

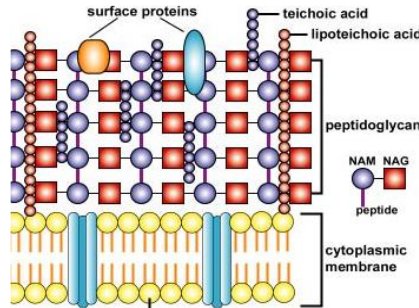
Bacterial Recycling of D-alanine during *Bacillus subtilis* Peptidoglycan synthesis

Fiyinfoluwa Babatunde, Supervisor: Richard Daniel
Centre for Bacterial Cell Biology, Newcastle University



Introduction

- ❖ *Bacillus Subtilis* cell wall consists of peptidoglycan monomers.
- ❖ This monomer consists of N-acetyl glucosamine (NAG) and N-acetyl muramic acid (NAM) with a peptide attached.
- ❖ Cleavage of D-alanine (a peptide component) is important for assembly of the cell wall and also occurs as part of cell wall maturation.
- ❖ However no evidence of D-alanine in culture medium leading to hypothesis that a reuptake system is in place.



AIMS

- ❖ Analyse the specificity of the uptake system
- ❖ Genetically identify the D-alanine transporter

Method

Synthetic Lethal strain

- ❖ AD1 was transformed with plasmid pLOSS AlrA to give strain AD2 and plated out on Kanamycin, X-gal and D alanine plates (KXD).

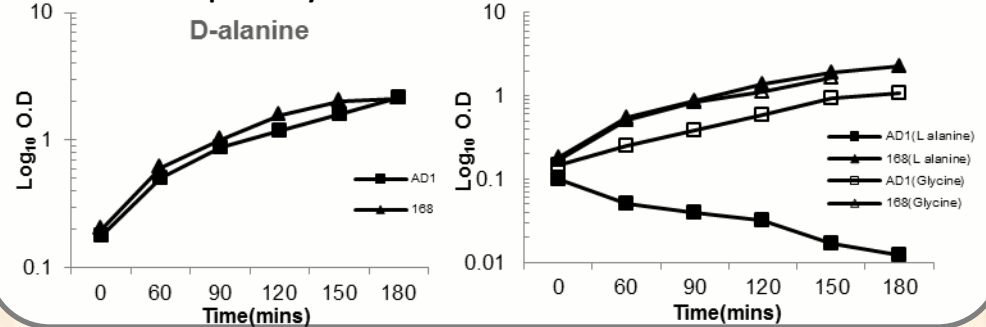
Disruption of genes involved in amino-acid uptake

- ❖ GlnH, TcyJ, TcyA, YcbK, YxeM and ArtP are proteins implicated in amino-acid uptake, the corresponding genes were disrupted.
- ❖ Amplification of the gene segment by Polymerase chain reaction (PCR) and ligation to a zeocin resistance cassette
- ❖ Transformation of *B. Subtilis* with resulting DNA
- ❖ Transformation of transformants with Ptsxc2 to recombine out the plasmid.

Specificity of the Uptake system

- ❖ A D-alanine dependent strain (AD1) and wild-type (WT) was used.
- ❖ An overnight culture of AD1 with 0.2 µg/ml D alanine and WT was set up.
- ❖ Amino-acids were added to test tubes to a final concentration of 0.6µg/ml.
- ❖ Test tubes were then inoculated using the growing culture prepared earlier. The O.D was taken every half hour.

Results: Specificity of D-alanine



Conclusion

- ❖ The results showed that transporter is fairly specific for alanine as even closely related amino acids do not seem to compete for transport.
- ❖ The number of blue colonies to screen through was about 10 times the number of white colonies seen on the plates making it impossible to find the stable blue colony. This suggested that either there are multiple transporter systems involved or that the specific transporter was essential for normal growth.

Reference:

Osborne SE, Tuinema BR, Mok MC, Lau PS, Bui NK, Tomljenovic-Berube AM, Vollmer W, Zhang K, Junop M, Coombes BK. 2012. Characterization of DalS, an ATP-binding cassette transporter for D-alanine, and its role in pathogenesis in *Salmonella enterica*. *J Biol Chem*. 4;287(19):15242-50
Kaiser, G. (2005) Doc Kaiser's Microbiology. Available at:
<http://faculty.ccbcmd.edu/courses/bio141/labmanua/lab16/diseases/mtuberculosis/u1fig11.html> (Accessed 13/11/2012).