Probing the bacterial cell wall of *E. coli* and *B. subtilis*

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Introduction and Aims

The cell wall of bacteria is composed of interlinked chains of peptidoglycan which both; maintain the shape of the bacteria and act as a barrier to protect the bacteria from their often harsh environments. Bacteria can be categorised by the arrangement of their peptidoglycan cell wall. There are two categories; Gram positive (a thick outer layer of peptidoglycan) and Gram negative (a thin layer of peptidoglycan between two cell membranes).

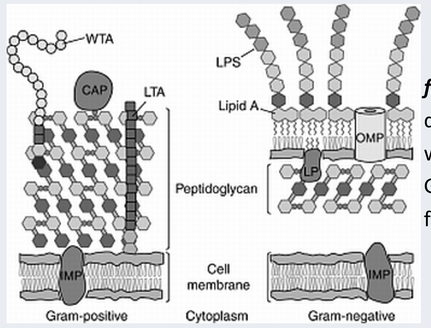


fig. 1 A visual comparison of the differences in the peptidoglycan cell wall structure of Gram positive and Gram negative bacteria. Adapted from Silhavy et al. (2010)

Koch and Demchick (1996) initially investigated the porosity of the bacterial cell wall. In their research they aimed to determine the relative cell wall porosity of both *E. coli* (Gram negative) and *B. subtilis* (Gram positive). From their results two conclusions were drawn:

- The size of the 'pores' in the cell walls of *B. subtilis* and *E. coli* were near identical
- The *E. coli* cell wall was equally porous as the *B. subtilis* cell wall

Aims:

- Develop a method which can be used assess the permeability of the *B. subtilis* and *E. coli* cell wall
- Examine the permeability of the *B. subtilis* cell wall in relation to that of *E. coli*
- Compare the results of this research to the results of Koch and Demchick (1996)

Method

Sacculi preparation:

B. subtilis and *E. coli* were grown in culture to late stationary phase. The culture was then centrifuged and the cells were treated to render the bacteria biochemically dead. These treatments included; SDS, DNase, RNase and Pronase.

Following treatments the cells were centrifuged and washed with water several times in order to remove the bacterial cell contents. The samples were then examined using light microscopy to determine that intact sacculi had been successfully extracted.

Diffusing in various sized fluorescent dextrans:

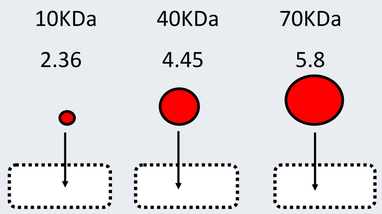


fig. 2 10KDa, 40KDa and 70KDa dextran were mixed with sacculi to determine what size of dextran was able to diffuse the cell wall. All of the dextran sizes used were able to diffuse into the sacculi, however 70KDa was found to have the slowest diffusion rate. Stoke radius for each dextran determined from pharmacosmos (2013)

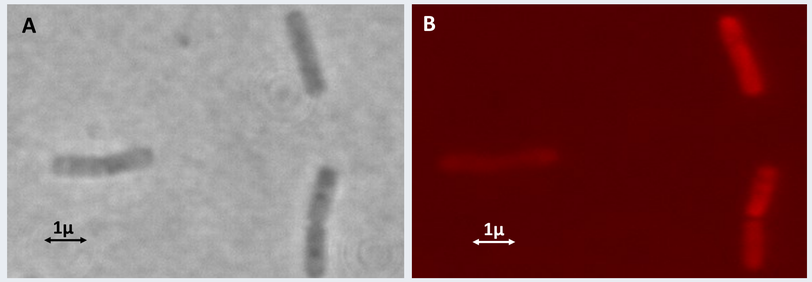


fig. 3 A) An image of sacculi created for the purpose of the experiment, observed using light microscopy. B) A representation of what to expect when using fluorescent microscopy to measure the level of fluorescence resulting from labelled dextran present within the sacculi

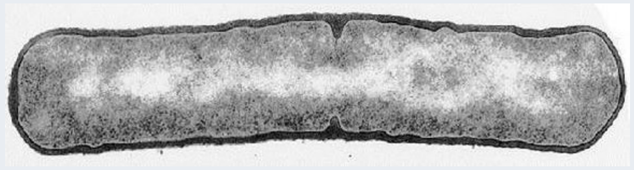


fig. 4 A Gram positive cell imaged using electron microscopy. It is predicted that the *B. subtilis* sacculi used in this experiment would appear identical to this, with the exception of having all cellular contents removed.

Monitoring dextran diffusion:

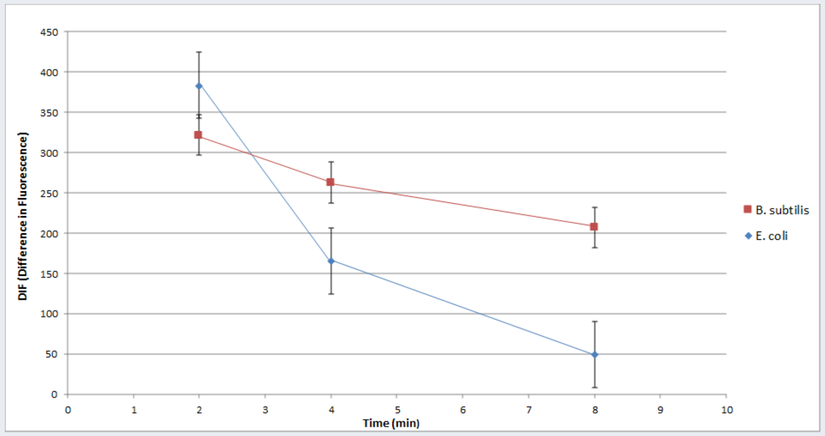
After allowing the 70KDa dextran to diffuse into the *E. coli* and *B. subtilis* sacculi. The dextran diffused sacculi were then suspended in a large volume of water from which a small sample was taken at set intervals of; 2 minutes, 4 minutes and 8 minutes.

These small samples were centrifuged and had their resulting supernatant discarded. The remaining pellet was then suspended in 5µl water and placed on top of an agarose bed set on top of a microscope slide. Fluorescent microscopy was then used for the samples of each time interval in order to locate and detect the level of fluorescence within the sacculi.

Using the microscopes software, a quantitative value was obtained for the fluorescence of the 'sacculi interior' and the fluorescence of the 'background'. The value obtained for the 'background' was subtracted from that obtained for the 'sacculi interior' which gave the Difference In Fluorescence (DIF) value for the sacculi. The mean DIF for the sacculi at each interval was then calculated.

Results

The change in the mean DIF values for *'70KDa dextran diffused sacculi' of B. subtilis* and *E. coli* over time



Conclusions

- It is possible to harvest intact sacculi from bacteria. These sacculi can then be used to assess the permeability of a given bacteria's cell wall in relation to the cell walls of bacteria from a different strain/species
- The relative permeability of a given cell wall can be determined, both by the rate at which a fluorescent molecule diffuses through the wall. And by what size fluorescent molecule is able to diffuse through the wall
- The cell wall of *B. subtilis* is less permeable than that of *E. coli*. The lower permeability of the *B. subtilis* cell wall compared to the wall of *E. coli* may be due to the cell wall of *B. subtilis* being roughly twenty times thicker than the wall of *E. coli*
- Our findings on the porosity of *E. coli* compared to *B. subtilis* differed to the findings of Koch and Demchick (1996)

References

1. Silhavy TJ, Kahne D, Walker S (2010) "The bacteria cell envelope" Cold Spring Harbor perspectives in biology **2**(5):a000414
2. Koch AL, Demchick P (1996) "The permeability of the wall fabric of *Escherichia coli* and *Bacillus subtilis*" Journal of bacteriology **178**(3):768-73
3. Pharmacosmos (2013) "Physical Properties of Dextran". Available at http://www.dextran.net/about-dextran/dextran-chemistry/physical-properties.aspx . Last accessed on 17/10/2013