Bacterial oligomers & polymers play opposite roles: MAMPs interact with each other and with host cell walls during induction of calcium signaling, which is suppressed by bacterial EPS

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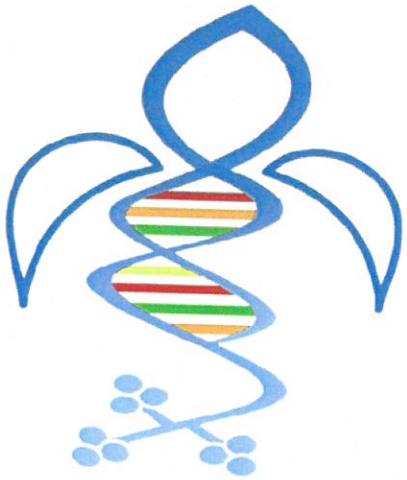

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Bacterial oligomers & polymers play opposite roles:MAMPs interact with each other and with host cell walls during induction of calcium signalling, which is suppressed by bacterial EPS .

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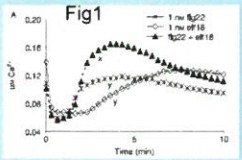
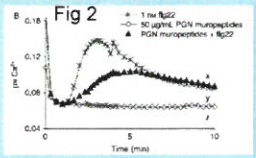
Triggering innate immunity

MAMP cocktails. Bacterial pathogens signal their presence by release of conserved, diverse MAMPs. These have been studied singly, but evidence reveals they are released as a cocktail. We combined pure MAMPs at non-saturating levels to challenge *Arabidopsis* & measure responses as Ca ion influx and generation of reactive oxygen species (ROS).

Bacterial MAMPS along with endogenous oligogalacturonides (OGA) showed **additivity** (eg flg22+elf18 FIG 1), **synergy** (flg22+LOS) and **interference** (flg22+OGA; flg+PGN peptides Fig 2). Interpretation is given in Aslam et al. (2009) but recognition of multiple MAMPS should ensure pathogen detection, as some MAMPs have evolved to avoid recognition.





MAMP combinations show additivity (Fig 1) and inter--ference (Fig 2) in Ca influx induction.

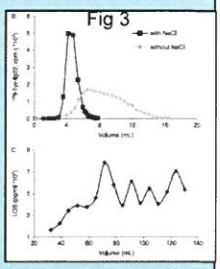


Fig 3.Influence of plant wall matrix on flg22 and LOS passage. *Tomato walls; 0.5x15cm column; radiolabelled flg; LAL assay for LOS*

Size matters. Macro- or supra-molecular MAMPs (PGN and LOS/LPS) are weak elicitors in plants (but potent in animals) compared with peptides flg22 and elf18. This may be explained by restricted access through the plant cell wall matrix. Fig 3 shows rapid permeation by flg22 but slow passage by LOS. The repeat pattern probably reflects size aggregates as LOS/LPS form micelles.

Suppressing innate immunity

Bacteria must prevent or suppress MAMP-triggered defences in order to invade. This is achieved in many ways but ***Type III effectors*** are clearly fundamental. Additionally, most bacterial plant pathogens require ***extracellular polysaccharides (EPS)*** for pathogenicity or full virulence (Fig 4). EPSs are multifunctional; protection from abiotic streses and host antimicrobials well known. But we sought a more fundamental role based on their structures: EPSs are **polyanionic** and **bind divalent cations**, notably the key signaling ion, **calcium** (Figs 5, 6)

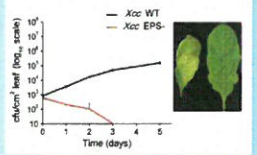
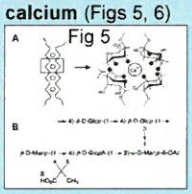
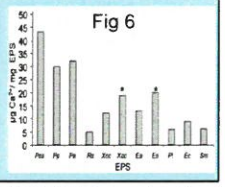


Fig 4. EPS-deficient mutant of *X. campestris* (and other xanthomonads) are non-pathogenic or reduced virulence

Fig 5. Calcium binding by poly--anionic alginate (*P. syringae*) & xanthan (*X. campestris*). Fig 6. All EPSs bound Ca eg pseudo-monads [P,Pss, Ps], Erwinia [Ea], X. campestris [Xcc].





EPSs bind apoplastic Ca ions and suppress defence responses

MAMPs trigger calcium influx from the **apoplast** to the **cytosol.** The calcium signature is dictated by speed, amplitude and duration of this influx. **Defence responses** are dependent on this influx, thus **removal of Ca ions suppresses defences.** EPS-mutants induce a larger Ca influx (Fig7) & oxidative burst (Fig 9). Pure EPS pre-treatments suppress both (Figs 8,10)

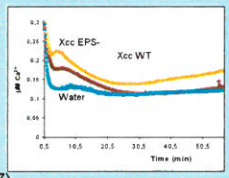


Figure 7. Comparison of bacterial induction of calcium influx. Aequorin-Arabidopsis leaf strips were infiltrated with *X. campestris* pv. *campestris* (Xcc) bacteria or water before calcium influx monitored by luminometry. Xcc WT = Xcc 8004, Xcc EPS- = Xcc 6397.

Ca

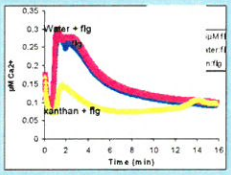


Figure 8. Suppression of MAMP-induced of calcium influx. Aequorin-Arabidopsis leaf discs were pre-infiltrated with 1 % xanthan or water 1 h before treatment with 500 nM flg22. Calcium influx was monitored by luminometry. This effect was mimicked by pre-treatment with 10 mM EGTA.

ROS

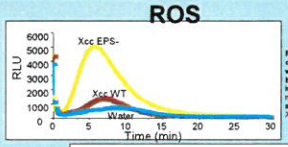


Figure 9. Comparison of oxidative burst induced by wild type vs. EPS- mutant bacteria. Arabidopsis leaf strips infiltrated w ROS production monitored w luminol based assay. WT = Xcc 8004, EPS- = Xcc 6397.

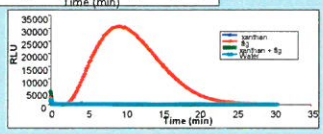
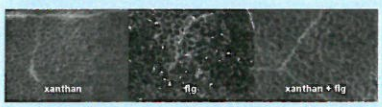


Figure 10. Suppression of MAMP-induced oxidative burst. Arabidopsis leaf discs pre-infiltrated w 1 % xanthan or water 1 h before 100 nM flg22. ROS production monitored w luminol based assay.

Callose deposition

Pre-treatment with pure EPS suppressed MAMP-induced callose deposition (Fig 11).



Comparison of callose deposition. Arabidopsis Col-0 plants were treated with 0.1 % xanthan and/ or 1 µM flg22. 24 h later treated leaves were decolourised and stained with aniline blue then viewed by UV microscopy.

Defence genes.

EPSs as WT bacteria and when pre-infiltrated pure suppress induction of defence-related genes: eg PR1, PDF1.2

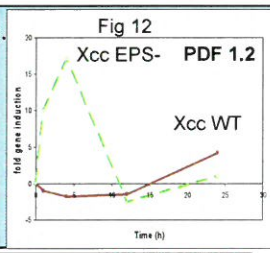


Fig 13. EPS does not suppress MAMP-receptor binding

The suppressive effect is not through physical blocking of MAMP access to receptors

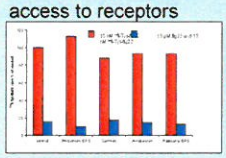
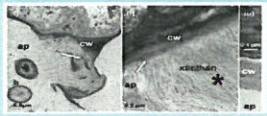


Figure 13 Effect of EPS on flg22 binding to FLS2. Arabidopsis cells expressing the FLS2 receptor were treated with 10 nM 125I-Tyr-flg22 alone or in combination with pure 0.1 %EPS and/ or 10 µM flg22. Radioactivity measured.

***In planta*:** Pure xanthan* resembles biofilms in planta and interacts with cell wall; Ca chelation? Fig 14



***In Planta* and in biofilms**: amounts of xanthan and alginate detected (GC-MS) would bind >5-10mM Ca; Ca levels in apoplast are 50-150 µM.

<u>Conclusion</u>:Specific suppression of defences by Ca chelation reveals a new fundamental role for EPSs in plant-pathogen compatibility

Refs from this work: Kemp et al (2004) PMPP **64,** 209; Erbs et al (2008) Chem Biol **15**, 438;Aslam et al (2008) Curr Biol **18**, 1078,Aslam et al (2009) Molec Plant Pathol **10**, 375.

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