Virus infection impairs fungal response to stress: effect of salt

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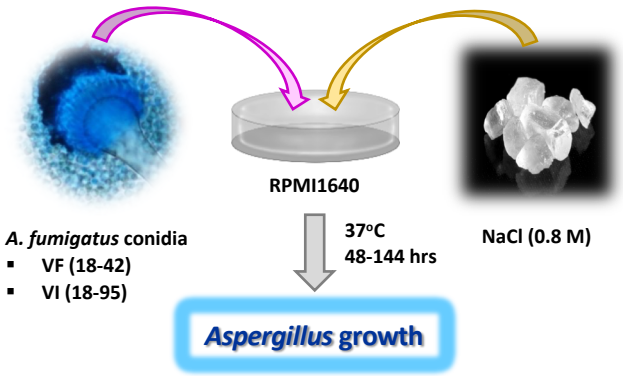
Introduction: There is little data on the effects of viral infections on fungal host physiology. Most studies have failed to demonstrate any effects, a few have shown effects on virulence. In previous studies we showed Aspergillus fumigatus polymycovirus-1 (AfuPmV-1)[1,2] impairs *A. fumigatus* (Af) in intermicrobial competition (largely in competition for iron), but also in effects of bacterial volatile organic molecules[3]. The former defect was related to temporal production of siderophores[4]. We now studied the effect of viral infection on another stress, hypertonic salt.

Methods: Reference Af strain Af293 is infected with AfuPmV-1, and was cured of infection with cycloheximide, generating isogenic virus-infected (VI, 18-95) and virus-free (VF, 18-42) lines. Growth (area) of both isogenic lines on RPMI1640 agar in the presence and absence (control) of 0.8 M NaCl was compared in 5 experiments (4 technical replicates/experiment), at 48 (when impaired radial growth could first be reliably measured), 72, 96, 120 and 144 hrs.



Results Summary: Salt stress impairs growth of VI and VF at all times sampled). VF control growth always exceeded and VF growth in salt always exceeded VI. Since VF growth exceeds VI in presence and absence of salt, we also examined growth in salt as a percentage of control growth. Initially, as a percentage of control, VI exceeded VF, but at 120 hrs VF began to exceed VI consistently, even by this measure, and persisted; thus at that time growth of VF in salt surges in relation to control growth, or, alternatively, its growth in salt persists compared to relative inhibition of VI.

Fig. 1

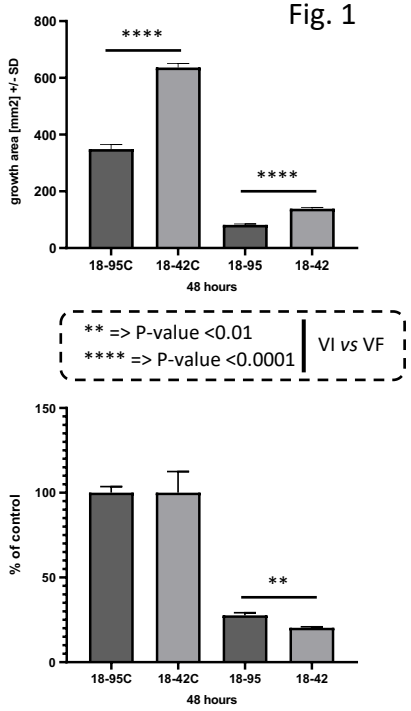


Figure 1. Experiment 4 (n=4) representative areas at 48 hrs. Top part, growth areas of the VF (18-42) and VI (18-95) isogenic Af strains. Left 2 bars, control growth (C) on RPMI agar; right 2 bars, growth in the presence of salt. Control growth for 18-42 is superior to 18-95 (p <0.0001); growth in salt is superior for 18-42 compared to 18-95 (p <0.0001). Salt inhibits growth of both strains in comparison to their controls (p <0.0001). Bottom part, growth in salt as percentage of control growth. At this time, by this measure, 18-95 is superior to 18-42 (p <0.002).

Fig. 2

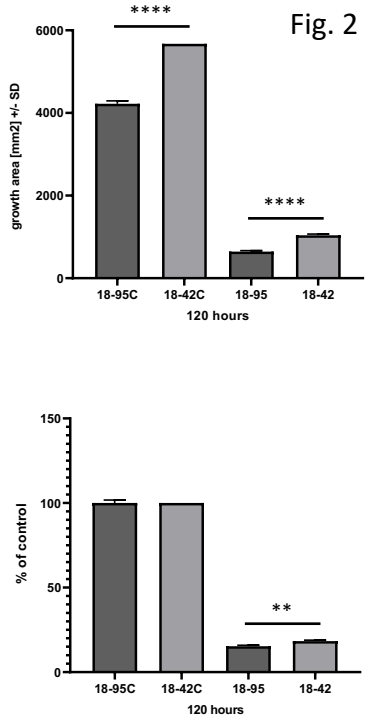


Figure 2. Experiment 4 (n=4) representative areas at 120 hrs. Top part, growth areas of the VF (18-42) and VI (18-95) isogenic Af strains. Left 2 bars, control growth (C) on RPMI agar; right 2 bars, growth in the presence of salt. Control growth for 18-42 is superior to 18-95, (p <0.0001); growth in salt is superior for 18-42 compared to 18-95 (p <0.0001). Salt inhibits growth of both strains in comparison to their controls (p <0.0001). Bottom part, growth in salt as percentage of control growth. At this time, by this measure, 18-42 growth is superior to 18-59, not only in absolute terms, but also as percentage of control growth (p= 0.003). In multiple experiments, 120 hrs was a transition time, and later observations indicated 18-42 growth always superior to 18-59, not only in absolute terms (as in every time point), but also as percentage of control growth.

Results (details):

Salt stress impairs Af growth; significant in 4/4 experiments (p<0.0001 at every time point, control growth *vs.* growth in salt) for both the VF and VI strains. Examples can be seen in the top half of Fig. 1 and 2.

Growth of the VF strain in the presence of salt is superior to the VI; significant at early time points in 3/5 experiments (48 hrs, p=0.01-<0.0001; 72 hrs, p=0.04-0.0004); consistently significant with further time of incubation in 4/4 experiments (96 hrs, p=0.02-<0.0001; 120 hrs, p=0.002-<0.0001) and 3/3 experiments (144 hrs, p=0.002-0.0002). Examples can be seen in the top half of Fig. 1 and 2, right pair of bars.

The VF strain control growth always exceeds that of the VI; significant in 4 experiments, at 48 hrs (p=0.005-<0.0001), 72 hrs (p=0.0007-0.0001), 96 hrs (p=0.002-<0.0001), 120 hrs (p=0.0003-<0.0001), and 144 hrs (n=3, p=0.0005-<0.0001). Examples can be seen in the top half of Fig. 1 and 2, left pair of bars.

Over time, the VF strain growth in the presence of salt, as a percentage of control growth, transitions from inferior to the VI, to superior. At the beginning of growth, the relative growth (as a percentage of control) of the virus-free strain was less than the virus-infected, in 4/4 experiments. The differences were significant in 3/4 (48 hrs, p= 0.03-0.002; 72 hrs, p=0.02-0.0002) and 2/4 (96 hrs, p=0.02-0.002). At 120 hrs, the growth of the VF in salt surges in relation to control growth, or, alternatively, its growth in salt persists compared to inhibition of the VI, indicating a transition. The differences were significant in 2/4 experiments (p=0.03-0.003). By 144 hrs, this phenomenon was consistent and significant in 3/3 experiments (p=0.02-0.0004). Examples can be seen in the bottom half of Fig. 1 and 2.

Conclusion: VF growth in several control media exceeded VI, as described here and in other media studied, although our prior studies indicated no differences in oxidative metabolism assays (XTT). VF growth in the presence of high salt was always superior to VI. Virus infection impairs response of Af to several different stresses. We report elsewhere VF-VI differences in production of siderophores[4] and recently, gliotoxins[5]. Temporal assays to document differences have been required, and will be, in future studies of mechanisms.

References: [1]Kanhayuwa, L., Kotta-Loizou, I., Özkan, S., Gunning, A.P., Coutts, R.H.A. A novel mycovirus from *Aspergillus fumigatus* contains four unique dsRNAs as its genome and is infectious as dsRNA. Proc. Natl. Acad. Sci. U. S. A. 112:9100-5, 2015. [2]Filippou, C., Coutts, R.H.A., Stevens, D.A., Sabino, R., Kotta-Loizou, I. Completion of the sequence of the Aspergillus fumigatus partitivirus 1 genome. Arch. Virol. 165:1891-4, 2020. [3]Nazik, H., Kotta-Loizou, I., Sass, G., Coutts, R.H.A., Stevens, D.A. Virus infection of *Aspergillus fumigatus* compromises the fungus in intermicrobial competition. Viruses 13:686, 2021. [4]Patil, R., Kotta-Loizou, I., Palyzova, A., Pluhacek, T., Coutts, R.H.A., Stevens, D.A., Havlicek, V. Freeing *Aspergillus fumigatus* of polymycovirus infection makes it more resistant to competition with *Pseudomonas aeruginosa* due to altered iron-acquiring tactics. J. Fungi 7:497, 2021. [5]Patil, R.H., Kotta-Loizou, I., Palyzová, A., Pluháček, T., Coutts, R., Stevens, D.A., Havlíček, V. Mycotoxin secretion by *Aspergillus fumigatus* as a response to mycovirus infection. Program of the 10th Advances Against Aspergillosis and Mucormycosis meeting, 2022, Abstract no. 24.