)** Analysis of functional gene content for genes differentially expressed between all pairs of samples. Gene sets from Table S2 are grouped by the predicted cellular function that they encode. Labels follow the COG ontology. **B)** Expression of biofilm associated genes in 12B01. Heatmap reflects the strength of gene expression, measured by the log of normalized transcripts (TPM, transcripts per million). 12B01 gene identifiers are shown above the heatmap, and boxes indicate groups of genes with related function. **C)** Antibiotic selection was sufficient to maintain expression of the multicopy plasmids used in this study. The growth and fluorescence of 12B01 expressing mKate from either pJAS2020.1 or pLL103 was monitored during growth on glucose minimal medium containing different concentrations of chloramphenicol. A no-plasmid control was included to assess intrinsic resistance of 12B01 to chloramphenicol. Three biological replicates are shown. **C)** Activity of the *tac* or *tad/flp* promoter in 10 mM glucose batch culture. Vertical dashed line indicates maximum OD of culture. Three biological replicates are shown.

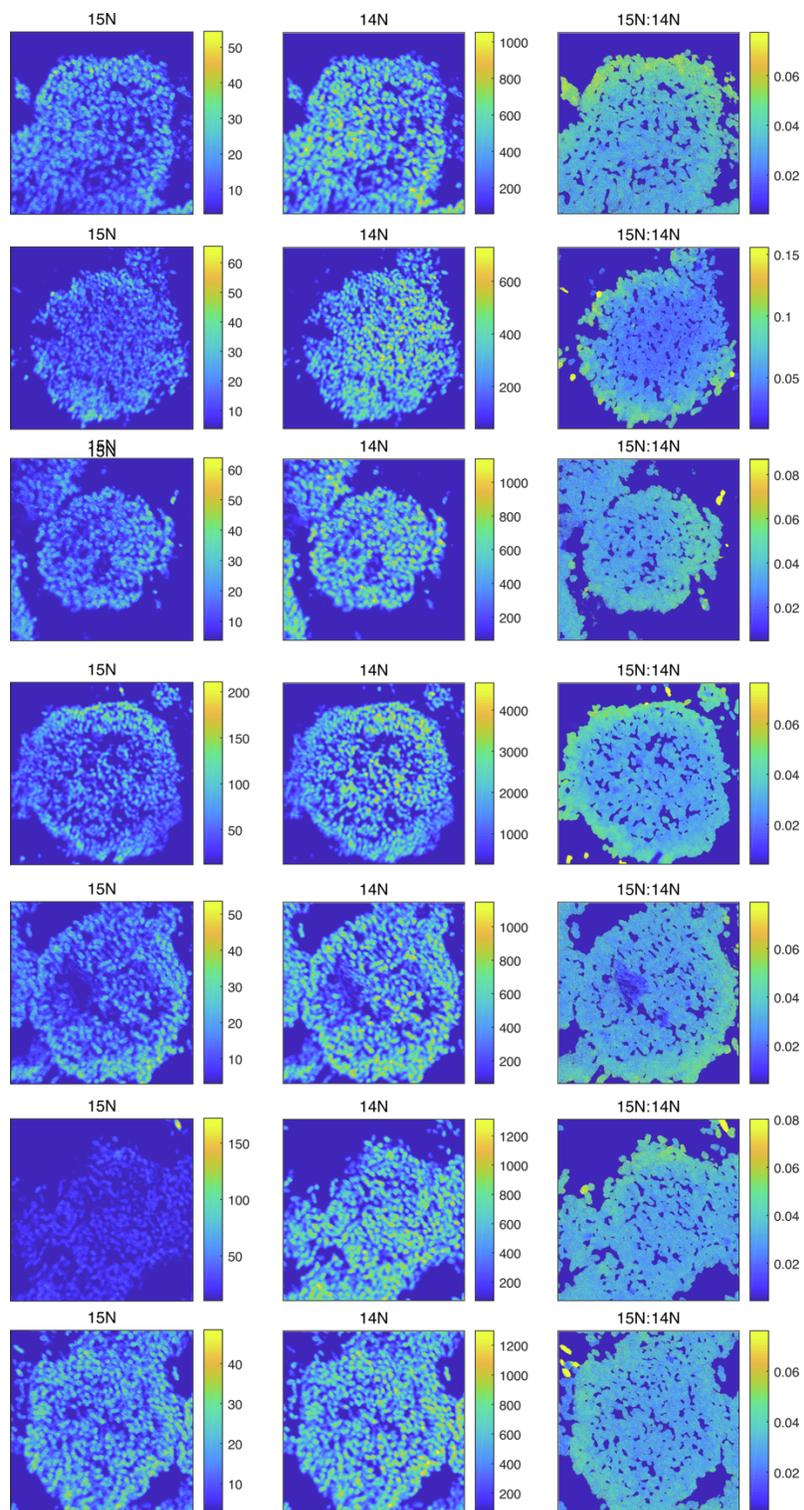


Figure S4. Quantification of ammonia assimilation by stage ii 12B01 clusters cultivated under slow shaking, related to Figure 5. Clusters were incubated for 4 h with ^{15}N ammonia. Incorporation of ^{15}N and ^{14}N into carbon containing biomass was quantified by secondary ion mass spectrometry. Left: $^{15}\text{N}^{12}\text{C}$ signal, middle, $^{14}\text{N}^{12}\text{C}$ signal, right enrichment of $^{15}\text{N}^{12}\text{C}$ ($^{15}\text{N}^{12}\text{C}/(^{15}\text{N}^{12}\text{C}+^{14}\text{N}^{12}\text{C})$). All measurements taken are shown.

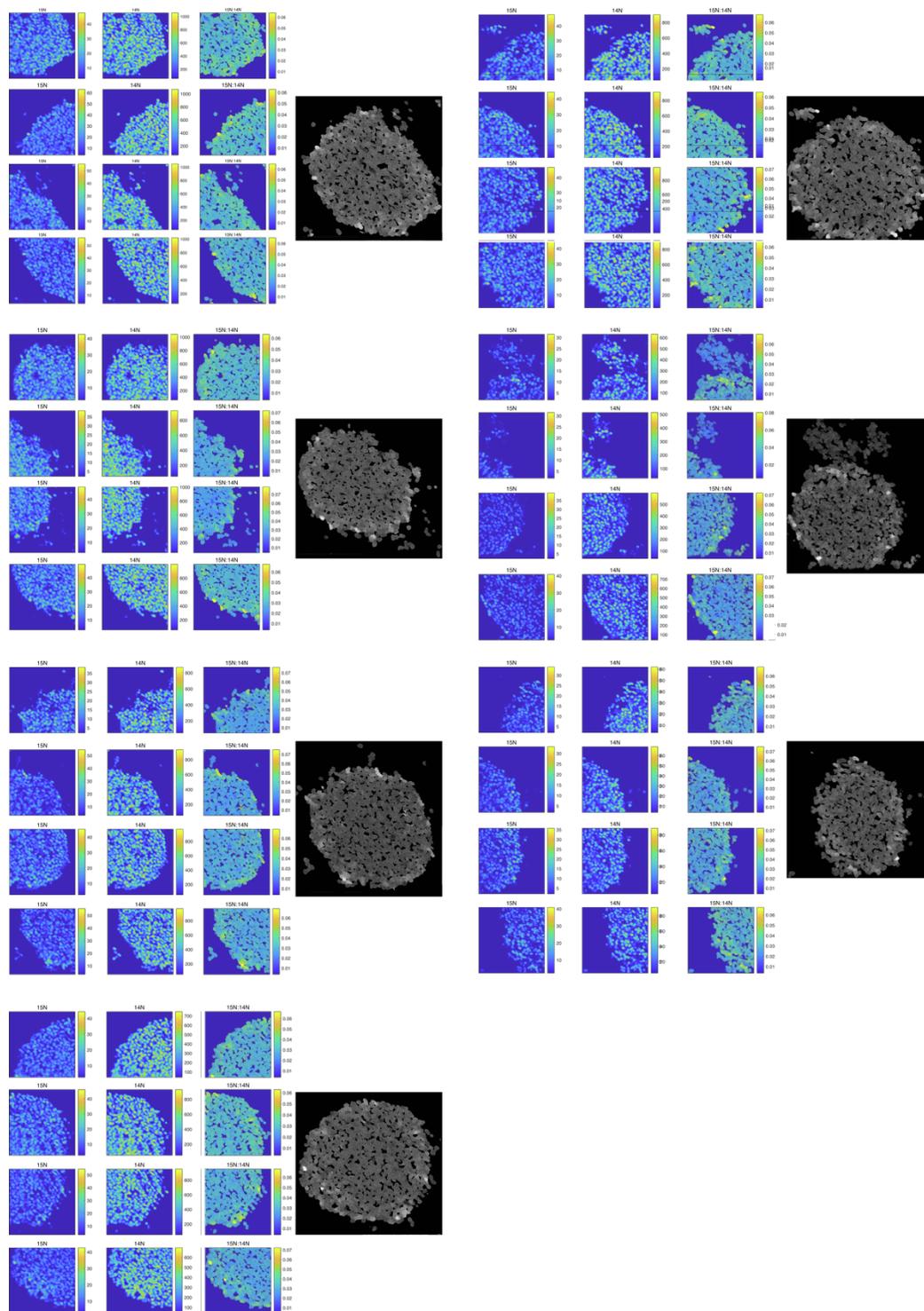


Figure S5. Quantification of ammonia assimilation by stage ii 12B01 clusters cultivated under faster shaking, related to Figure 5. As in Figure S4, clusters in stage ii of formation were incubated for 4 h with ^{15}N ammonia. Incorporation of ^{15}N and ^{14}N into carbon containing biomass was quantified by secondary ion mass spectrometry. Left: $^{15}\text{N}^{12}\text{C}$ signal, middle, $^{14}\text{N}^{12}\text{C}$ signal, right enrichment of $^{15}\text{N}^{12}\text{C}$ ($^{15}\text{N}^{12}\text{C}/(^{15}\text{N}^{12}\text{C}+^{14}\text{N}^{12}\text{C})$). Stitched image is shown to the far right. All measurements taken are shown.