Report on microbial aggregation (clustering) by 2QR-complex polysaccharides

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Adhesion of microorganisms to host tissues is the first essential step in infection

Pathogenic microorganisms have developed tools to breach the natural host defense system to colonize and invade our tissues. The majority of microbial problems are initiated by adhesion of harmful microorganisms to human epithelial surfaces (skin and mucosa) (1, 2). To effectively bind to host tissue surfaces, many microorganisms possess multiple adhesin proteins on their outer surface. These adhesins bind to polysaccharide groups that are present on receptor molecules at the surface of our epithelial cells (3).

Polysaccharides as anti-adhesive agents in microbial infections

Anti-adhesive strategies are aimed at blocking the interaction between microbial adhesins and host epithelial cell receptors in order to prevent infection at an early stage (4–6). The human mucosal surfaces are decorated with a mucin layer which acts as an anti-adhesive strategy and also human mother's milk contains anti-adhesive polysaccharides (7–12). Polysaccharides from several natural sources are known for their anti-adhesive (also described as 'anti-adhesion') activity. Blocking microbial adhesion by anti-adhesive strategies effectively renders pathogens harmless. The protective effect of anti-adhesive polysaccharides has been demonstrated convincingly in a variety of in vitro studies and in vivo models with different pathogenic microorganisms (4, 6, 13).

Importance of polyvalent interactions and aggregative properties of anti-adhesive polysaccharides

Many anti-adhesion compounds are large molecules with multiple adhesin binding sites (polyvalency). The presence of multiple binding sites on the anti-adhesive polysaccharides and the fact that many adhesins are present on microbial surfaces allows multiple interactions between microbes and anti-adhesives. Such polyvalent interactions have stronger neutralizing capacity than individual adhesin-polysaccharide interactions (14–16). In addition, such polyvalent interactions can cause aggregation (clustering) of microorganisms (14–17). The microorganisms trapped in such large aggregates are unable to reach and bind epithelial surfaces.

The example below presented by Almant *et al*. (16) nicely shows the aggregative effect of an experimental polyvalent anti-adhesive compound for *E. coli* bacteria.

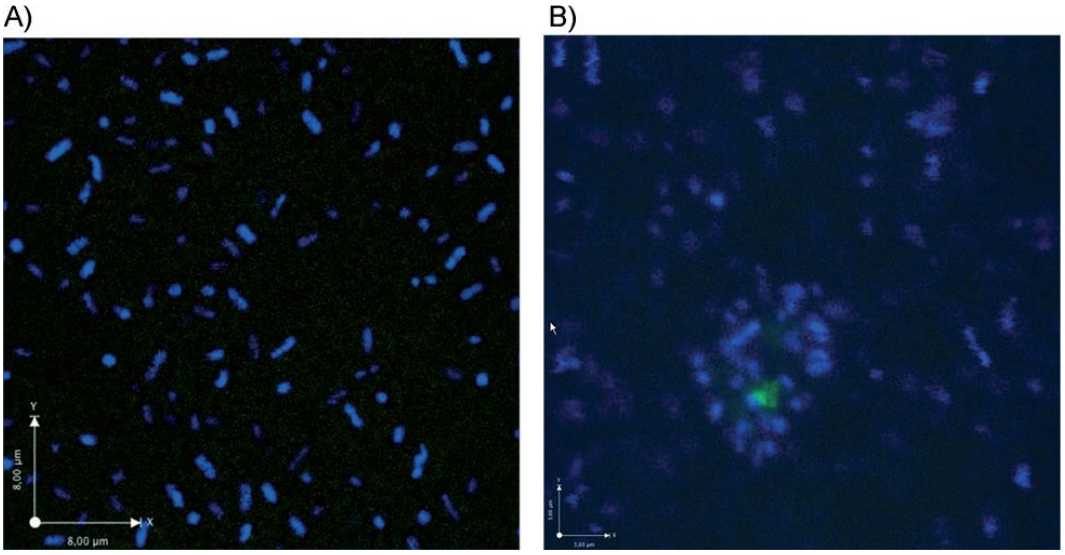

Figure 1. Microscopy images of A) Uropathogenic *E. coli* in solution fluorescently labeled with Hoechst stain 33258. Individual bacteria are observed without clustering; B) Bacterial cluster (in blue) co-localizes with a FITC-labeled experimental anti-adhesive compound (in green). Image reproduced from Almant et al., 2011 (16).

What is 2QR-complex?

2QR-complex is a mixture of anti-adhesive polysaccharides derived from the inner gel of Aloe barbadensis leaves, which is patented and used in many products of the BioClin product lines. It consists of diverse large multi-branched polysaccharides that contain a large array of different structures that can bind to adhesins of different microbial pathogens. Due to its diverse and large polysaccharide structures, 2QR-complex can bind and trap adhesins of various pathogens through polyvalent interactions.

Aggregative properties of 2QR-complex

In a research project performed at the Amsterdam Dental University (ACTA), The Netherlands, the aggregative (clustering) effect of 2QR-complex was investigated with *Staphylococcus aureus*. The bacterium was incubated in a microwell plate the absence or presence of 2QR-complex, and at different time point a picture was taken. In this experiment, only the bacteria but not 2QR-complex were labelled, so only *S. aureus* was visualized. Figure 2 clearly demonstrates that *S. aureus* bacteria strongly aggregate in the presence of 2QR-complex. As only the bacteria present on the bottom of the well are in-focus, it takes a while before bacteria start to sediment and aggregate.

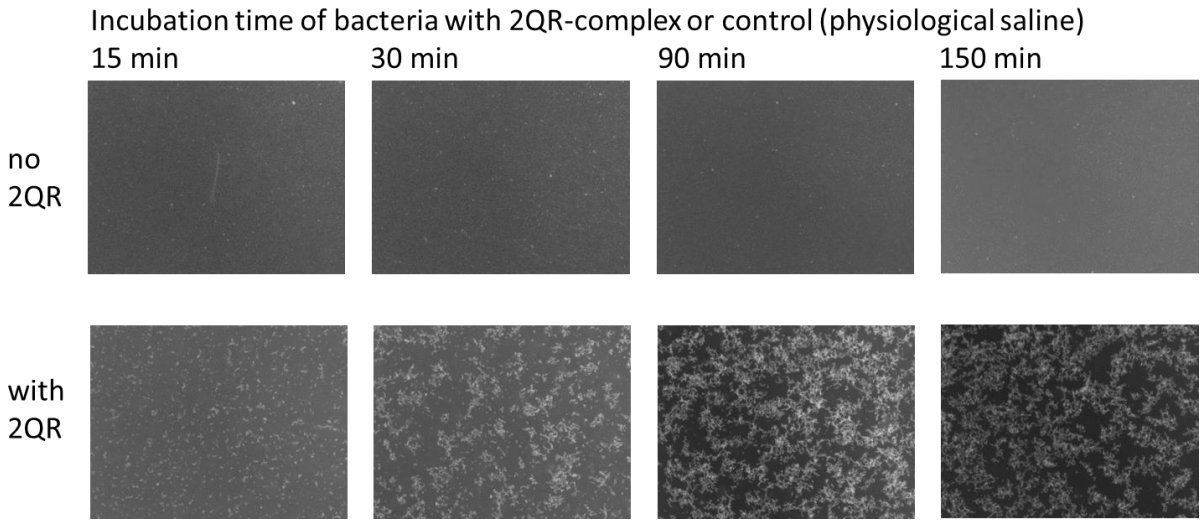

Figure 2. Incubation of FITC-labelled *S. aureus* in the absence or presence of 2QR-complex in a microwellplate. The camera only presents grey-scale images, so the green fluorescent color of the FITC-labelled bacteria is not represented in this picture.

In the absence of 2QR-complex, no bacterial aggregation was present. Figure 3 shows a magnification of the incubations of *S. aureus* with and without 2QR-complex after 30 min. For incubation without 2QR-complex, only individual bacteria are present, which are almost undiscernible to the eye at the magnification used (640x). In the presence of 2QR-complex, the large clusters of *S. aureus* bacteria are prominently present.

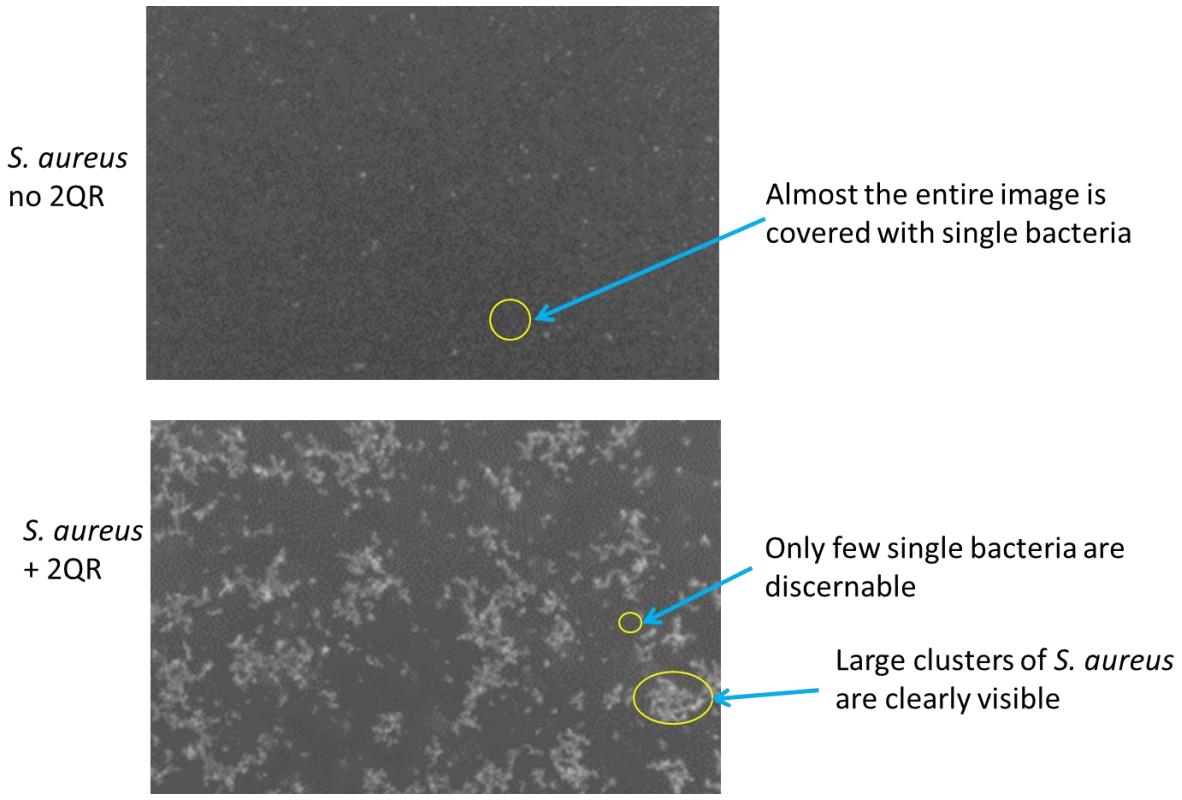

Figure 3. Enhanced magnification of images shown in Figure 2 for the 30 min. incubation.

In a subsequent experiment performed by Ron Legerstee (a Dutch microscopist and wound healing consultant) one drop of 2QR-complex was mixed with one drop of FITC-labelled (green) *S. aureus* cells on a glass microscope slide. In this setup a 400x magnification was used, but the use of a sensitive full-color camera greatly improved the visibility of individual bright green-fluorescent *S. aureus* cells.

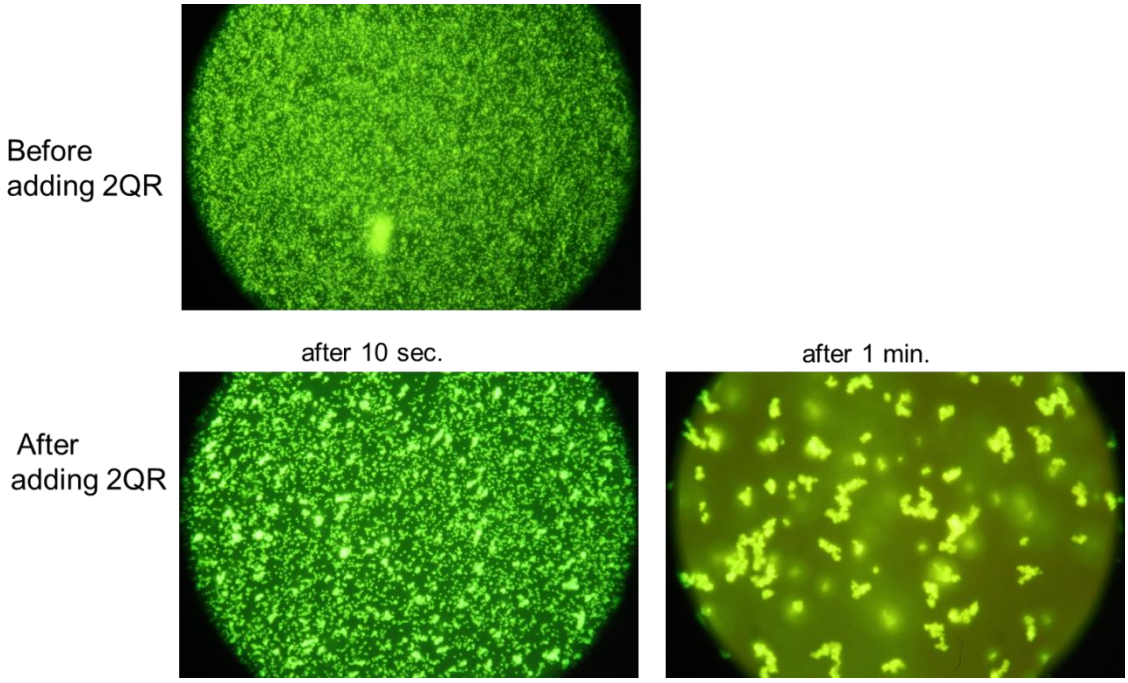

Figure 4. Incubation of FITC-labelled (green) *S. aureus* in the presence of 2QR-complex on a glass microscope slide.

Within seconds, the *S. aureus* bacteria started to aggregate in the presence of 2QR-complex (Figure 4). In a control incubation without 2QR-complex, no aggregation occurred. The dynamics of bacterial aggregation in the presence of 2QR-complex, and absence of such aggregation in the absence of 2QR-complex, was nicely visualized in a movie generated by Ron Legerstee. The conditions were similar as described for Figure 4, and in this setup the camera was set to produce a movie of about 3 minutes of the process of microbial aggregation instead of taking still photo. An edited version of this movie was produced with a side-by-side comparison of incubations with and without 2QR-complex to show the most prominent elements of the bacterial clustering process caused by 2QR-complex. [filename of the time-lapse movie: 2QR vs without 2QR - clustering Side by Side comparison].

As a result of aggregation, the adhesive properties of microbial cells are neutralized, and in addition, the clusters of microbes are more easily removed from the human body. Therefore, these *in vitro* experiments clearly demonstrate the potential of 2QR-complex to neutralize microbial pathogens.

References

1. **Finlay BB**. 1997. Exploitation of Mammalian Host Cell Functions by Bacterial Pathogens. Science (80-) **276**:718–725.

2. **Ofek I**, **Hasty DL**, **Doyle RJ**. 2003. Bacterial Adhesion to Animal Cells and Tissues. American Society for Microbiology Press, Washington, DC.

3. **Varki A**, **Cummings RD**, **J.D. E**. 2009. Essentials of glycobiology, p. . *In* Esko, JD, Sharon, N (eds.), Microbial lectins: Hemaglutinins, Adhesins and Toxins2nd editio. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

4. **Ofek I**, **Hasty DL**, **Sharon N**. 2003. Anti-adhesion therapy of bacterial diseases: prospects and problems. FEMS Immunol Med Microbiol **38**:181–191.

5. **Kelly CG**, **Younson JS**. 2000. Anti-adhesive strategies in the prevention of infectious disease at mucosal surfaces. Expert Opin Investig Drugs **9**:1711–21.

6. **Sharon N**. 2006. Carbohydrates as future anti-adhesion drugs for infectious diseases. Biochim Biophys Acta **1760**:527–37.

7. **Loke MF**, **Lui SY**, **Ng BL**, **Gong M**, **Ho B**. 2007. Antiadhesive property of microalgal polysaccharide extract on the binding of Helicobacter pylori to gastric mucin. FEMS Immunol Med Microbiol **50**:231–238.

8. **Lee J-H**, **Shim JS**, **Chung M-S**, **Lim S-T**, **Kim KH**. 2014. Inhibition of Pathogen Adhesion to Host Cells by Polysaccharides from Panax ginseng. Biosci Biotechnol Biochem **73**:209–212.

9. **Lee J-H**, **Shim JS**, **Lee JS**, **Kim JK**, **Yang IS**, **Chung M-S**, **Kim KH**. 2006. Inhibition of pathogenic bacterial adhesion by acidic polysaccharide from green tea (Camellia sinensis). J Agric Food Chem **54**:8717–23.

10. **Ganan M**, **Collins M**, **Rastall R**, **Hotchkiss a T**, **Chau HK**, **Carrascosa a V**, **Martinez-Rodriguez a J**. 2010. Inhibition by pectic oligosaccharides of the invasion of undifferentiated and differentiated Caco-2 cells by Campylobacter jejuni. Int J Food Microbiol **137**:181–5.

11. **Lee J**, **Shim J**, **Chung M**. 2009. In vitro anti-adhesive activity of green tea extract against pathogen adhesion. Phyther Res **466**:460–466.

12. **Peterson R**, **Cheah WY**, **Grinyer J**, **Packer N**. 2013. Glycoconjugates in human milk: protecting infants from disease. Glycobiology **23**:1425–38.

13. **Kelly CG**, **Medaglini D**, **Younson JS**, **Pozzi G**. 2001. Biotechnological Approaches to Fight Pathogens at Mucosal Sites. Biotechnol Genet Eng Rev **18**:329–347.

14. **Bernardi A**, **Jiménez-Barbero J**, **Casnati A**, **De Castro C**, **Darbre T**, **Fieschi F**, **Finne J**, **Funken H**, **Jaeger K-E**, **Lahmann M**, **Lindhorst TK**, **Marradi M**, **Messner P**, **Molinaro A**, **Murphy P V**, **Nativi C**, **Oscarson S**, **Penadés S**, **Peri F**, **Pieters RJ**, **Renaudet O**, **Reymond J-L**, **Richichi B**, **Rojo J**, **Sansone F**, **Schäffer C**, **Turnbull WB**, **Velasco-Torrijos**

T, **Vidal S**, **Vincent S**, **Wennekes T**, **Zuilhof H**, **Imberty A**. 2013. Multivalent glycoconjugates as anti-pathogenic agents. Chem Soc Rev **42**:4709–27.

15. **Pieters RJ**. 2009. Maximising multivalency effects in protein-carbohydrate interactions. Org Biomol Chem **7**:2013–25.

16. **Almant M**, **Moreau V**, **Kovensky J**, **Bouckaert J**, **Gouin SG**. 2011. Clustering of Escherichia coli type-1 fimbrial adhesins by using multimeric heptyl alpha-D-mannoside probes with a carbohydrate core. Chem - A Eur J **17**:10029–10038.

17. **Krachler AM**, **Orth K**. 2013. Targeting the bacteria-host interface: strategies in anti-adhesion therapy. Virulence **4**:284–94.