Adhesion of peptides to S.pneumoniae – Pulldown with cells

Cultivation

100 ml of THB media were inoculated 1/10 with an overnight culture of *S. penumoniae* R6 and incubated at 37 °C without agitation. The OD_{600} was measured frequently, aiming to the highest possible concentration of around OD_{600}=0.8, since the R6 strain of *S.penumoniae* undergoes autolysis at an OD_{600} of about1.0. At an OD_{600} Of 0.560 (after autolysis already started, see Fig 1) the cells were split into five tubes of 20 ml, centrifugated(3500 g, 5 minutes, 4 °C) and put on ice. 20 ml of the *E. coli* strains (carrying the plasmid coding for the peptides C4P, CSP and A5P) were cultured in 2xYT media with 100µg/ml Ampiciliin, resuspended in 1 ml of R-buffer and lysed as previously described. Additionally, a strain carrying the empty vector with the his-tagged MBP only was cultured. The final OD_{600} of the cells was very similar and around 2.8, allowing for a comparability of the lysates.

Incubation of *S. pneumoniae*

The pelleted S. pneumonia cells were resuspended in either 1 ml of buffer alone or in the different supernatants of the lysed E. coli cells and incubated for 30 minutes on a tilting table at 4 °C. The cells were then pelleted, the supernatant pipetted of and the pellet resuspended in 200 µl of the R-buffer resulting in roughly equivalent cell density as the lysed *E. coli* cells had a five-fold OD.

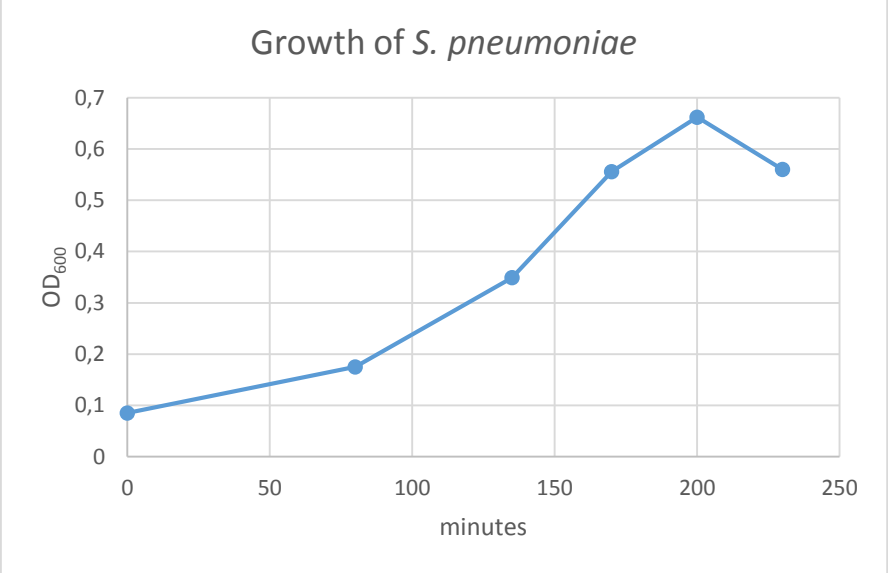

Figure 1: Growth of the S. pneumonia culture. The growth corresponds to the literature with a doubling time of roughly 40 minutes. The decreasing OD_{600} after 3.5 hours and an OD_{600} of 0,662 indicates the typical autoloysis of the R6 strain which usually starts at an OD_{600} of approximately 1.0.

Analysis by SDS-Page and Western Blot

75 µl of the fractions of the *E. coli* supernatant, the supernatant of the S. pneumonia cells after incubation with the supernatent and the resuspended cell pellet were incubated with 6x Laemmli Loading dye for 10 minutes at 95 °C and analysed by SDS-Page, loaded with 3 µl or 10 µl of the sample. Two gels were loaded parallel to enable subsequent analysis by Western-Blot.

The two gels with 3 µl and 10 µl were blotted onto a nitrocellulose membrane for 1 hour and 5 min at 400 mA and blocked overnight in 1xTBS/ 3% BSA solution at 4 °C. The membranes were then incubated for 1 hour in 5 ml 1xTBS/3% BSA with 1:2000 Quiagen Anti-His antibody produced in mouse, washed

twice for 10 minutes with 1xTBS/ 0.1% Tween and once with 1xTBS, and incubated for 1 hour with the secondary antibody, anti-mouse IgG HRP (Promega W4028), diluted 1:2000 in 1XTBS/ 10% milk. The membranes were then washed 4 times for 10 minutes each with 1xTBS/ 0.1% Tween followed by a final wash step for 5 minutes in 1xTBS. The membrane was then developed with chemiluminescence agents and the luminescence was photographed in a photo-chamber.

Media & Buffer

2xYT Media	For 1 liter: 16g peptone, 10g yeast, 10g NaCl
R-Buffer	50 mM Tris, 100 mM NaCl, 5% v/v glycerol, pH 7.5
THB Media	Todd-Hewitt Broth, was prepared from OXOID THB mixture (CMO189), but can otherwise be prepared from: Heart Infusion (dehydrated) 3.1 g Yeast Enriched Peptone 20 g Dextrose 2 g Sodium Chloride 2 g Disodium Phosphate0.4 g Sodium Carbonate 2.5 g

Protocol generously provided by the lab

Prof. Thorsten Mascher

Großhadernerstr. 2-4

82152 Planegg-Martinsried

www.syntheticmicrobe.bio.lmu.de