Sialic acid is involved in the differential binding of streptococcal species to milk and salivary glycoproteins

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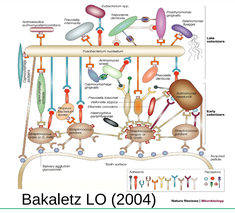
Introduction

The mouth is first entry point for pathogenic organisms and saliva is potentially the first line of defence against infection. Oligosaccharides in milk have been postulated as providing protection against bacterial infection in infants [1]. Proteins in human milk and saliva have similar glycoproteins with different attached sugars. Sialic acids found on human milk glycoproteins have been reported to have an immune protective role for the infant against bacterial infections [2]. However, whether this monosaccharide in both secreted fluids mediates streptococcal infection of the mouth remains unknown. The aims of this project are

- **To develop methods to visualise and quantitate binding of oral pathogens *Streptococcus gordonii* and *S. mutans* to salivary and milk glycoproteins**
- **To show whether sialic acids are involved in binding of human saliva and milk glycoproteins to common oral pathogens**
- **To confirm sialic acid as being part of the bacterial binding epitope in this interaction**



Bakaletz LO (2004)

Methods

Proteins contained in human saliva and milk were separated from other components by precipitation. Bacterial binding to glycoproteins was measured by

(A) Hydroxyapatite crystals (HA) → Coat with HMW fraction of saliva → Add fluorescently labelled bacteria → wash → Visualise by microscopy and quantitate fluorescence

(B) Milk → Methanol/ Chloroform precipitation [3] → Saliva → Filtered → Coat PVDF membrane of 96 well plate with precipitated milk or saliva proteins → Add fluorescently labelled bacteria → wash → Quantitate fluorescence

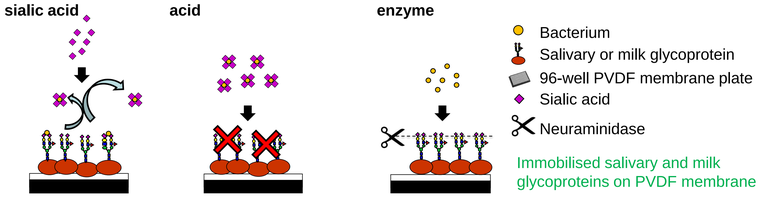
Oral pathogens *Streptococcus gordonii* F552, *S. mutans* LT11 and laboratory strain *Escherichia coli* DH5α were compared.

Effect of sialic acid (◆) on differential bacterial (●)binding to glycoproteins (🖱)

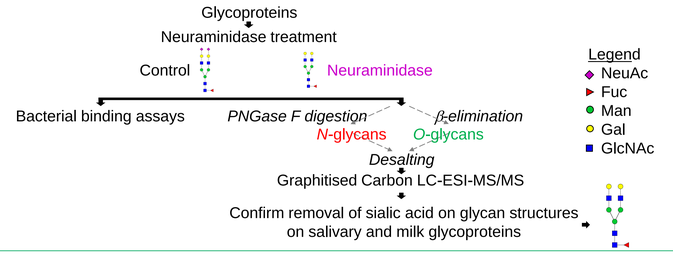
(1) Washing bound bacteria with free sialic acid

(2) Pre-incubating bacteria with free sialic acid

(3) Removing sialic acid with enzyme

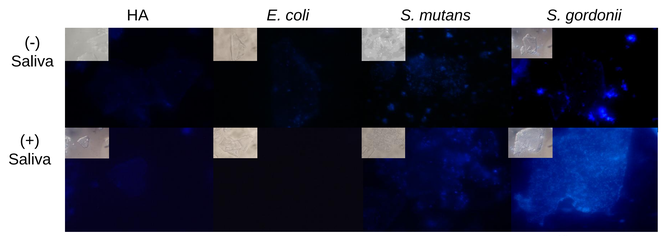


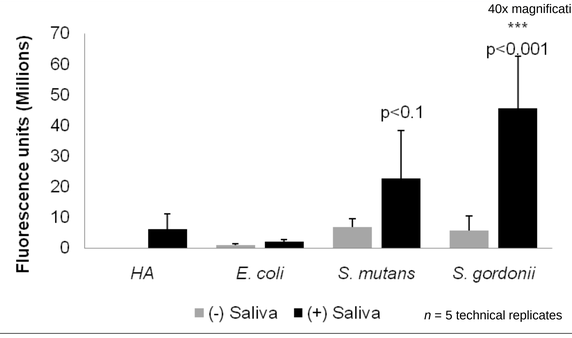
Confirmation that terminal sialic acids are removed



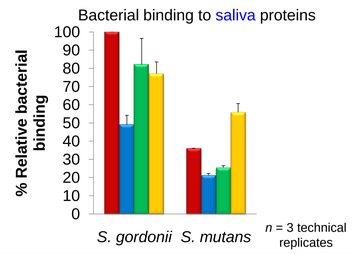
Results

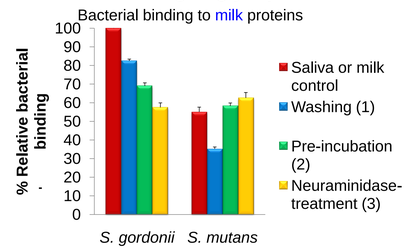
(A) *Streptococcus gordonii* binds better than *S. mutans* to saliva-coated hydroxyapatite





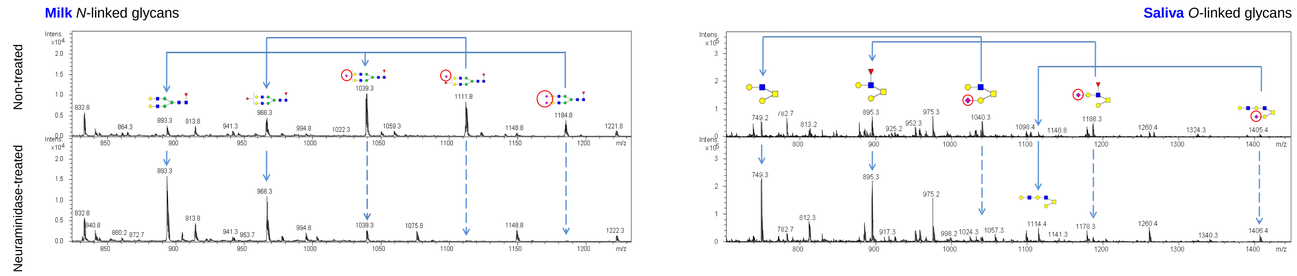
(B) Effect of sialic acids on bacterial binding to glycoproteins





- ***Streptococcus gordonii* binds more strongly than *S. mutans* to proteins in saliva and milk**
- **Sialic acid washing after bacterial binding reduces amount of bound *S. gordonii* and *S. mutans***
- **Pre-incubation of sialic acid with bacteria showed streptococcal binding has decreased except for *S. mutans* binding to milk proteins**
- ***S. gordonii* binding is reduced after removal of sialic acids but *S. mutans* binding is increased in both saliva and milk**

Confirming sialic acids removal from human milk and salivary glycoproteins



Loss of terminal sialic acid (◆) to form corresponding desialylated structures was shown after 16 hours neuraminidase digestion.

Conclusions

- ***Streptococcus gordonii* and *S. mutans* bound differently to glycoproteins in both saliva and milk**
- **Interaction of *S. gordonii* with saliva and milk glycoproteins was shown to be partially reliant on sialic acid residues for binding**
- ***S. mutans* did not appear to use sialic acid containing epitopes for binding and bound more strongly with the removal of sialic acid and consequent exposure of other sugar epitopes**

Future work

Q1 How do gastrointestinal pathogens bind to human milk glycoproteins? Do these pathogens bind differently to different fractions of milk? How does this compare to bovine milk?

Q2 Does the glycoprotein-pathogen interaction change if human milk glycosylation pattern is altered? Does the removal of specific sugars change the milk glycoprotein binding properties to gastrointestinal bacteria?

Impact to the dairy industry

Can we add human-like glycan structures to bovine milk products to mimic the protection properties of human milk?

References

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