**SUPPLEMENTARY INFORMATION**

belonging to the manuscript

# Production of poly-β-1,6-*N*-acetylglucosamine by MatAB is required for hyphal aggregation and hydrophilic surface adhesion by *Streptomyces*

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**Table S1: Similarities between *matB* and characterized glycosyltransferases type 2 and carbohydrate esterase type 4 as found on the Cazy database by BlastP.** Based on the annotation of the GT2 and CE4 domain found in *matA* its translated protein sequence was compared with similar typed proteins for which there is experimental evidence as tracked by the CAZY database. Two blast databases were made of the 266 GT2 proteins and the 58 CE4 proteins. The top 10 hits and the scores for both databases are given in the table.

Blastp verus characterized GlycosylTransferase Family 2

| Uniprot | Gene | Organism | Disciption | Score | E-value |
|---|---|---|---|---|---|
| P75905 | *pgaC* | *Escherichia coli (K12)* | Poly-beta-1,6-N-acetyl-D-glucosamine synthase | 157 | 3E-43 |
| C8YYH7 | *pgaC* | *Acinetobacter baumannii* | Poly-beta-1,6-N-acetyl-D-glucosamine synthase | 151 | 2E-41 |
| Q5HKQ0 | *icaA* | *Staphylococcus epidermidis* | Poly-beta-1,6-N-acetyl-D-glucosamine synthase | 150 | 6E-41 |
| Q5VJB2 | *aagC* | *Aggregatibacter actinomycetemcomitans* | Poly-beta-1,6-N-acetyl-D-glucosamine synthase | 140 | 2E-37 |
| Q5QFG3 | *aagC* | *Actinobacillus pleuropneumoniae* | Poly-beta-1,6-N-acetyl-D-glucosamine synthase | 137 | 2E-36 |
| Q84GC8 | *hasA* | *Streptococcus equi subsp. zooepidemicus* | Hyaluronan synthase | 89 | 2E-20 |
| O50201 | *hasA* | *Streptococcus dysgalactiae subsp. equisimilis* | Hyaluronan synthase | 89 | 2E-20 |
| Q9LJP4 | CSLC4 | *Arabidopsis thaliana* | Xyloglucan glycosyltransferase 4 | 87 | 1E-19 |
| P74165 | sll1377 | *Synechocystis sp. (PCC 6803)* | Beta-monoglucosyldiacylglycerol synthase | 86 | 3E-19 |
| Q8YMK0 | all4933 | *Nostoc sp. (PCC 7120)* | Beta-monoglucosyldiacylglycerol synthase | 85 | 4E-19 |

Blastp verus characterized Carbohydrate Esterase Family 4

| Uniprot | Gene | Organism | Disciption | Score | E-value |
|---|---|---|---|---|---|
| Q81AF4 | BC_3618 | *Bacillus cereus (ATCC 14579)* | Peptidoglycan N-acetylglucosamine deacetylase | 140 | 3E-40 |
| Q8RBF4 | *Cda1* | *Caldanaerobacter subterraneus* | chitin deacetylase | 133 | 2E-36 |
| Q81EK9 | BC_1960 | *Bacillus cereus (ATCC 14579)* | Peptidoglycan N-acetylglucosamine deacetylase | 123 | 2E-33 |
| Q8Y9V5 | lmo0415 | *Listeria monocytogenes serovar* | Peptidoglycan N-acetylglucosamine deacetylase | 125 | 5E-33 |
| P72333 | *nodB* | *Rhizobium sp. (N33)* | Chitooligosaccharide deacetylase | 120 | 7E-33 |
| P02963 | *nodB* | *Rhizobium meliloti* | Chitooligosaccharide deacetylase | 119 | 1E-32 |
| Q1M7W8 | *nodB* | *Rhizobium leguminosarum* | Chitooligosaccharide deacetylase | 118 | 3E-32 |
| P04339 | *nodB* | *Rhizobium leguminosarum bv. viciae* | Chitooligosaccharide deacetylase | 118 | 3E-32 |
| P50355 | *nodB* | *Rhizobium sp. (NGR234)* | Chitooligosaccharide deacetylase | 118 | 3E-32 |
| P50354 | *nodB* | *Rhizobium galegae* | Chitooligosaccharide deacetylase | 118 | 3E-32 |

**Table S2: Strains used in the study**

| Strain or plasmid | Description and genotype | Reference |
|---|---|---|
| **Strain** | | |
| ***Streptomyces lividans 66 (1326)*** | SLP2+ SLP3+ | [51] |
| Δ***cslA*** | *S. lividans* 66 Δ*cslA* | [25, 62] |
| ***GAD05*** | *S. lividans* 66 Δ*matAB* | [29] |
| ***GAD06*** | *S. lividans* 66 Δ*matB* | this work |
| ***GAD07*** | *S. lividans* 66 Δ*matA* | this work |
| ***GAD08*** | *S. lividans* 66 Δ*matAB* + pMAT7 | this work |
| ***GAD09*** | Δ*cslA* + pMAT7 | this work |
| **Plasmids** | | |
| **pSET152** | *oriT* RK2, pUC18 replicon, $Apra^R$ | [49] |
| **pMAT7** | pSET152::$P_{gapA}$-*matAB* | this work |

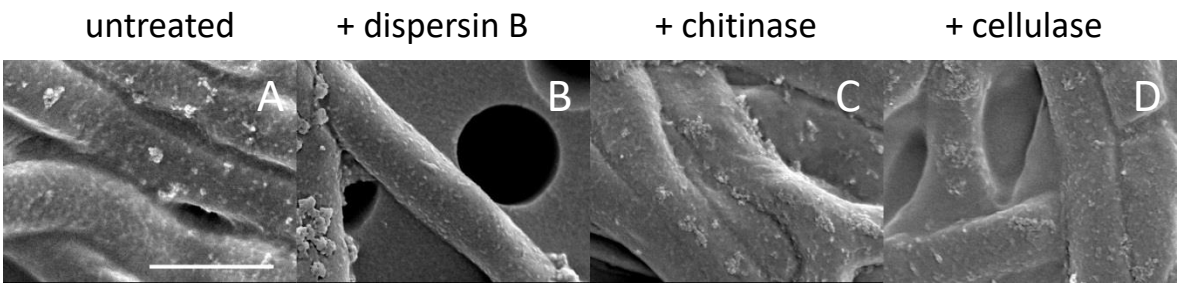


***Figure S1. Effect of hydrolytic enzymes on the accumulation of EPS on hyphae of S. lividans 66.*** Mycelia of wild-type *S. lividans* 66 were treated with either 50 µg/ml dispersin B, 0.5 U/ml chitinases or 2 U/ml cellulases for 4 h. Untreated hyphae were used as the control. The samples were then imaged with SEM to visualize the extracellular matrix. Note that treatment with dispersin B, which degrades PNAG, resulted in a smooth hyphal surface. Bar, 1 µm.

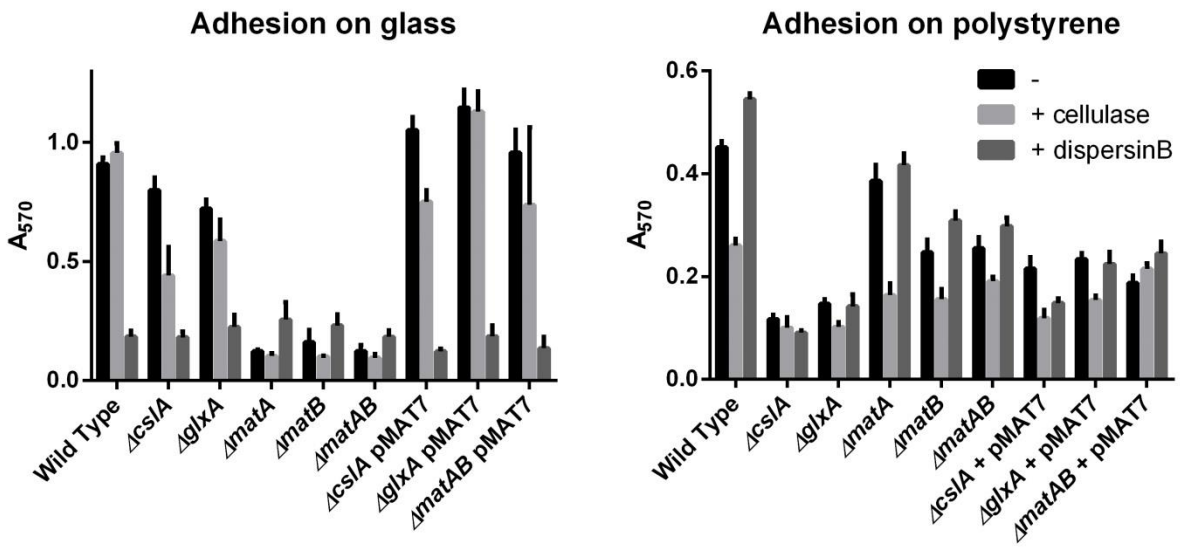


***Figure S2. Quantification of attachment to solid surfaces.*** Surface attachment was quantified for *S. lividans* 66 and its respective *cslA, glxA*, *matA* or *matB* mutants with and without added dispersin B or cellulase. Also shown are the strains harboring the pMAT7 construct, which overexpresses the *matAB* genes. Quantification was performed by staining attached cells with crystal violet and measuring dissolved crystal violet spectrophotometrically at 570 nm. The average and standard deviation of five independent wells are given. Left: surface attachment on glass from overnight growth. Right: surface attachment to polystyrene after 7 days of growth.