GENE ASSOCIATION ANALYSIS

Details of relevant TFs:

**ColR** – Part of the ColR/S two component system, ColR is the seemingly positive regulator of genes involving metal ion homeostasis and membrane functionality, ColS is the sensor that somehow detects metal ions (mainly Zinc, lesser degree Fe, Mn, Cd) and activates ColR[1, 2].

**LexA** – Negative regulator likely acting via dimerization. Constantly expressed and active – deactivated by autocatalytic cleavage induced by RecA in response to DNA damage. When active, represses genes for DNA repair, error checking, etc[3, 4].

**AmrZ** – A.K.A AlgZ, performs both positive and negative regulation in Aeruginosa, upregulates genes for alginate production and twitching motility whilst downregulating flagella production. Seemingly constantly active when expressed – expression mediated by Sigma factor AlgT which is sequestered by mucA when alginate content exceeds an unspecified amount (seemingly when the alginate content of the *capsule* exceeds x amount), when alginate drops, AlgT is freed and AmrZ expressed[5-7].

**Ptxs** – Transcriptional repressor the seems constantly active, bound to a region overlapping the RNA pol binding site until 2KGA binds to it, causing dissociation. Commonly found as part of gad and kgu operon regulation as these operons promote 2KGA uptake and production from gluconic acid, thus ptxs acts as a “switch” for 2KGA metabolism[8, 9].

**LasR** – Transcriptional activator of las genes (virulence associated proteases) activated by P.Aeruginosa autoinducer “OdDHL”, a molecule involved in quorum sensing; LasR genes seemingly activate when population density exceeds “x” amount[10, 11].

**Fur** – Transcriptional repressor; Upon Fe2+ binding, dimerises and activates to prevent transcription of Fe2+ uptake genes. Also indirectly promotes expression of non-essential iron storage/binding molecules and ROS-detoxifying enzymes (Fe2+ catalyses ROS production) by repressing expression of relevant small RNAs[12].

**MexT** – Transcriptional activator of MexEF-Oprn drug efflux system(confers AB resistance and repress quorum sensing/virulence) among other genes. Seemingly activated by oxidation inducing oligomerization[13, 14].

**Anr** – Transcriptional activator of various genes revolving around anaerobic cell function (e.g fermentation, denitrification, redox homeostasis, oxidative stress resistance…) and *possibly* a repressor of “cio” genes. Activated by low aqueous O2 (4Fe-4S and/or 2Fe-2S bind O2 at high O2 conc, when O2 drops it instead binds Anr, enabling dimerization and activation)[15].

**VqsM** – Transcriptional activator of >203 Aeruginosa genes, >50% being involved in quorum sensing. Unclear/unspecified activation condition[16]. Potential autoinducer regulated given involvement in quorum sensing?

**PtxR** – Transcriptional activator of genes involved in virulence and quorum sensing. Seems constitutively active and regulated through expression which is likely regulated by PvdS(active in iron starvation) and Vfr[17, 18].

**PcaR** – Transcriptional activator AND repressor – induces expression of the pca group of genes of the B-ketoadipate pathway, represses own expression. Unclear activation mechanism – potentially just always active and expressed just represses itself when concentration gets too high?[19].

**CueR** – Transcriptional activator of genes involving Cu2+ homeostasis, seemingly focused on lowering cytoplasmic Cu2+ in response to an excess by upregulating efflux and Cu2+ sequestering proteins. Activated by Cu2+ binding. Exists as homodimer bound to DNA, seemingly regardless of if Cu2+ bound or not[20].

**ArgR** – Transcriptional activator AND repressor, though seems to vary significantly between species. In P.Aeruginosa it acts as both and is activated by Arginine binding, serving to repress genes involved in Arginine biosynthesis whilst simultaneously upregulating Arginine catabolism genes, essentially serving as a negative feedback system for cell arginine concentration[21-24].

**Zur** – Transcriptional repressor of genes involving Zinc influx. Activates upon zinc binding inducing dimerization. Potentially an activator of genes in certain species and may indirectly promote zinc efflux via the czcRS system through some mechanism[25].

SIDE-NOTE: A lot more research exists for Aeruginosa vs Putida. Given that TF function should be highly conserved and these are species of the same genus, the research on Aeruginosa TFs will be assumed valid for Putida TFs. Likewise for gene function if papers on Putida cant be found.

fecA

Cluster\_109793 ColR 116 62.931 8.333

Cluster\_82679 LexA 116 4.31 8.333

fecA is an Fe2+ dicitrate transporter found in the outer membrane of various species including P.Aeruginosa. It is involved in Iron uptake during periods of iron deprivation, transporting it in the form of iron dicitrate seemingly due to the fact citrate readily chelates around iron[26].

*Hypothesis: ColR is a positive regulator and known to be activated by Fe, if to a lesser extent, so it makes sense to have it as a regulator of fecA given it should be expressed to prevent iron deficiency, and presumably a countermeasure to ColR prevents over-uptake of iron. LexA is a repressor activated by DNA damage among other factors. It could be that the species where fecA falls under the LexA regulon exists in a high iron environment thus preventing iron uptake is more crucial than promoting it most of the time. Alternatively since Fe2+ is a known catalyst for ROS production [12] its possible that fecA falling under LexA regulon is so that if ROS induced DNA damage occurs, further Fe2+ uptake is suppressed by repressing fecA expression thus limiting further DNA damage.*

czcR\_3

Cluster\_66703 ColR 182 17.033 1.613

Cluster\_68396 CueR 182 0.549 1.613

czcR\_3 is the effector component of a two component system for metal ion resistance – when the corresponding czcRS detects high concentrations of ions like Zn2+, Cu2+,Co2+ and Cd2+ it phosphorylates and activates czcR\_3 allowing it to promote expression of genes for the efflux pump czcCBA that exports said ions[27, 28].

*Hypothesis: Both ColR and CueR perform positive regulation with the only major difference being the cause of activation, with ColR being activated in response to Zn2+(and Fe/Mn/Cd to lesser extent) whilst CueR responds to Cu2+. All of these are ions that the czcCBA pump exports and czcR\_3 is activated in response to. This idea is purely a theory with weak basis, but its possible that czcR\_3 falls under the ColR regulon in a strain where Cu2+ is ubiquitous thus poses major risk to the cell whilst it falls under the CueR regulon where Cu2+ is more scarce so as to avoid Cu2+ being exported from the cell in response to Zn/Fe/Co/Cd like it would’ve been under ColR regulation. However this raises the issue of how the metal ions are handled in the czcR\_3 CueR regulon strain given the cell would only respond if Cu2+ was high, ignoring if any of the other ions were high.*

fumC\_1

Cluster\_44208 Fur 204 16.176 2.174

Cluster\_80552 AmrZ 204 26.961 2.174

fumC\_1 encodes fumarase, a component of the TCA cycle which converts fumerate to malate using H2O. For an unclear reason, fumC\_1 is upregulated in P.Aeruginosa cells under iron deprivation, a paper hypothesised that upregulating fumC may be to increase energy production for alginate production which seems to promote iron uptake but no conclusion could be reached[29].

*Hypothesis: Fur is a repressor, AmrZ is a repressor and activator. Fur represses in response to Fe2+ whilst AmrZ in response to excessive alginate capsule content. Both conditions are major factors in the paper describing fumC, Fur seems to line up with what the paper said of iron deprivation promoting fumC expression given that if iron is high concentration then Fur would be actively repressing fumC\_1 in our data. Alginate induced repression of fumC\_1 may simply be an alternative mode of regulation without advantage or disadvantage but this seems unlikely. Perhaps the Fur fumC\_1 regulon occurs in a strain where Fe2+ concentrations are low or fluctuating, whereas the AmrZ fumC\_1 regulon occurs in a strain where Fe2+ is constantly high or low thus cannot be used as a regulator of alginate production so the cell instead relies on measuring the alginate content itself. The issues with these hypotheses is we don’t know why fumC expression is reliant on Fe2+ concentrations in the first place, and I do not know how alginate production is terminated in Fur fumC regulon cells if Fe2+ were to remain constantly low concentration.*

oprD\_6

Cluster\_4421 ArgR 213 7.042 0.98

Cluster\_11358 ColR 213 0.469 0.98

Cluster\_97363 Zur 213 0.469 0.98

oprD\_6 encodes an outer membrane porin(or subunit thereof) called oprD whose endogenous function is to act as a channel for basic amino acids and select small peptides. However it is also a means for antibiotics like carbapenem to enter the cell – downregulation or select mutations to oprD confers resistance to such antibiotics[30].

*Hypothesis: ArgR and Zur are repressors whilst ColR seems to be an activator. ArgR and Zur differ in activation requirements – the former activated by binding Arginine, the latter by binding Zinc. ColR is activated by ColS in presence of Zn and other ions. Given that oprD is an antibiotic means of entry, it could be that oprD falls under the ColR regulon in a strain not exposed to such antibiotics thus didn’t adapt or has a means of adaptation outside of differing transcription factor regulation. It makes sense that it falls under the ArgR regulon given that arginine is a basic amino acid, which oprD would transport, therefore once sufficient Arginine is obtained oprD would be downregulated until further arginine is needed. I’m uncertain of the reason it falls under the Zur regulon, its possible that zinc could enter the cell through oprD, given its small size and positive charge, and could therefore serve as a means of negative feedback on oprD expression. Zur could also serve as a “more strict” form of repression compared to ArgR as due to its small size and alternate routes of entry, zinc will enter the cell more rapidly than arginine and possibly reach higher concentrations, resulting in longer periods of oprD repression that could serve as an antibiotic resistance mechanism, though this reasoning seems unlikely.*

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