Multidrug and efflux transporters of the model microbe *Dictyostelium discoideum*

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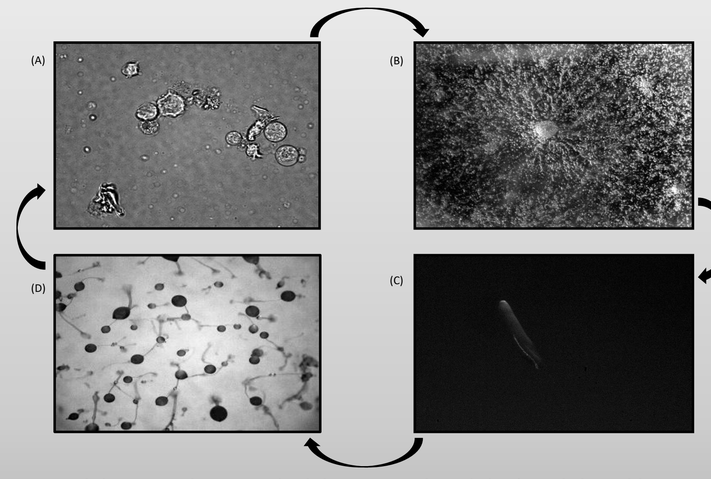
Introduction

Dictyostelium discoideum is a social amoeba predominantly found in the soil and woodland floor, where it engulfs bacterial prey in its unicellular life-stage. That ability to phagocytose other cells, and its aggregation and differentiation in its multicellular life cycle stage, make it an ideal biomedical model microbe. Its genome revealed many orthologues of genes of higher eukaryotes and thus it has been used in many studies of basic biology and disease, including:

- Cell differentiation
- Cell motility
- Chemotaxis
- Phagocytosis



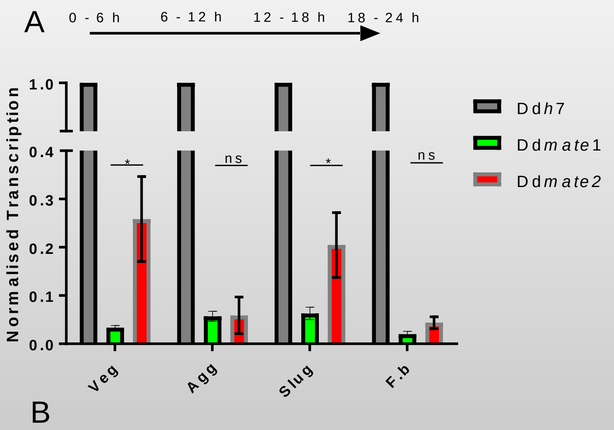
(A) Vegetative growth; (B) Aggregation; (C) Slug; (D) Fruiting body

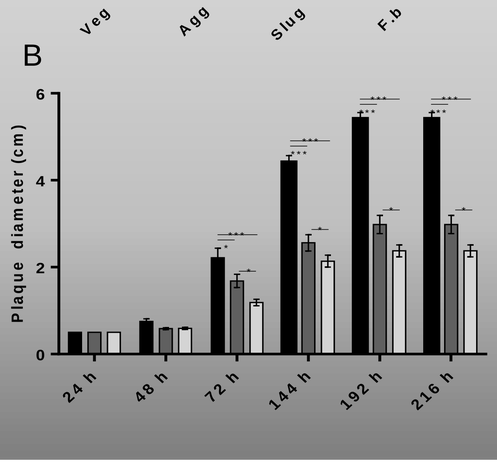
The evolutionary conserved MATE proteins were identified here in *D. discoideum,* whose role as a model microbe for development and as a preclinical vehicle for drug transport, put it at the centre of the interesting dichotomy between transports' biological activity and toxin efflux. Whereas bacterial MATEs mediate antibiotic resistance, in plants examples such as *A. thaliana* TT12 sequester flavonoids in seedcoat endothelium that alters seed dormancy (Marinova *et al.,* 2007), or JAT-1 of *N. tabacum* transports the alkaloid nicotine for its role in plant defence (Morita *et al.,* 2009).

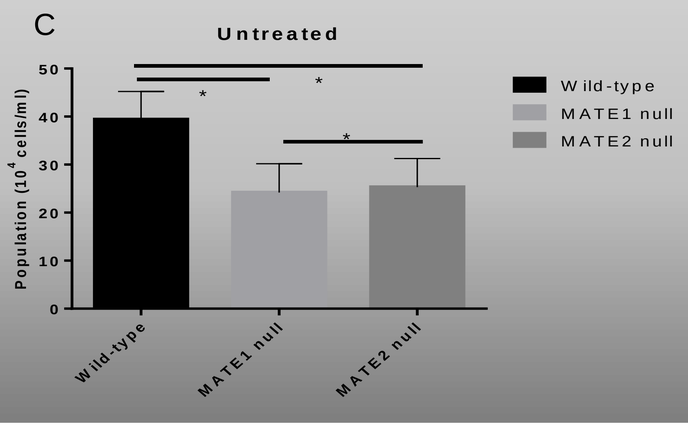
Flavonoids are polyphenolic compounds, found in many of the plant-derived products we eat and drink, whose many physiological and developmental effects suggest they could be useful therapeutics. Biological evidence at a cellular level is gradually being published including work using *Dictyostelium* (Waheed *et al.,* 2014; Ferrara and Thompson, 2019) and supports the idea that some of these secondary metabolites might act on specific targets in particular diseases, improving our understanding beyond the widely cited 'anti-oxidant, anti-inflammatory, anti-proliferation and anti-cancer properties'.

These *D. discoideum* MATEs may usefully model the human MATEs, aid understanding of flavonoids' effects, and should be considered when using this model eukaryote to screen drugs.

Novel MATEs in *D. discoideum*





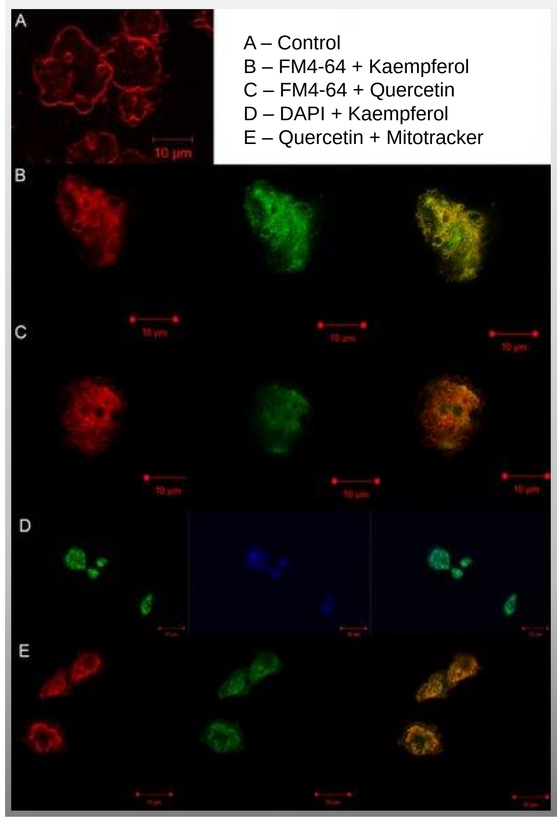


When comparing the transcription levels for Dd*mate*1 and Dd*mate*2, Dd*mate*2 transcription was significantly higher in vegetative and slug forming stages (A). The pattern of regulation was quite distinct in each, and reproducible across biological repeats. It is worthy to note; therefore, transcription suggests unique and non-redundant functions.

Phagocytosis is conserved throughout evolution including mammalian immune response (Dunn *et al.,* 2018), and is essential for *D. discoideum* survival. Notably, there is a significant reduction in plaque diameter after 48 h for both mutant lines, with a marginal different between both mutant lines (B).

Axenic growth is also markedly reduced in both mutants after 24 h (C).

Flavonoid sub-cellular localisation



A – Control
B – FM4-64 + Kaempferol
C – FM4-64 + Quercetin
D – DAPI + Kaempferol
E – Quercetin + Mitotracker

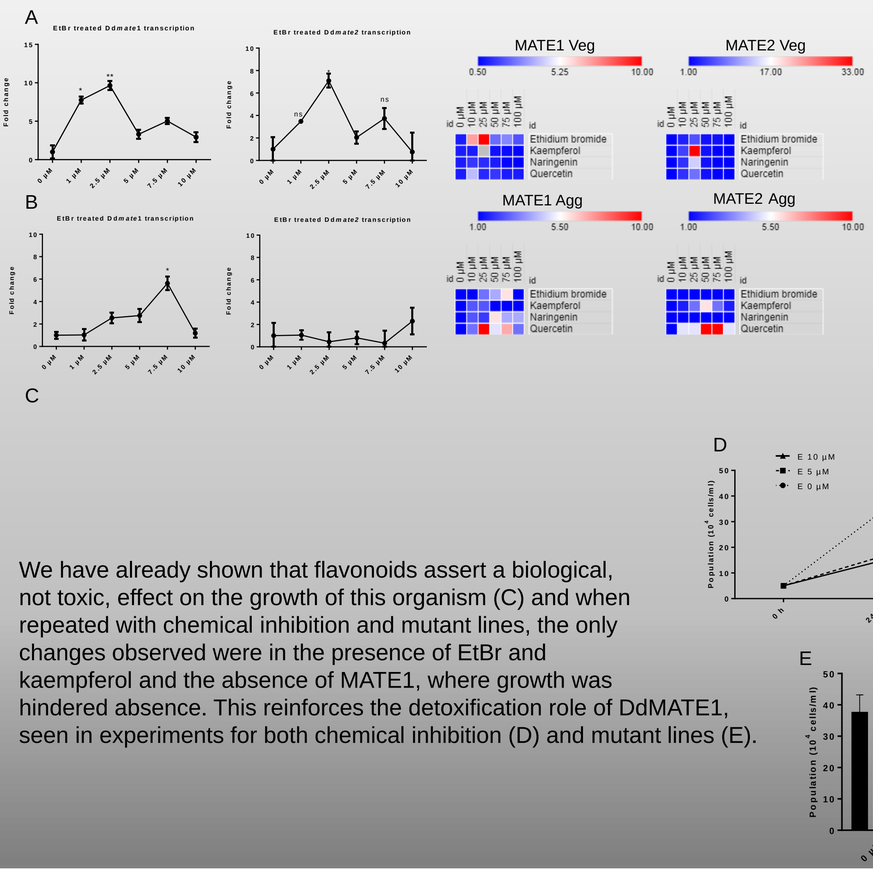
Confocal microscopy was used to determine the sub-cellular localisation of these "model drugs" in *D. discoideum* using a method previously described (Ferrara and Thompson, 2019).

FM4–64 stained the plasma membrane and the contractile vacuole. The merged kaempferol and FM4–64 staining overlapped, in agreement with high kaempferol levels confirmed by LCMS in cell extracts.

Of the flavonoids tested, no signal was detected within the nucleus (D) contrary to previous reports in cancer cell lines (Cai *et al.,* 1997; Hadi *et al.,* 2007).

MitoTracker–flavonoid colocalization was in agreement with the purported anti-oxidant properties of flavonoids (E): both were previously suggested to be cytotoxic to cancer cell lines by stimulating the mitochondria to overproduce ATP (Chen *et al.,* 2014; Sak, 2014).

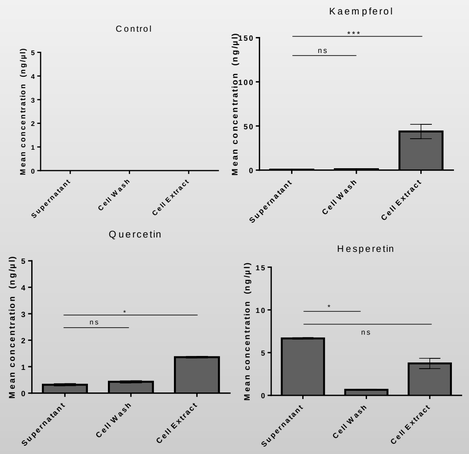
Toxin or biological response?



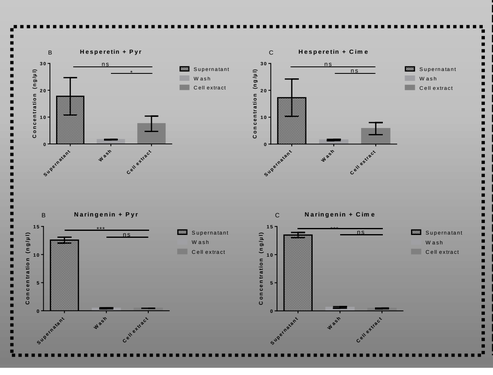
Transcriptional regulation suggested that Dd*mate*1 and 2 may be induced with EtBr. Considering that both *mates* were induced, for the hypothetical efflux of this toxin, (A) but in developing cells Dd*mate*2 showed no changes (B), this may be indicative of a detoxification role for Dd*mate*1 and something more complex for Dd*mate*2 (C).

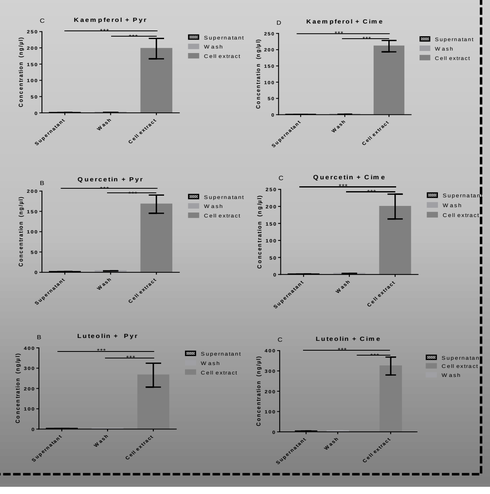
We have already shown that flavonoids assert a biological, not toxic, effect on the growth of this organism (C) and when repeated with chemical inhibition and mutant lines, the only changes observed were in the presence of EtBr and kaempferol and the absence of MATE1, where growth was hindered absence. This reinforces the detoxification role of DdMATE1, seen in experiments for both chemical inhibition (D) and mutant lines (E).

Roles in transport



If we inhibit MATE efflux, what happens to flavonoids that enter cells? we can see above that kaempferol and quercetin enter cells, whereas hesperetin and naringenin remain outside, perhaps exerting their effects at the cell surface. Upon inhibition (and in mutant lines, not shown) their internal concentrations increase significantly, Implicating both MATEs in the transport of these compounds (at least those that are internalised).





We have shown that flavonoids exert a biological effect, and aren't toxic per se. But do they enter cells? We can visualise the sub-cellular localisation of flavonoids in *D. Discoideum* (Ferrara and Thompson, 2018). We observed differential transport of flavonoids seen in plants, using LC-MS in parallel to quantify the flavonoid concentrations in and out of cells.

Summary

- *Ddmate1* and *2* are both transcribed, *Ddmate2* more so, with peaks in vegetative and slug life-cycle stages.
- Removing MATE function by inhibitor or mutation increased intracellular levels of various compounds, confirming these as efflux transporters.
- *MATE1* and *MATE2* phenotypes indicated roles beyond detoxification: on *Klebsiella* lawns these mutants produced significantly smaller plaques than WT, and their axenic growth rates were also lower.
- Increased flavonol intracellular concentrations confirmed that efflux not import was impeded in *MATE1* and *MATE2*, and kaempferol therefore further reduced *MATE1*-cells' growth.

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