Pseudomonas aeruginosa virulence analyzed in a *Dictyostelium discoideum* model of infection

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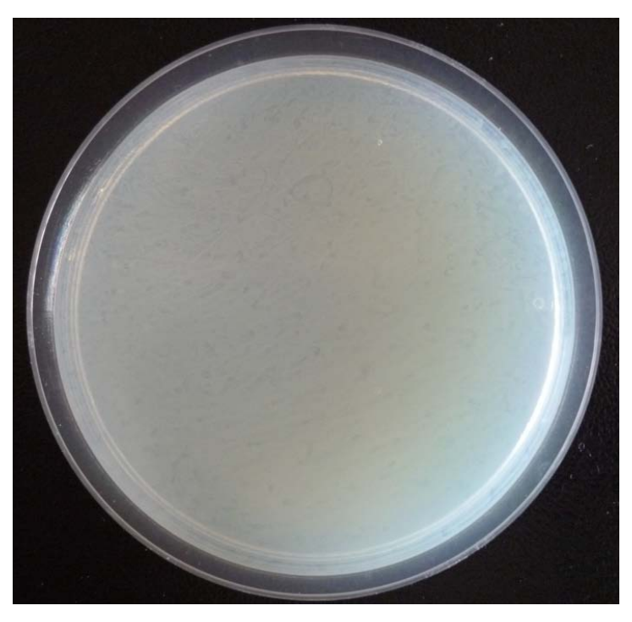
Introduction

Pseudomonas aeruginosa is a major opportunistic human pathogen which produces a large variety of secreted and cell-associated virulence factors. Since *P. aeruginosa* infections are difficult to treat due to the emergence of highly antibiotic resistant strains, alternative drug targets including virulence factors are currently being under investigation. Recently, it has been shown that *P. aeruginosa* uses similar virulence factors when infecting mammalian systems or non-mammalian hosts like the social amoeba *Dictyostelium discoideum*, the nematode *Caenorhabditis elegans* or the fruit fly *Drosophila melanogaster* (Hilbi et al., 2007; Alibaud et al., 2008).

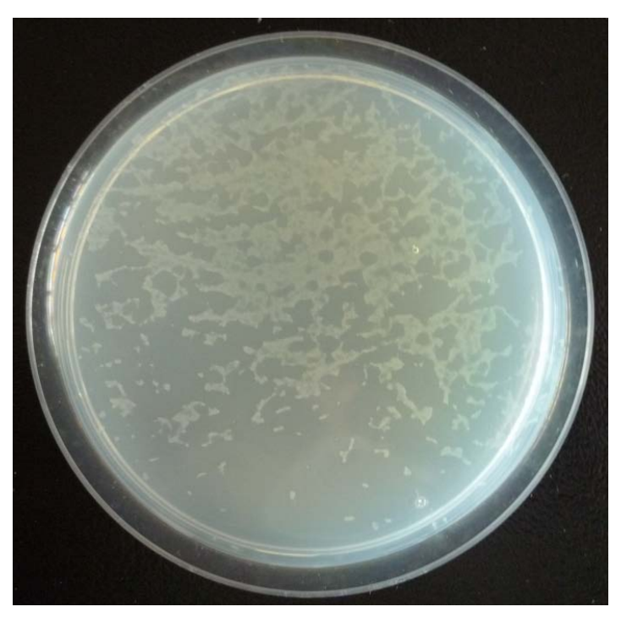
In this study, we used a comprehensive *P. aeruginosa* PA14 transposon mutant library (Liberati et al., 2006) to screen for mutants with reduced virulence. Furthermore, microarray analyses were performed to gain a deper insight into the interaction of *P. aeruginosa* with *D. discoideum*.

Microarray analysis of *P. aeruginosa* PA14 – *D. discoideum* interaction

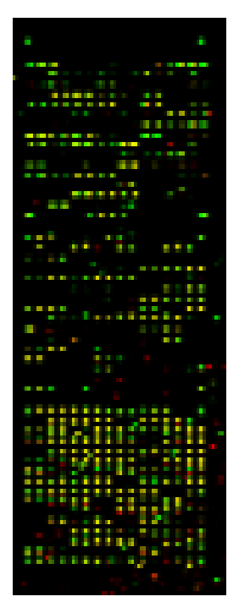
P. aeruginosa PA14



P. aeruginosa PA14 - *D. discoideum*



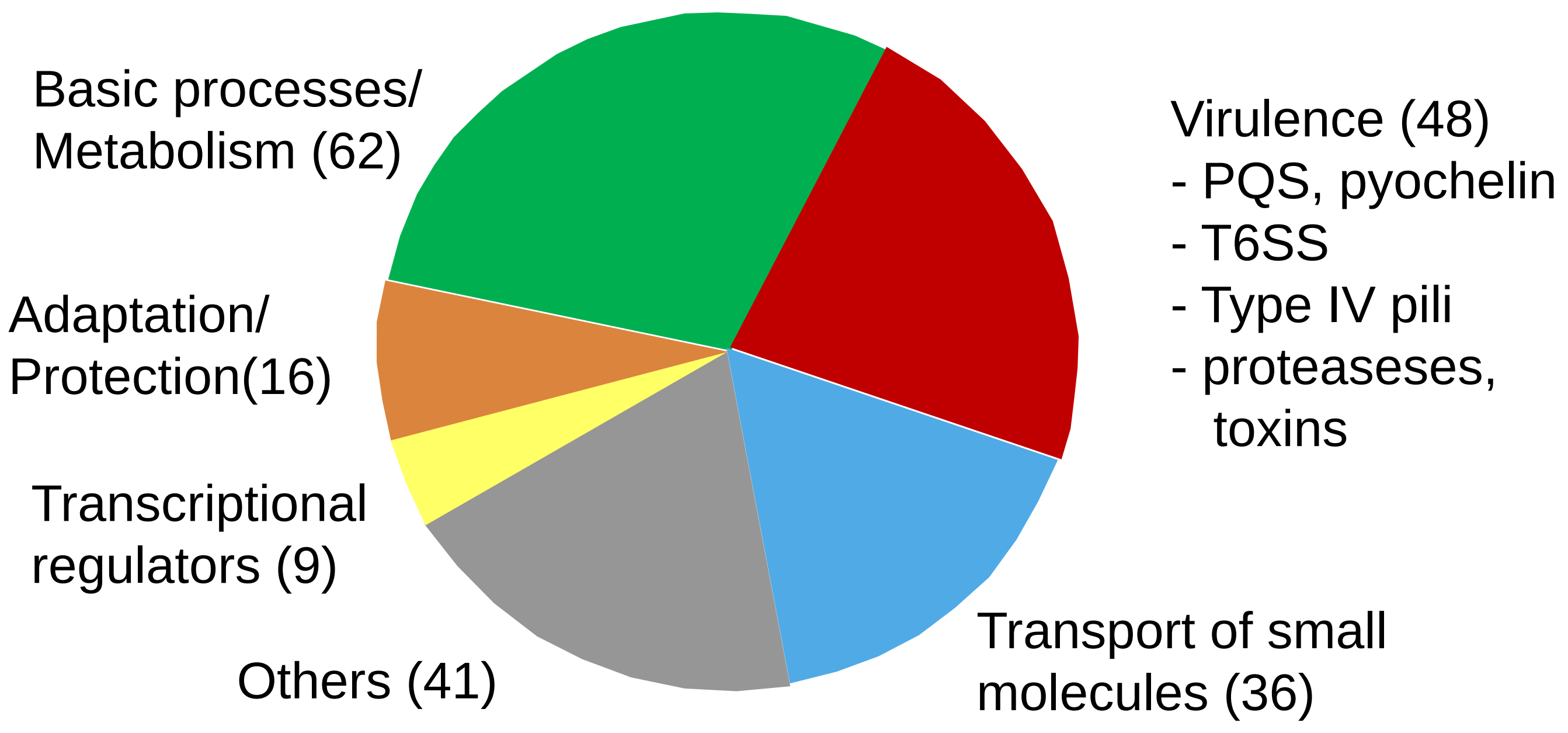
- M9 medium
- 40 hours
- 23°C



505 Genes up- or down-regulated (>3fold)

- 317 up (~105 hypothetical)
- 188 down (~ 97 hypothetical)

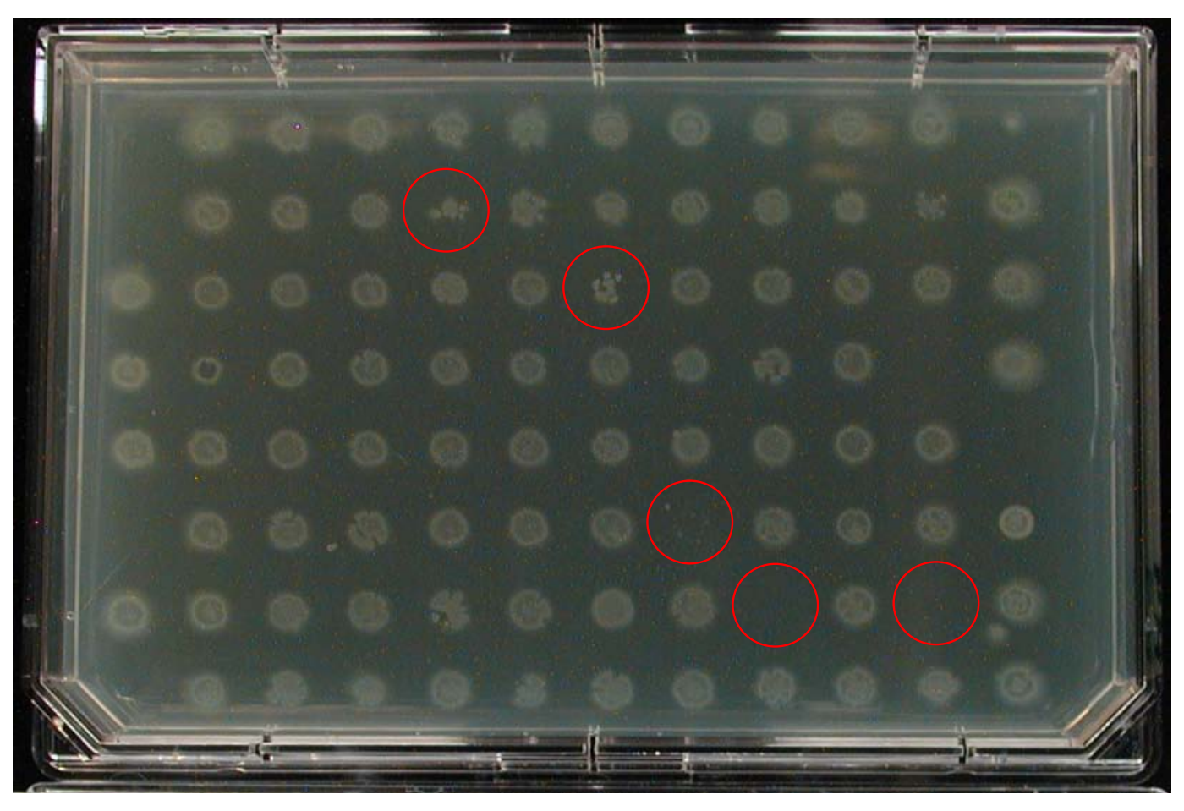
Genes up-regulated in response to *D. discoideum*



Global screening of a *P. aeruginosa* PA14 mutant library for decreased virulence

Preliminary screening

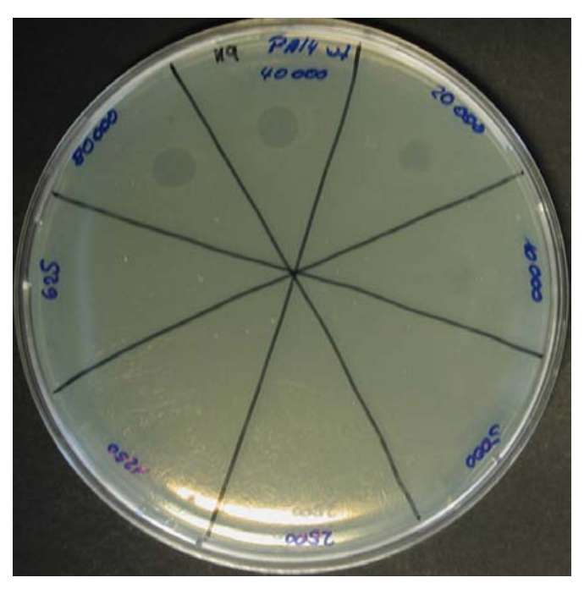
~ 6500 *Tn*-mutants
(M9-medium, 4 days)



Identification of mutants with
altered virulence phenotype

Verification

wildtype

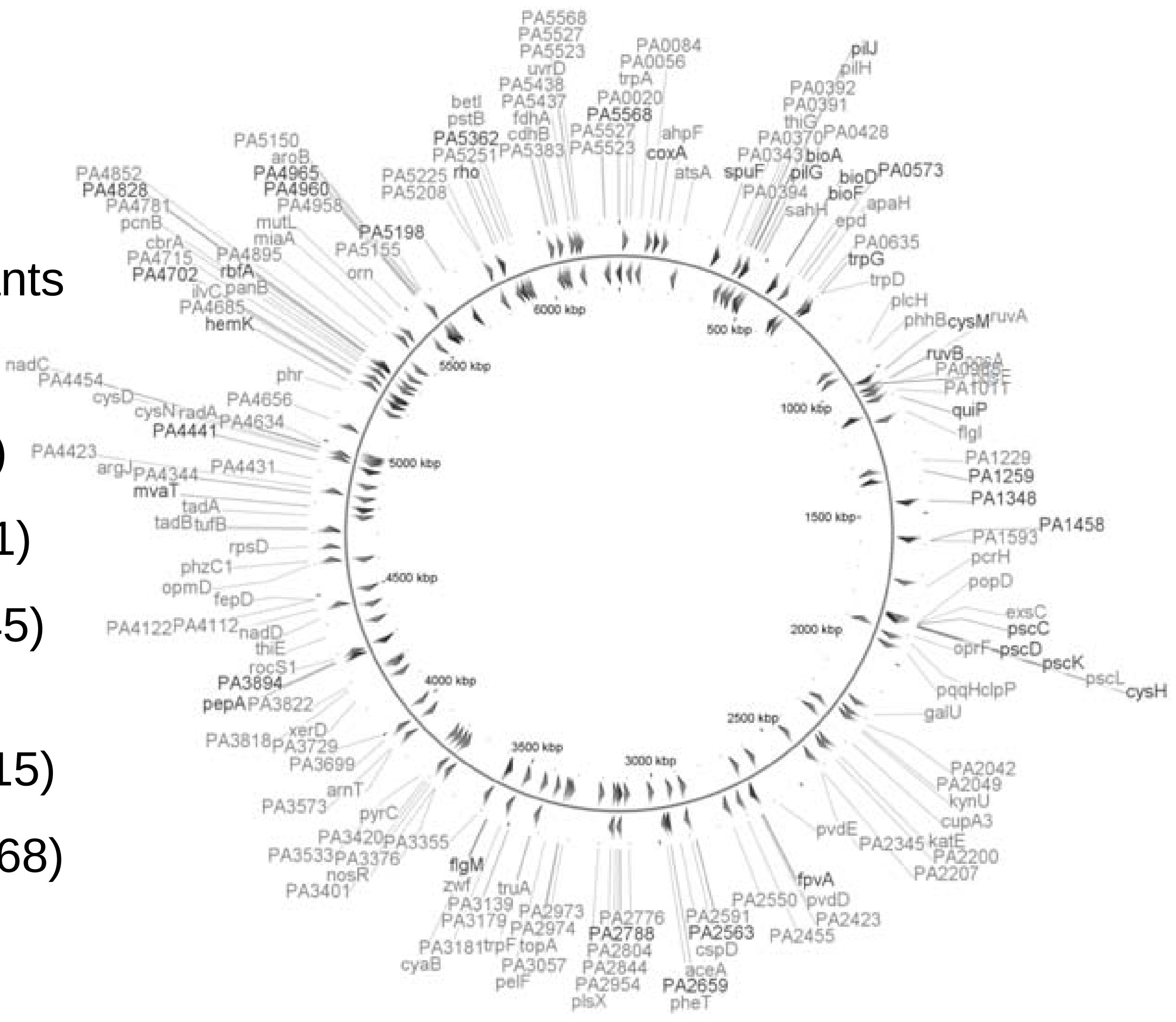


Tn-mutant

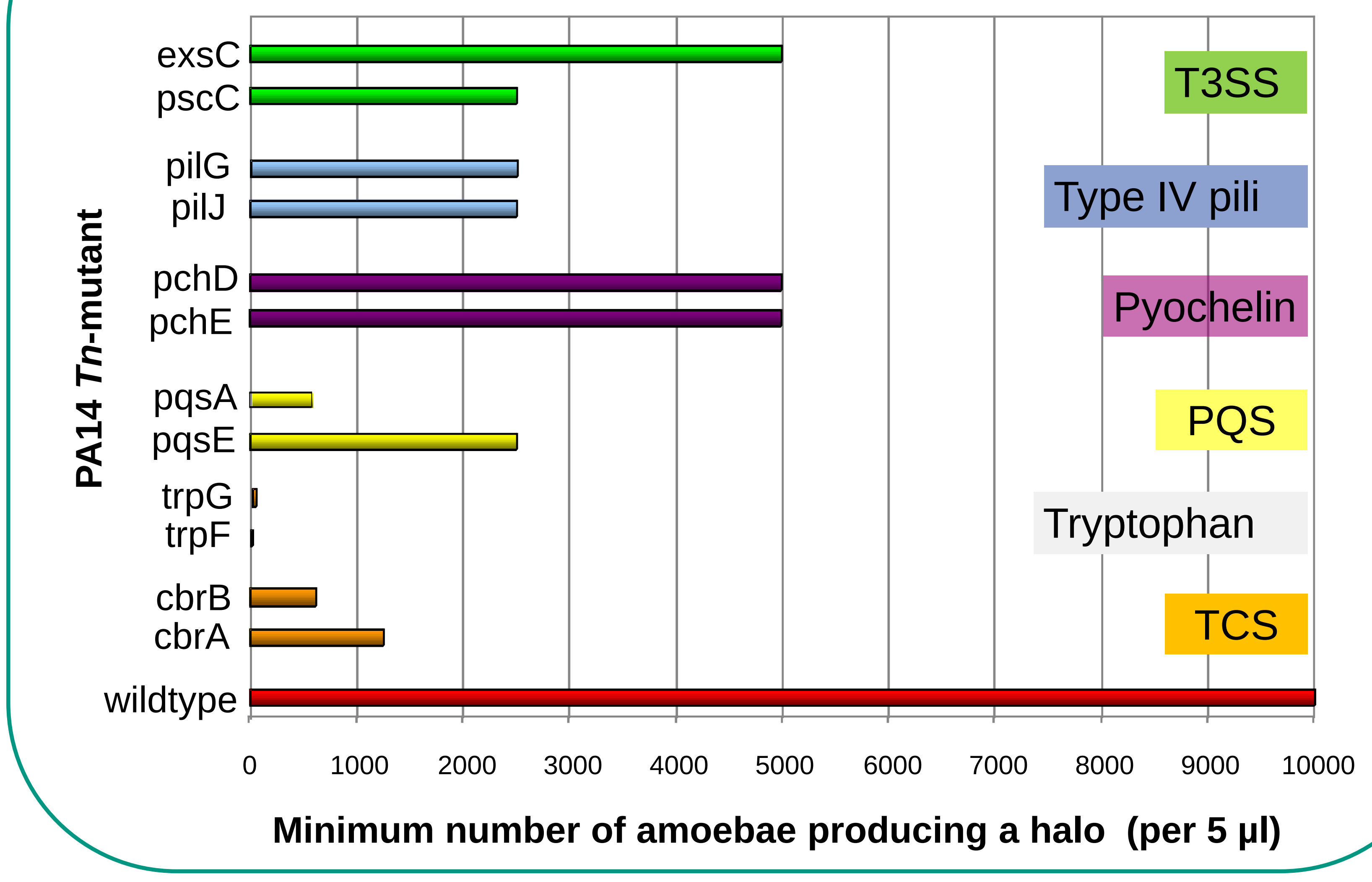
Transposon mutants with decreased virulence

Summary

- ~200 Tn-mutants
- T3SS (9)
- Type IV pili (7)
- Regulators (21)
- Metabolism (45)
- Secondary metabolites (15)
- Hypothetical (68)



Virulence of selected PA14 mutants



Summary

- Microarray analysis revealed ~500 genes differentially regulated in response to *D. discoideum*.
- Various virulence related genes including secondary metabolites, T6SS, Type IV pili and extracellular proteases and toxins were highly up-regulated.
- Type IV pili, secondary metabolites (e.g. PQS or pyochelin) and the two-component system cbrAB which is involved in the regulation of nitrogen/ carbon metabolism are crucial factors for *P. aeruginosa* to circumvent protozoan grazing.

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