
Identification of novel pneumococcal adherence factors by a combination of genome-wide approaches
 Hester Bootsma
 Laboratory of Pediatric Infectious Diseases
 Radboud University Medical Centre
 Nijmegen, The Netherlands
 h.bootsma@cukz.umcn.nl

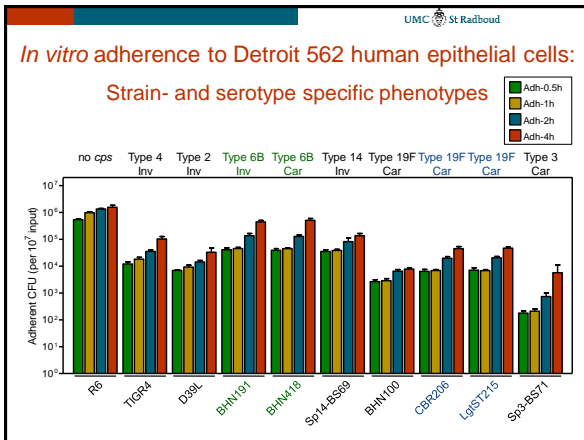
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Streptococcus pneumoniae: colonization and infection

- Study molecular pathogenesis of colonization and infection *in vitro*
 - Exploit various *in vitro* cell culture models in a standard fashion: adherence, invasion, phagocytosis, killing, chemotaxis, ciliary beating
 - Identify species- and strain-specific characteristics of pneumococcal isolates

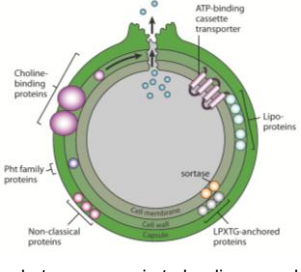
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In vitro adherence to human pharyngeal epithelial Detroit 562 cells



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Several pneumococcal factors affecting adherence are known...



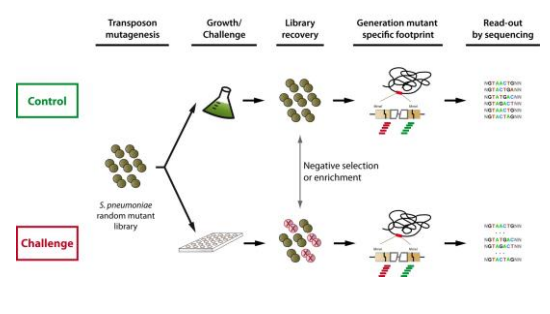
...but more remain to be discovered

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Identify novel adherence factors by two genomic approaches

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Identification of genes essential for adherence using TnSeq



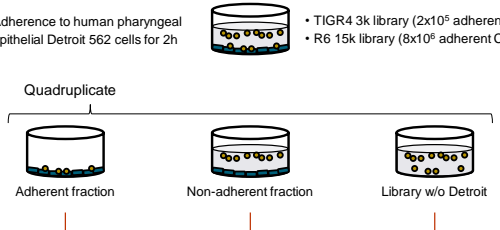
van Opijnen et al. 2009, Peter Burghout

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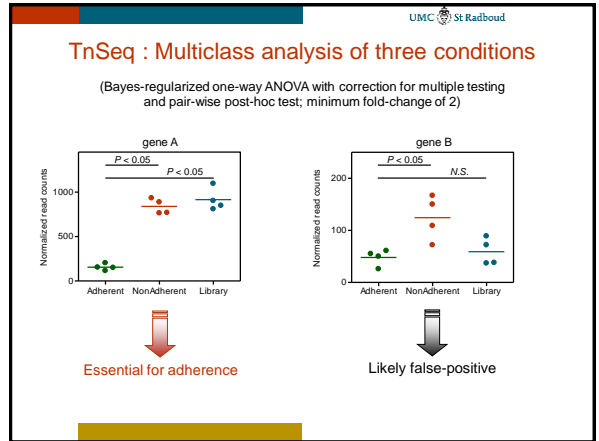
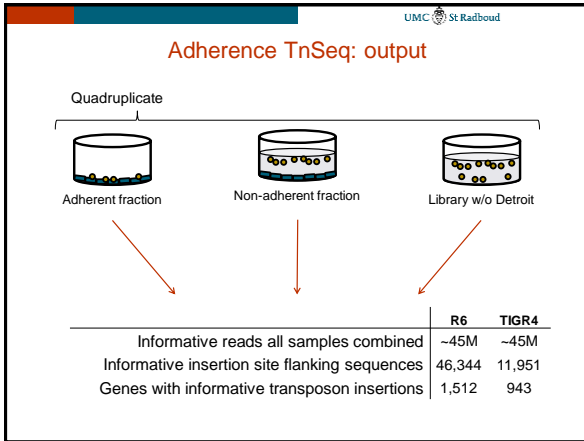
Adherence TnSeq: experimental setup

Adherence to human pharyngeal epithelial Detroit 562 cells for 2h

- TIGR4 3k library (2x10⁸ adherent CFU)
- R6 15k library (8x10⁸ adherent CFU)



Equivalent fractions are expanded for DNA isolation and analyzed by TnSeq

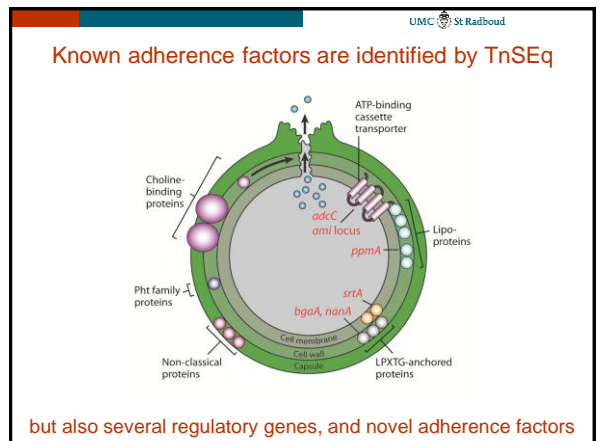
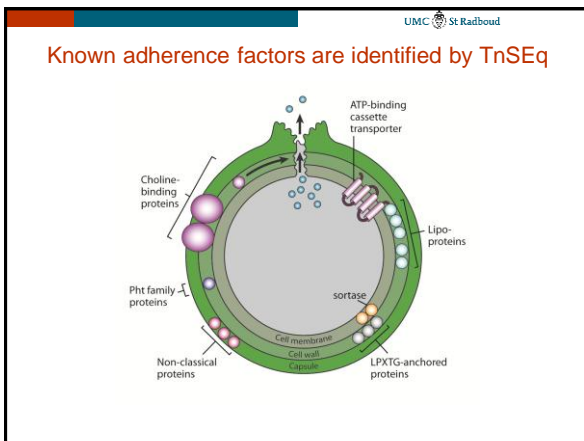
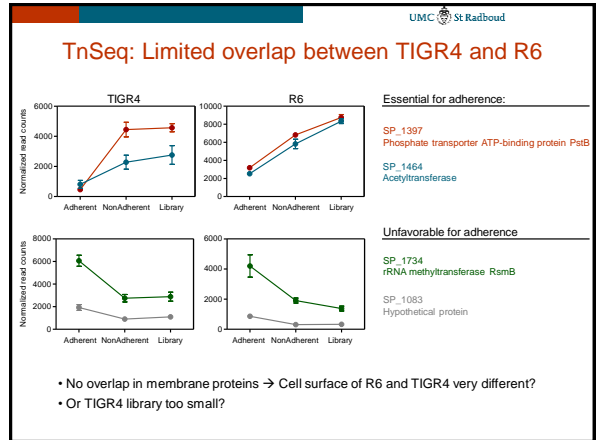


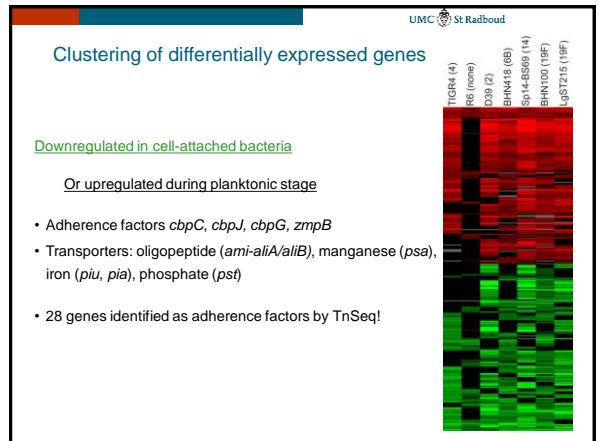
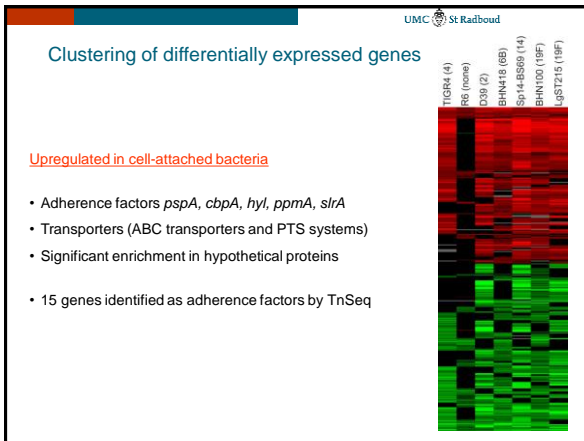
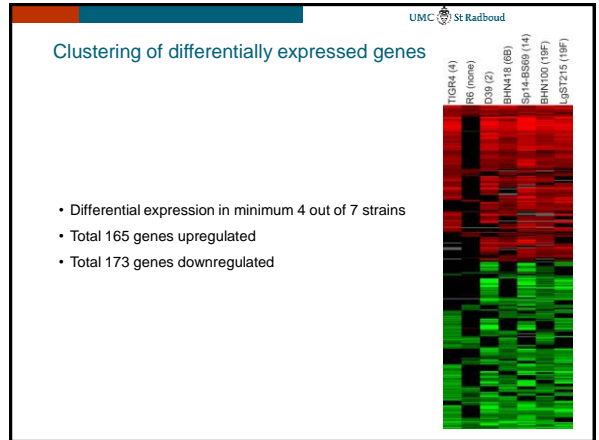
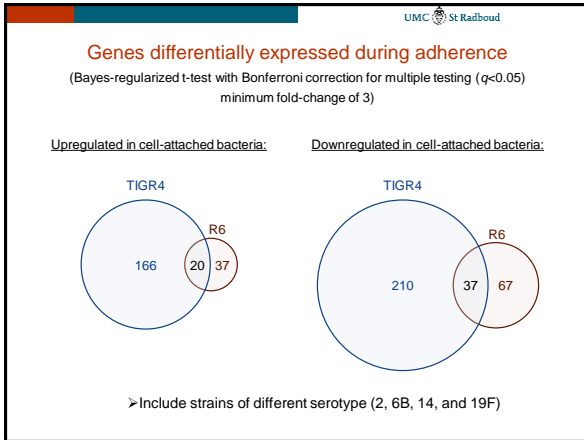
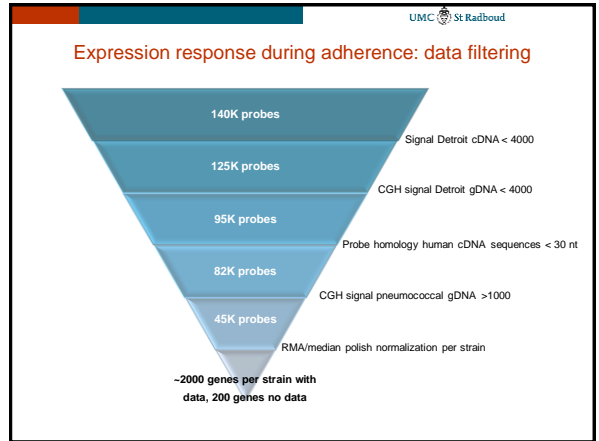
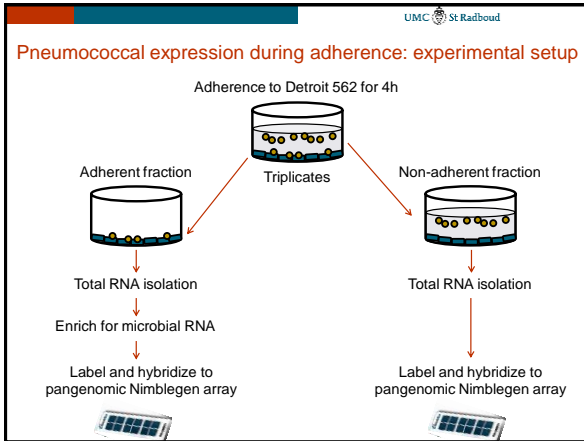
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TnSeq results in numbers

	Essential for adherence				Total
	Cytoplasmic	Cell membrane	Cellwall	Unknown	
R6	27	20	4	12	63
TIGR4	27	24	1	7	59

	Unfavorable for adherence				Total
	Cytoplasmic	Cell membrane	Cellwall	Unknown	
R6	7	5	3	1	16
TIGR4	12	9	2	3	26

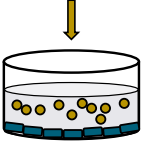




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Working Hypothesis

- Pneumococcus senses the presence of eukaryotic cells
- Priming of adherence happens during planktonic stage

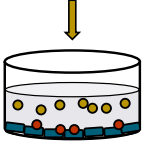


A yellow arrow points down to a petri dish containing several yellow dots, representing pneumococci in a planktonic stage.

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Working Hypothesis

- Pneumococcus senses the presence of eukaryotic cells
- Priming of adherence happens during planktonic stage



A yellow arrow points down to a petri dish containing several yellow dots and a few red dots, representing pneumococci sensing eukaryotic cells. A red arrow points down from the dish.

- When bound, mRNA expression of certain adhesion factors is switched off, while others are turned on

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Concluding remarks

- ✓ Tn-Seq rapidly identifies known and new adhesion factors
- ✓ Various cell envelope proteins and regulatory proteins are involved in adherence
- ✓ Importance of nutritional balance and adherence/colonization is underscored
- ✓ Certain cell adhesion factors are upregulated in the planktonic fraction

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THANKS TO

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Pneumopath



SEVENTH FRAMEWORK PROGRAMME

EUROPEAN UNION