ABC transporters with a lipoyl-domain are regulated by pneumococcal DLDH

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Introduction

Streptococcus pneumoniae (the pneumococcus) is one of the main causes of respiratory tract infections worldwide. Our earlier studies have shown that pneumococci require the regulatory enzyme dihydrolipoamide dehydrogenase (DLDH) to survive in the host. In pneumococci, DLDH does not act in its usual role as the E3 component of 2-oxo acid dehydrogenases. Instead it is required for full import of certain substrates, such as galactose and raffinose, through ATP-binding cassette (ABC) transporters. In this study we have further characterized the transport events that are potentially regulated by DLDH.

What affect does DLDH have on the virulence of *Streptococcus pneumoniae*?

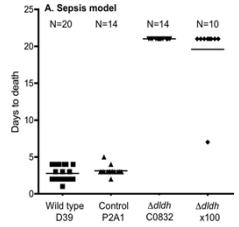

Figure 1. Virulence-attenuation of DLDH-negative *S. pneumoniae*.
Mice were injected in the tail-vein with 300 colony forming units (CFUs) of wild type D39 pneumococci, a mutant with an insertion of the mutagenesis-plasmid downstream of *dldh* (P2A1, control mutant), or the *dldh*-mutant (C0832:1). x100 were injected with 30,000 CFUs. The mice were monitored for survival and the day of death was recorded.

DLDH-negative bacteria were unable to infect the host, and were quickly cleared, suggesting that the DLDH enzyme is required for full virulence.

Which sugars does DLDH play a role in transporting into the *S. pneumoniae* cell?

Carbohydrate	Growth D39	Growth D39Δdldh
Glucose	+++	+++
Maltose	+++	+++
Galactose	+++	-
Raffinose	+++	+
Lactose	+++	+++

Figure 2. Growth determination of wild-type and DLDH-negative pneumococcal strains on different carbon sources.
Growth using the above carbon sources as sole carbon sources was recorded as (–) no detectable growth, (+) growth between OD600 0.1 and 0.3 after 72 h, (++) growth between 0.3 and 0.6 within 48 h, (+++) growth ≥ 0.6 within 48 h.
The D39Δdldh mutant showed a growth deficiency in the presence of galactose or raffinose as sole carbon sources, suggesting that the Mgl (galactose) and Msm/Raf (raffinose) ABC transporters were regulated by DLDH. Bioinformatics analyses indicated that these transporters contain either a lipoyl motif or a lipoyl domain, the substrate for DLDH. We have shown that DLDH binds directly to the lipoyl motif of each ATP-binding protein

What other ATP binding cassette (ABC) transporters carry a lipoyl domain/motif?

ORF (putative function)	Start (aa)	Lipoyl-motif*	Mismatch
		N A WD C A VQ F F IV S M F M MY AI F F N V V VF TA V N V D I H I ID ST I S I	
SP0846 (mglA, Galactose-ATP BP)	464	DEILNVSDRIAVIHDGKIQGIVSPETTNKq	1
SP2171 (adcC, zink ATP BP)	31	fVTLTGENGAAKtTLIKASLGILQPRIGKV	2

* The lipoyl-binding motif as reported by Prosite (PDOC00168). Non-capital letters in red indicate mismatch.

Figure 3A. Pneumococcal ABC transport proteins with possible lipoyl-binding sites.
A lipoyl motif is present in the AdcC (zinc transporter) open reading frame, suggesting that DLDH may be responsible for zinc transport

MalK of *E. coli*

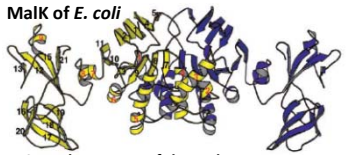

Figure 3B. Crystal structure of the maltose ATP transport protein MalK from *E. coli* (adapted from Bohm et al 2002; 277: 3708) showing a model of the central ATPase core and the regulatory domain that includes a domain with lipoyl-like fold of the "dihydrolipoamide acetyltransferase" superfamily (CATH 2.40.50.100, http://www.cathdb.org/), the substrate for DLDH.

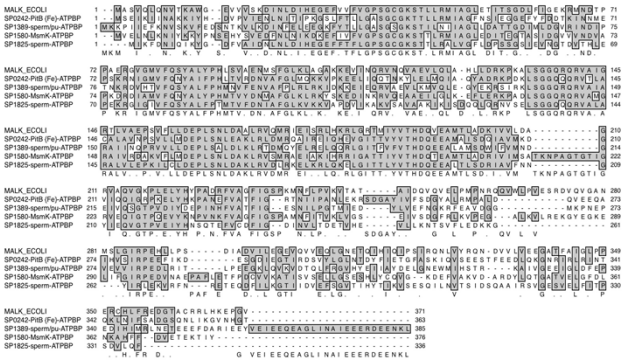

Figure 3C. Formatted sequence alignment of lipoyl-domain carrying ATP binding proteins in *Streptococcus pneumoniae* genome from strain TIGR4 compared to the maltose ATP-binding protein MalK from Escherichia coli.
Boxed and highlighted amino acids are similar or identical in a majority of the sequences.

Besides MsmK, the iron transport protein PitB, the polyamine transport protein PotD and the uncharacterized transport protein SP1825 carry a domain with a lipoyl-like fold, to which DLDH can potentially bind.

Are PotD, PitB and AdcC transporters regulated by DLDH?

What effect does zinc deficient media have on D39ΔDLDH growth?

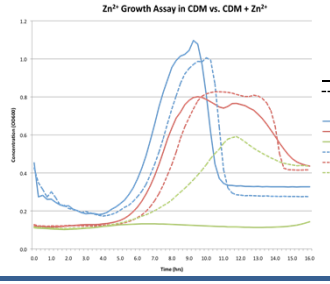

Figure 4. Zn^{2+} Growth Assay in CDM with and without added Zn^{2+}
Bacterial growth was compared between synthetic media that is zinc deficient (solid line) and the same media with zinc added (hatched line). Growth of wild type D39 pneumococci was compared with D39 Δdldh and CT21 (AdcC-negative mutant) by following the optical density of the suspension over time.

D39 grew equally well in both media. The AdcC-negative mutant did not grow in the synthetic media unless zinc was added. The DLDH mutant grew equally well as D39, indicating that DLDH does not play a role in zinc transport and therefore does not regulate AdcC.

Does DLDH regulate iron transport?

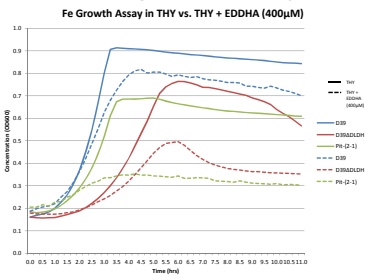

Figure 5. Fe^{3+} Growth Assay in THY vs. THY + EDDHA (400μM)
Bacterial strains (D39, D39 Δdldh, and Pit-(2-1), lacking the expression of PitB) were grown to 0.6 OD in THY. The cells were centrifuged, washed with PBS and resuspended in THY without (solid line) or with (hatched line) the addition of the iron cheloator EDDHA. Cell growth was measured by following the optical density of the suspension over time.

D39 pneumococci grew equally well in both media. The PitB negative mutant showed a drastically decreased growth in the presence of EDDHA. The DLDH-mutant strain showed a similar reduced growth as the PitB mutant in the presence of EDDHA. This data suggests that DLDH does play a role in iron transport by potentially regulating PitB.

Does DLDH play a role in polyamine uptake?

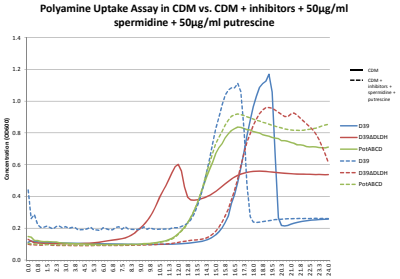

Figure 6A. Polyamine growth assay
Bacterial strains (D39, D39 Δdldh, and TIGR4 ΔPotABCD) were grown either in CDM (solid line) or in CDM containing inhibitors of polyamine biosynthesis as well as the polyamines spermidine and putrescine. Cell growth was measured by following the optical density of the suspension over time.

There was no clear phenotype seen in this experiment, even in the strain lacking the polyamine transporter. This led us to pursue radioactive data to get more definitive results.

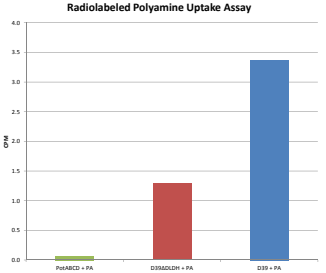

Figure 6B. Radiolabeled Polyamine Uptake Assay
Bacterial strains (D39, D39 Δdldh, and TIGR4 ΔPotABCD) were grown to an OD600 of 0.6 OD in THY. The cells were centrifuged, washed with PBS and resuspended in PBS with radiolabeled spermidine and putrescine added. After a 1 hour incubation period, the cells were filtered through .45μm filters. Radioactivity of the bacteria captured on the filter was measured using liquid scintillation counter.

The PotABCD mutant, lacking the polyamine transporter, showed no polyamine uptake. The DLDH mutant showed uptake 2.5 times smaller than the wild type D39, suggesting that DLDH does regulate polyamine transport and PotD.

Conclusions

- ***DLDH regulates galactose and raffinose transport through a direct binding of the protein to a lipoyl-motif/domain on the ATP-binding protein of the respective transporters.***
- ***Four additional transporters were shown to carry similar domains.***
- ***We have collected data that suggests that DLDH regulates both iron and polyamine transport, but likely not zinc transport.***
- ***Future studies will be conducted to specifically characterize the interaction of DLDH with the PitB iron transporter and the PotD polyamine transporter as well as with the SP1825 ORF, that is as of yet uncharacterized.***