

Supplementary Information Figures

Microbial lag phase can be indicative of, or independent from, cellular stress

Philip G. Hamill¹, Andrew Stevenson¹, Phillip E. McMullan¹, James P. Williams¹, Abiann D. R. Lewis¹, Sudharsan S², Kath E. Stevenson³, Keith D. Farnsworth¹, Galina Khroustalyova⁴, Jon Y. Takemoto⁵, John P. Quinn¹, Alexander Rapoport⁴, and John E. Hallsworth¹

¹*Institute for Global Food Security, School of Biological Sciences, Queen's University Belfast, 19 Chlorine Gardens, Belfast, BT9 5DL, Northern Ireland.*

²*Department of Chemistry, PGP College of Arts and Science, NH-7, Karur Main Road, Paramathi, Namakkal, Tamil Nadu, 637 207, India*

³*Special Collections and Archives, McClay Library, Queen's University Belfast, 10 College Park Avenue, Belfast, BT7 1LP, Northern Ireland.*

⁴*Laboratory of Cell Biology, Institute of Microbiology and Biotechnology, University of Latvia, Jelgavas Str., 1-537, LV-1004 Riga, Latvia.*

⁵*Utah State University, Department of Biology, 5305 Old Main Hill Logan, UT 84322, USA.*

Supplementary Figures

Fig. S1. Progress of spore germination for four strains of xerophilic fungi: (a) *Aspergillus penicillioides* JH06THH; (b) *Xeromyces bisporus* FRR 3443; (c) *X. bisporus* FRR 1522; and (d) *Xerochrysium xerophilum* FRR 0530. Percentage germination was determined on malt-extract yeast-extract phosphate agar (MYPiA) supplemented with diverse stressor(s) and incubated at 30°C for up to 50 days. Media were supplemented with: glycerol (red lines), at 7.0 and 7.1 M, with water-activity values of 0.707 and 0.664, respectively; glycerol (5.5 M)+NaCl at 0.5, 1.0, 1.5 and 1.6 M (green lines), with water-activity values of 0.765, 0.741, 0.709 and 0.692, respectively; glycerol (5.5 M)+sucrose at 0.25, 0.50, 0.65 and 0.80 M (blue lines), with water-activity values of 0.734, 0.699, 0.674 and 0.637, respectively; glycerol (5.5 M) + glucose at 0.8 and 1.0 M + fructose at 0.80 and 1.0 M (yellow lines), with water activities of 0.694 and 0.649, respectively; and glycerol (5.5 M)+NaCl (0.5 M)+sucrose at 0.3 and 0.5 M (black lines), with water-activity values of 0.701 and 0.685, respectively (see Table S2). Two hundred spores, and 50 individual germinated spores were measured for germ-tube length, mean values are plotted, and grey bars indicate standard errors. Modified from Stevenson *et al.*¹⁴ with permission from Wiley Online Library.

Fig. S2. Length of lag phase versus maximum rate of growth (optical density arbitrary units h⁻¹) for *Bacillus subtilis* in Belitzki minimal medium broth that had been supplemented with a range of stressors: (a) glycerol at 2.72 (◆), 2.26 (■), 1.81 (▲), 1.36 (×), 0.91 (-) and 0.45 M (●), and without glycerol (control; +); (b) proline at 2.61 (◆), 2.18 (■), 1.74 (▲), 1.30 (×), 0.87 (-), 0.43 M (●), and without proline (control; +); (c) glucose at 1.94 (◆), 1.62 (■), 1.30 (×), 0.97 M (-), 0.65 M (●), and 0.32 M (□); (d) MgCl₂ at 1.37 (◆), 1.14 (■), 0.91 (▲), 0.68 M (×), 0.46 (-), 0.23 nM (●), and without MgCl₂ (control; +); (e) sucrose at 1.31 (◆), 1.10 (■), 0.88 (▲), 0.66 (×), 0.44 (-), 0.22 M (●), and without sucrose (control; +); and (f) ammonium sulphate at 0.76 (◆), 0.51 (■), 0.25 M (▲), and without ammonium sulphate (control; +). All controls and treatments were carried out in triplicate. Data were obtained from Fig. S13 (see *Experimental procedures*), and trend lines showing a strong correlation between data are solid and black (b; c; d; e; f), and a weak correlation is indicated by the dashed black line (a) (Table S5).

Fig. S3. Growth curves for *Saccharomyces cerevisiae* at 23°C: strain 14 in salt-supplemented nutrient broth (SSNB) (a) and yeast extract peptone dextrose broth (YPDB) (b); and strain 77 in SSNB (c) and YPDB (d). The water content of these cells was reduced to 8-10% w/w, and sterile distilled water or a 1 M xylitol solution (10 ml) was added to 50 mg of dried cells for determinations of cell viability (see *Experimental procedures*). Cells suspended in water (not subjected to any dehydration treatment) (pink dots), and cells suspended in 1 M xylitol (no dehydration treatment) (orange dots), were used as controls. For dehydrated cells, rehydration treatments were either rapid, using water (grey dots); rapid, using 1 M xylitol (brown dots); gradual, using water vapour then rapid in water (purple dots); or gradual rehydration with water vapor, then rapid in 1 M xylitol (turquoise dots). Mean values are plotted, and grey bars indicate standard errors.

Fig. S4. Growth curves for *Saccharomyces cerevisiae* at 30°C: strain 14 in salt-supplemented nutrient broth (SSNB) (a) and yeast extract peptone dextrose broth (YPDB) (b); and strain 77 in SSNB (c) and YPDB (d). The water content of these cells was reduced to 8-10% w/w and for determinations of cell viability, sterile distilled water or a 1 M xylitol solution (10 ml) was added to 50 mg of dried cells (see *Experimental procedures*). Cells suspended in water (not subjected to any dehydration treatment) (pink dots), and cells suspended in 1 M xylitol (no dehydration treatment) (orange dots), were used as controls. For dehydrated cells, rehydration treatments were either rapid, using water (grey dots); rapid, using 1 M xylitol (brown dots); gradual, using water vapour then rapid in water (purple dots); or gradual rehydration with water vapor, then rapid in 1 M xylitol (turquoise dots) or for xylitol dehydrated cells; gradual, using water vapour then rapid in water (×); and gradual, using water vapour then rapid in 1 M xylitol (▲). All conditions were assessed in triplicate, mean values were plotted, and grey bars indicate standard error.

Fig. S5. Growth curves for *Mrakia frigida* (DSM 70883) on malt-extract yeast-extract phosphate agar (MYPiA) at 1.7°C, supplemented with a range of stressors: (a) sucrose (1.5 M); (b) glucose (2.8 M); (c) glycerol (4.3 M); (d) glycerol (2.7 M); (e) NaCl (2.5 M); and (f) MgCl₂ (1 M) and combined solute-treatment 2 (20% glycerol, 10% sucrose, 1% KCl plus 5% NaCl [w/v]). For each treatment, there were six replicates; these are

shown in red, blue, orange, green, purple, and turquoise on each plot.

Fig. S6. Maximum growth rate for *Escherichia coli* BL21 (syn. DE3) versus stressor concentration in Luria–Bertani (LB) broth (23°C) supplemented with: (a) ethanol; (b) butanol; and (c) urea. The last three datapoints of each curve (corresponding to the three highest stressor concentrations) were used to determine the theoretical maximum concentration that would permit growth for each stressor, using a linear trend line (dotted line) for (d) ethanol; (e) butanol; and (f) urea.

Fig. S7. Growth curves for *Bacillus subtilis* 168 in Belitzki minimal medium broth, supplemented with a range of stressors: (a) betaine at 2.56 (♦), 2.13 (■), 1.71 (▲), 1.28 (×), 0.85 (-) and 0.43 M (●), and without betaine (control; +); (b) guanidine hydrochloride at 150 (♦), 125 (■), 100 (▲), 75 (×), 50 (-) and 25 nM (●), and without guanidine hydrochloride (control; +); (c) ammonium sulphate at 0.76 (♦), 0.51 (■), 0.25 M (▲), and without ammonium sulphate (control; +); and (d) polyethylene glycol 600 at 400 (♦), 333 (■), 267 (▲), 200 (×), 133 (-) and 67 nM (●), and without polyethylene glycol 600 (control; +). All conditions were assessed in triplicate, mean values are plotted, and grey bars indicate standard errors.

Fig. S8. Length of lag phase versus maximum rate of germination for eight strains of extremophilic fungi: (a) *Aspergillus penicillioides* JH06GBM; (b) *A. penicillioides* JH06THJ; (c) *Eurotium amstelodami* FRR 2792; (d) *Eurotium echinulatum* FRR 5040; (e) *Eurotium halophilicum* FRR 2471; (f) *Eurotium repens* JH06JPD; (g) *Xeromyces bisporus* FRR 0025; and (h) *X. bisporus* FRR 2347. Germination was assessed on malt-extract yeast-extract phosphate agar (MYPiA) supplemented with diverse stressor(s) and incubated at 30°C for up to 50 days. Media were supplemented with: glycerol (red dots), at 7.0 and 7.1 M, with water-activity values of 0.707 and 0.664, respectively; glycerol (5.5 M)+NaCl at 0.5, 1.0, 1.5, and 1.6 M (green dots), with water-activity values of 0.765, 0.741, 0.709 and 0.692, respectively; glycerol (5.5 M)+sucrose at 0.25, 0.50, 0.65, and 0.80 M (blue dots), with water-activity values of 0.734, 0.699, 0.674, and 0.637, respectively; glycerol (5.5 M) + glucose at 0.8 and 1.0 M + fructose at 0.80 and 1.0 M (yellow lines), with water activities of 0.694 and 0.649, respectively; glycerol (5.5 M)+NaCl (0.5 M)+sucrose at 0.3 and 0.5 M (black dots), with water-activity values of 0.701 and 0.685, respectively. Data were obtained from Fig. S13

(see *Experimental procedures*), and trend lines showing a strong correlation and moderate correlation between data are solid and black (**a**; **c**; **e**; **f**; **h**), a weak correlation is indicated by a dashed black line (**b**), while dashed grey lines indicate inconsistency between lag and growth rates (i.e. no correlation was observed) (**d**; **g**) (Table S5).

Fig. S9. Length of lag phase versus maximum rate of growth *Mrakia frigida* (DSM 70883) on malt-extract, yeast-extract phosphate agar (MYPiA) at 1.7°C, supplemented with a range of stressors: (**a**) sucrose (1.5 M); (**b**) glucose (2.8 M); (**c**) glycerol (4.3 M); (**d**) glycerol (2.7 M); (**e**) NaCl (2.5 M); and (**f**) MgCl₂ (1 M) and combined solute-treatment 2 (20% glycerol, 10% sucrose, 1% KCl plus 5% NaCl [w/v]). Trend lines showing strong correlations between data are solid and black (**a**; **d** and **e**), while moderate correlations are indicated by a dashed black line (**b**; **c**; and **f**) (Table S5).

Fig. S10. Length of lag phase versus maximum rate of growth for *Escherichia coli* BL21 (syn. DE3) in Luria–Bertani (LB) broth, supplemented with a range of stressors: (**a**) ethanol at 600 (orange x), 650 (grey x), 700 (yellow x), 750 (blue x), 800 (green x) and 850 nM (black x); (**b**) butanol at 70 (purple x), 80 (orange x), 90 (grey x), 100 (yellow x), 110 (blue x) and 120 nM (green x); and (**c**) urea at 295 (purple x), 389 (orange x), 482 (grey x), 574 (yellow x), 663 (blue x), 751 (grey x), and 838 nM (black x). Data were obtained from Fig. S14 (see *Experimental procedures*), and trend lines showing a strong correlation between data are solid and black (**b** and **c**), while a moderate correlation is indicated by a dashed black line (**a**) (Table S5).

Fig. S11. Growth curves for *Bacillus subtilis* 168 in Belitzki minimal medium broth, supplemented with a range of stressors: (**a**) glycerol at 2.72 (◆), 2.26 (■), 1.81 (▲), 1.36 (×), 0.91 (-) and 0.45 M (●), and without glycerol (control; +); (**b**) proline at 2.61 (◆), 2.18 (■), 1.74 (▲), 1.30 (×), 0.87 (-), 0.43 M (●), and without proline (control; +); (**c**) glucose at 1.94 (◆), 1.62 (■), 1.30 (×), 0.97 M (-), 0.65 M (●), and 0.32 M (□); (**d**) MgCl₂ at 1.37 (◆), 1.14 (■), 0.91 (▲), 0.68 M (×), 0.46 (-), 0.23 nM (●), and without MgCl₂ (control; +); (**e**) sucrose at 1.31 (◆), 1.10 (■), 0.88 (▲), 0.66 (×), 0.44 (-), 0.22 M (●), and without sucrose (control; +); and (**f**) polyethylene glycol 6000 at 50 (◆), 42 (■), 33 (▲), 25 (×), 17 (-) and 8 nM (●) and without polyethylene glycol 6000 (control; +).

Fig. S12. Length of lag phase versus specific growth rate for: *Pseudomonas fluorescens* (ATCC 17400) (a, f and k), *Salmonella typhimurium* (ATCC 13311) (d, i and n), *Staphylococcus aureus* (ATCC 13566) in nutrient broth (e, j and o), *Brochothrix thermosphacta* (ATCC 12706) (b, g, and l), and *Enterococcus faecalis* (ATCC 7080) in brain heart infusion broth (c, h and m). In each case, media were supplemented with glycerol, NaCl, or sucrose (Li and Torres, 1993): (a) *P. fluorescens* with NaCl to a range of water activities: 0.980 at 4°C (◆), 0.982 at 8°C (Ж), 0.956 at 8°C (X), 0.981 at 16°C (●), 0.956 at 16°C (■), 0.981 at 26°C (-) and 0.955 at 26°C (▲); (b) *B. thermosphacta* with NaCl to a range of water activities: 0.968 at 4°C (▲), 0.952 at 4°C (x); 0.968 at 8°C (◆), 0.952 at 8°C (■), 0.968 at 16°C (-), 0.952 at 16°C (Ж), 0.967 at 26°C (+), and 0.952 at 26°C (●); (c) *E. faecalis* with NaCl to a range of water activities: 0.978 at 12°C (▲), 0.982 at 16°C (◆), 0.958 at 16°C (x), 0.981 at 20°C (●), 0.956 at 20°C (■), and 0.953 at 26°C (Ж); (d) *S. typhimurium* with NaCl to a range of water activities: 0.983 at 12°C (■), 0.967 at 12°C (x), 0.984 at 16°C (Ж), 0.965 at 16°C (▲), 0.980 at 20°C (●), 0.965 at 20°C (◆), 0.984 at 26°C (+) and 0.964 at 26°C (-); (e) *S. aureus* with NaCl to a range of water activities: 0.968 at 12°C (■), 0.950 at 12°C (x), 0.967 at 16°C (◆), 0.950 at 16°C (▲), 0.968 at 20°C (●), 0.950 at 20°C (Ж), 0.950 at 26°C (-), and 0.970 at 26°C (+); (f) *P. fluorescens* with glycerol to a range of water activities: 0.978 at 4°C (■), 0.956 at 4°C (x), 0.980 at 8°C (◆), 0.956 at 8°C (▲), 0.981 at 16°C (-), 0.956 at 16°C (Ж), 0.979 at 26°C (+), and 0.955 at 26°C (●); (g) *B. thermosphacta* with glycerol to a range of water activities: 0.972 at 4°C (■), 0.950 at 4°C (x), 0.972 at 8°C (◆), 0.950 at 8°C (▲), 0.972 at 16°C (●), 0.949 at 16°C (Ж) 0.973 at 26°C (+) and 0.948 at 26°C (-); (h) *E. faecalis* with glycerol to a range of water activities: 0.968 at 12°C (◆), 0.946 at 12°C (x), 0.968 at 16°C (Ж), 0.946 at 16°C (■), 0.968 at 20°C (-), 0.944 at 20°C (▲) and 0.944 at 26°C (●); (i) *S. typhimurium* with glycerol to a range of water activities: 0.982 at 12°C (▲), 0.968 at 12°C (x), 0.983 at 16°C (◆), 0.964 at 16°C (■), 0.983 at 20°C (●), 0.969 at 20°C (Ж), 0.969 at 26°C (-) and 0.982 at 26°C (+); (j) *S. aureus* with glycerol to a range of water activities: 0.977 at 12°C (■), 0.975 at 16°C (▲), 0.955 at 16°C (x), 0.974 at 20°C (Ж), 0.950 at 20°C (◆), 0.970 at 26°C (-), and 0.952 at 26°C (●); (k) *P. fluorescens* with sucrose to a range of water activities: 0.980 at 4°C (x), 0.980 at 8°C (■), 0.980 at 16°C (Ж), 0.960 at 16°C (▲), 0.978 at 26°C (●), and 0.955 at 26°C (◆); (l) *B. thermosphacta* with sucrose to a range of water activities: 0.969 at 4°C (x), 0.969 at 8°C (Ж), 0.958 at 8°C (■), 0.972 at 16°C (●), 0.957 at 16°C (▲), 0.972 at 26°C (-) and 0.951 at 26°C (◆); (m) *E. faecalis*

with sucrose to a range of water activities: 0.979 at 12°C (x), 0.978 at 16°C (♦), 0.980 at 20°C (ж), 0.954 at 20°C (■), 0.981 at 26°C (●) and 0.950 at 26°C (▲); (n) *S. typhimurium* with sucrose to a range of water activities: 0.974 at 12°C (▲), 0.967 at 12°C (X), 0.978 at 16°C (ж), 0.967 at 16°C (■), 0.977 at 20°C (●), 0.964 at 20°C (♦), 0.975 at 26°C (+) and 0.964 at 26°C (-); (o) *S. aureus* with sucrose to a range of water activities: 0.980 at 12°C (■), 0.977 at 16°C (▲), 0.953 at 16°C (x), 0.978 at 20°C (●), 0.950 at 20°C (♦), 0.980 at 26°C (-) and 0.947 at 26°C (ж). Trend lines showing a strong correlation between data are solid and black (a-g; i-k and n-o) while moderate correlations are indicated by a dashed black line (h; l and m) (Table S5).

Fig. S13. Progress of spore germination for eight strains of extremophilic fungi: (a) *A. penicillioides* JH06GBM; (b) *A. penicillioides* JH06THJ; (c) *Eurotium amstelodami* FRR 2792; (d) *E. echinulatum* FRR 5040; (e) *E. halophilicum* FRR 2471; (f) *E. repens* JH06JPD; (g) *X. bisporus* FRR 0025; and (h) *X. bisporus* FRR 2347. Germination was assessed on malt-extract yeast-extract phosphate agar (MYPiA) supplemented with diverse stressor(s) and incubated at 30°C for up to 50 days. Media were supplemented with: glycerol (red lines), at 7.0 and 7.1 M, with water-activity values of 0.707 and 0.664, respectively; glycerol (5.5 M) + NaCl at 0.5, 1.0, 1.5 and 1.6 M (green lines), with water-activity values of 0.765, 0.741, 0.709 and 0.692, respectively; glycerol (5.5 M) + sucrose at 0.25, 0.50, 0.65 and 0.80 M (blue lines), with water-activity values of 0.734, 0.699, 0.674 and 0.637, respectively; glycerol (5.5 M) + glucose at 0.8 and 1.0 M + fructose at 0.80 and 1.0 M (yellow lines), with water activities of 0.694 and 0.649, respectively; and glycerol (5.5 M) + NaCl (0.5 M) + sucrose at 0.3 and 0.5 M (black lines), with water-activity values of 0.701 and 0.685, respectively (Table S2). Two hundred spores, and 50 spores that had germinated (and were spatially separate from others), were used to determine mean germ-tube length: these mean values were plotted, and grey bars indicate standard errors. Modified from Stevenson *et al.*¹⁴, with permission from Wiley Online Library.

Fig. S14. Growth curves for *Escherichia coli* BL21 (syn. DE3) in Luria–Bertani (LB) broth, supplemented with a range of stressors: (a) ethanol at 600 (orange line), 650 (grey line), 700 (yellow line), 750 (blue line), 800 (green line) and 850 nM (black line); (b) butanol at 70 (purple line), 80 (orange line), 90 (grey line), 100 (yellow line), 110 (blue line) and 120 nM (green line); (c) urea at 295 (purple line), 389 (orange line),

482 (grey line), 574 (yellow line), 663 (blue line), 751 (grey line), and 838 nM (black line).

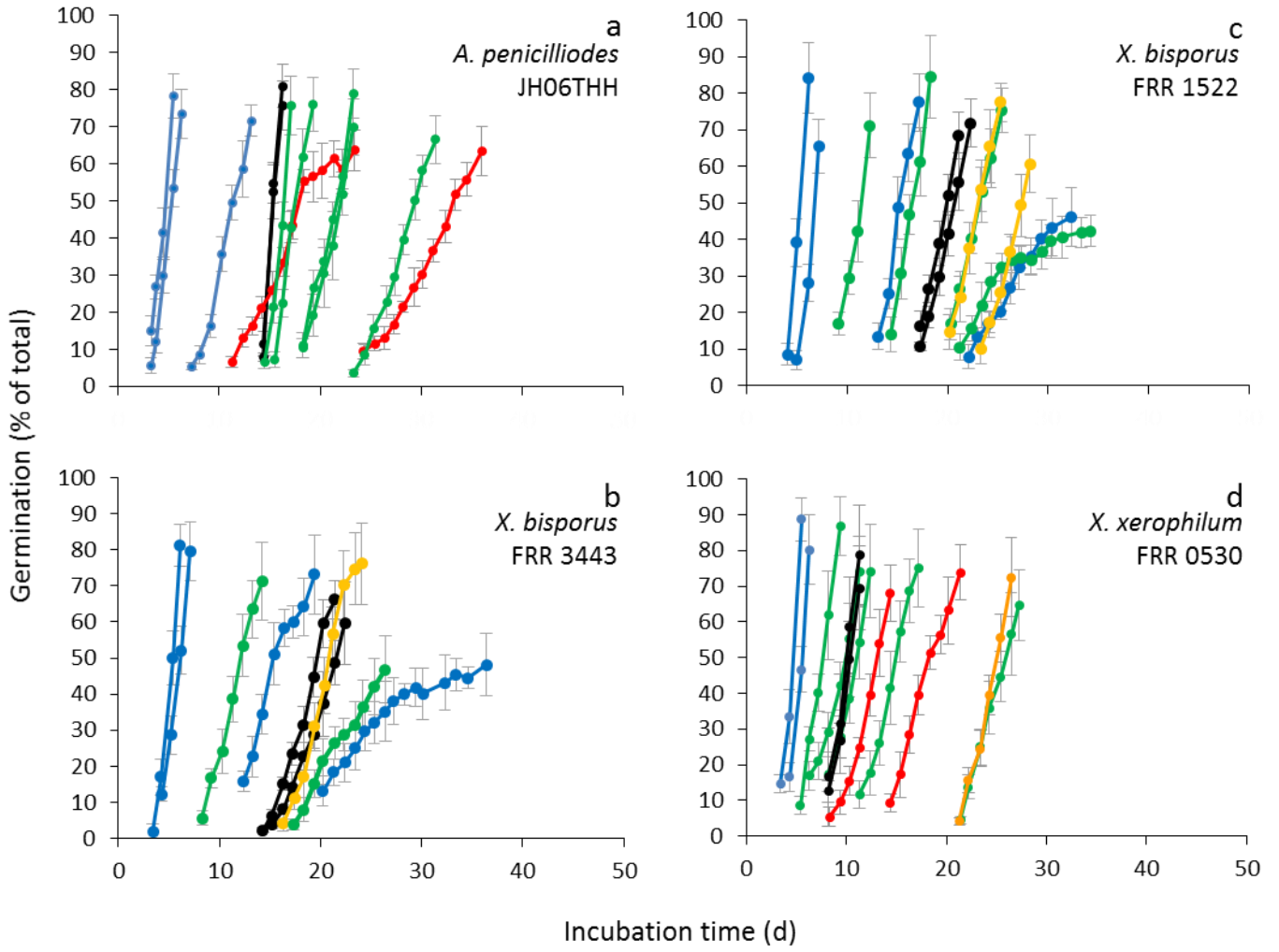


Figure. S1

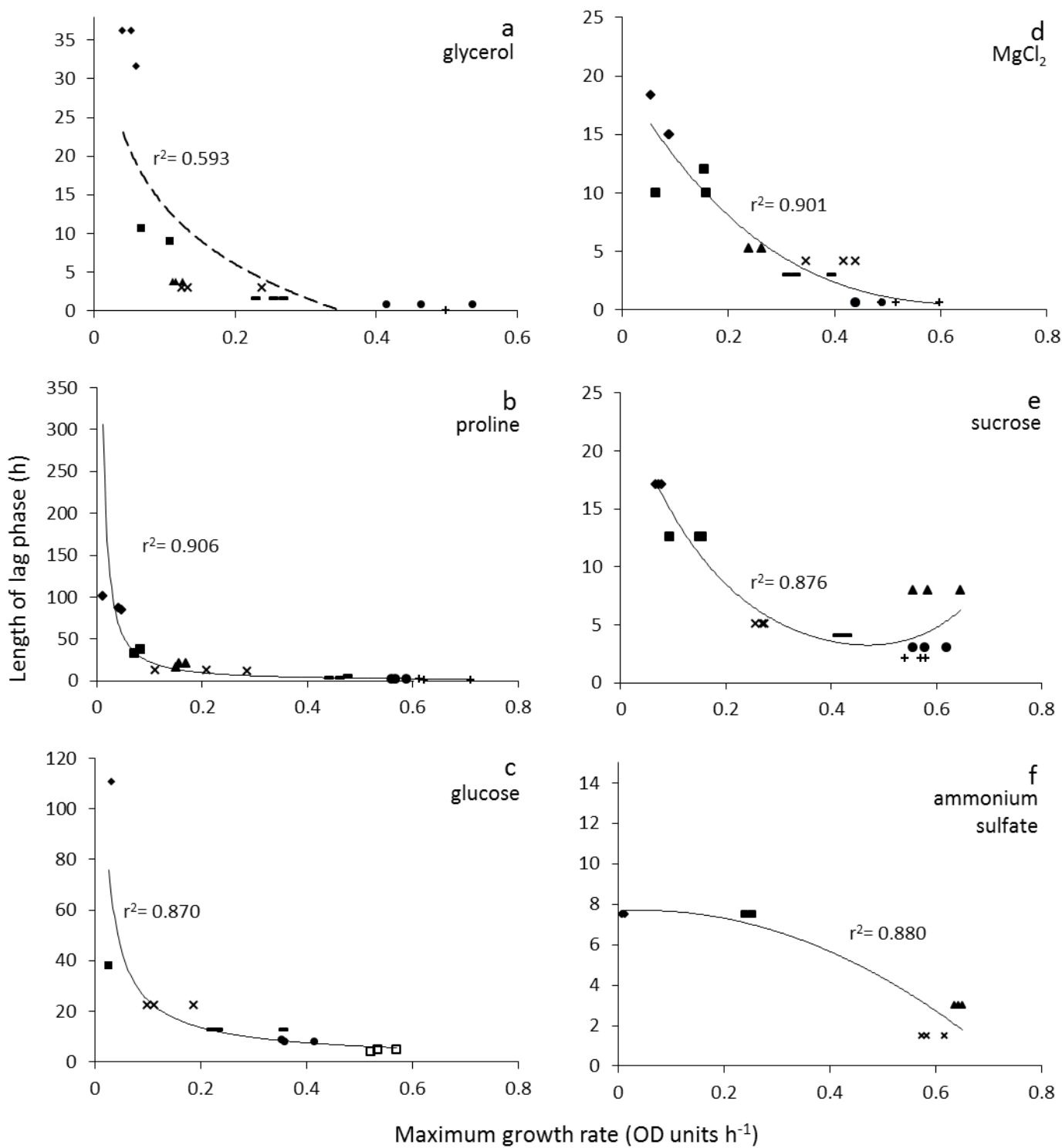


Figure. S2

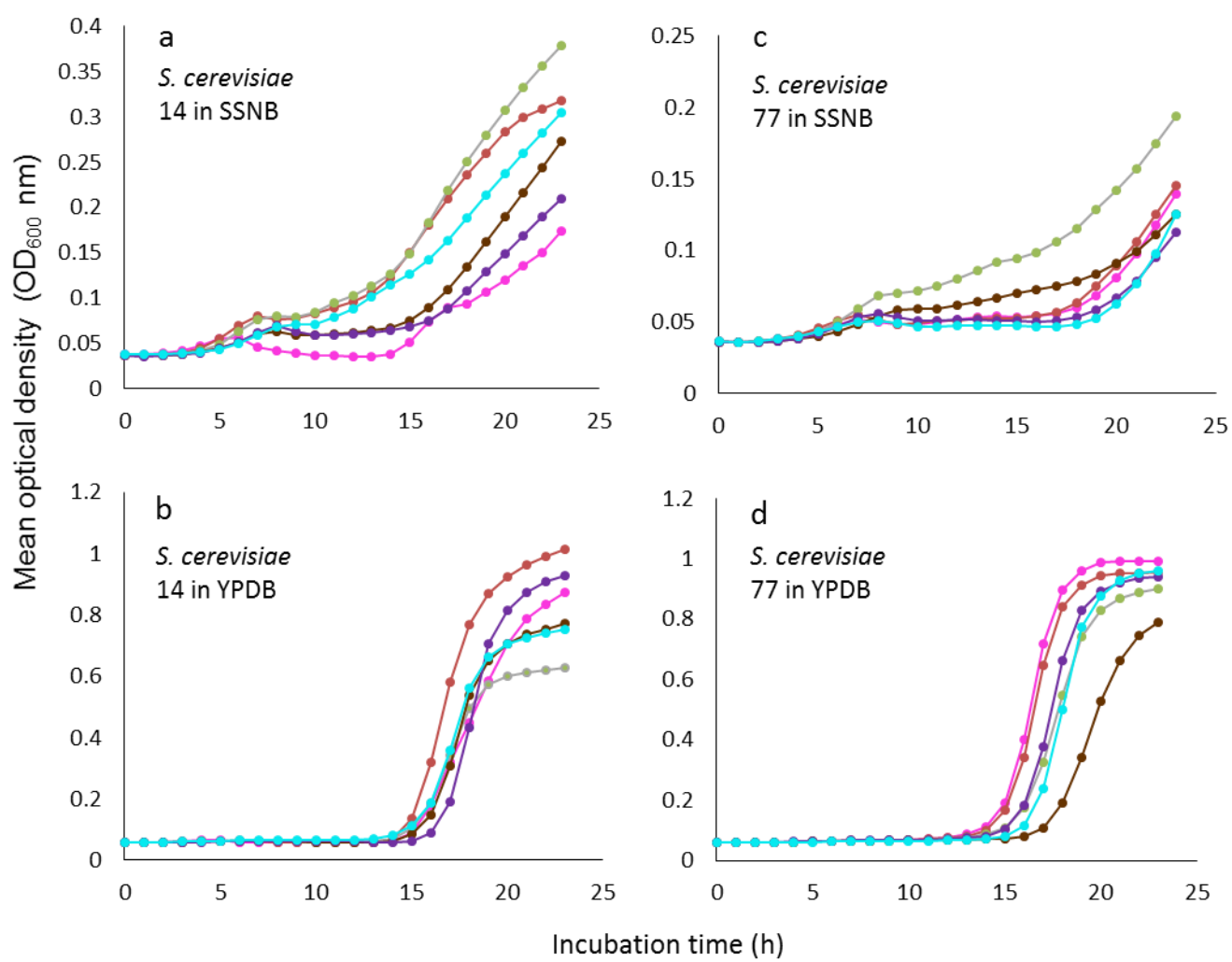


Figure. S3

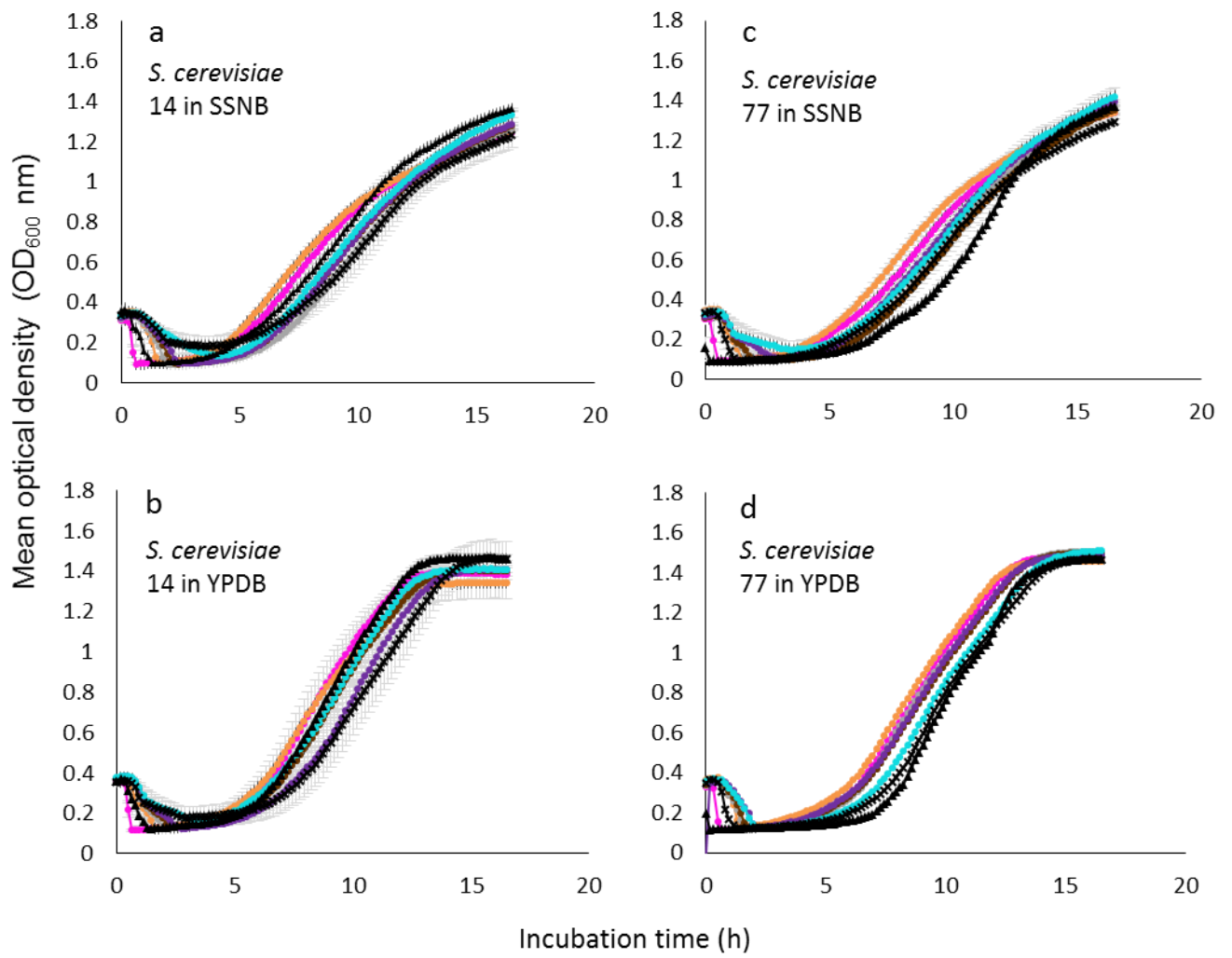


Figure. S4

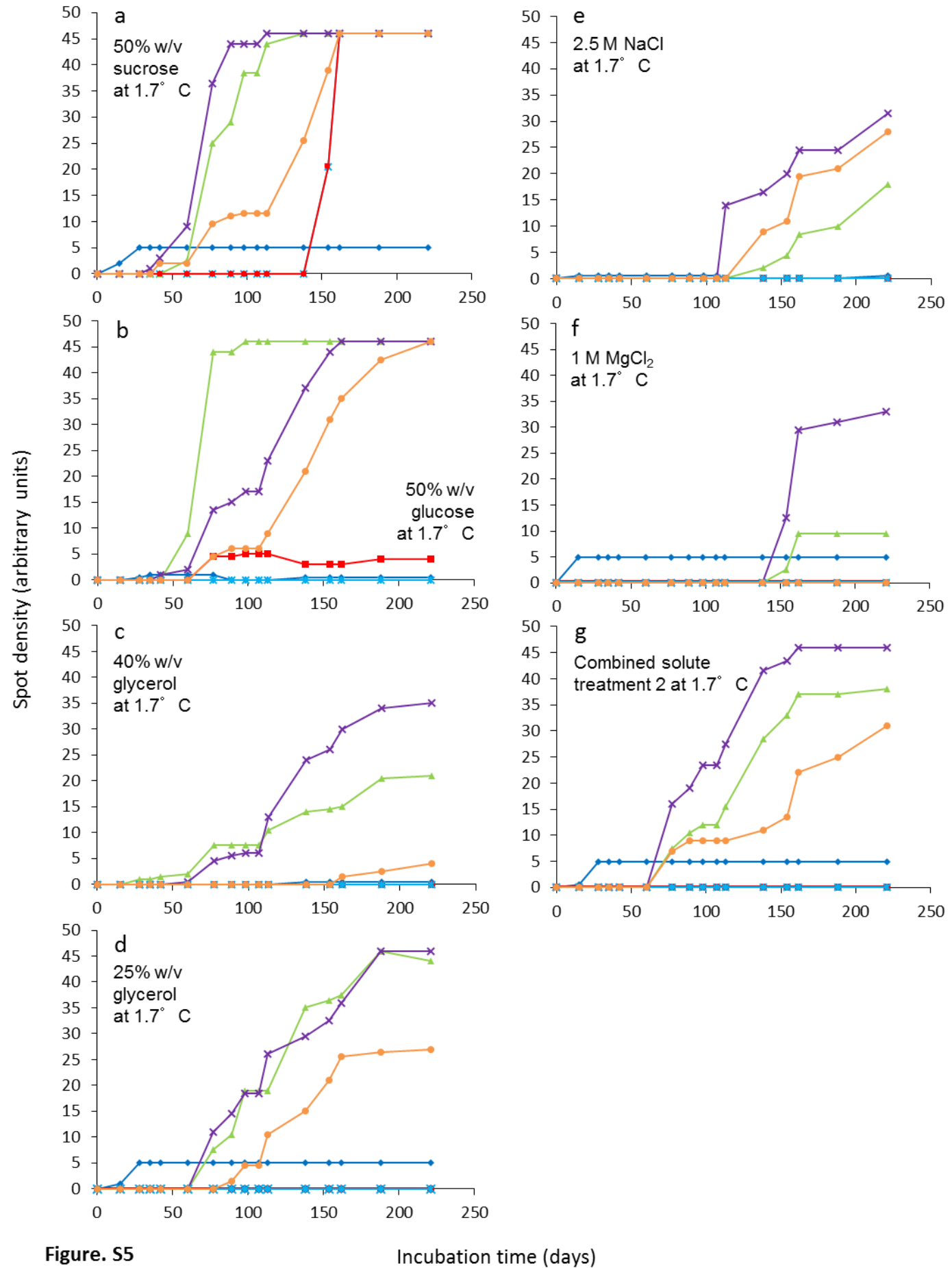


Figure. S5

Incubation time (days)

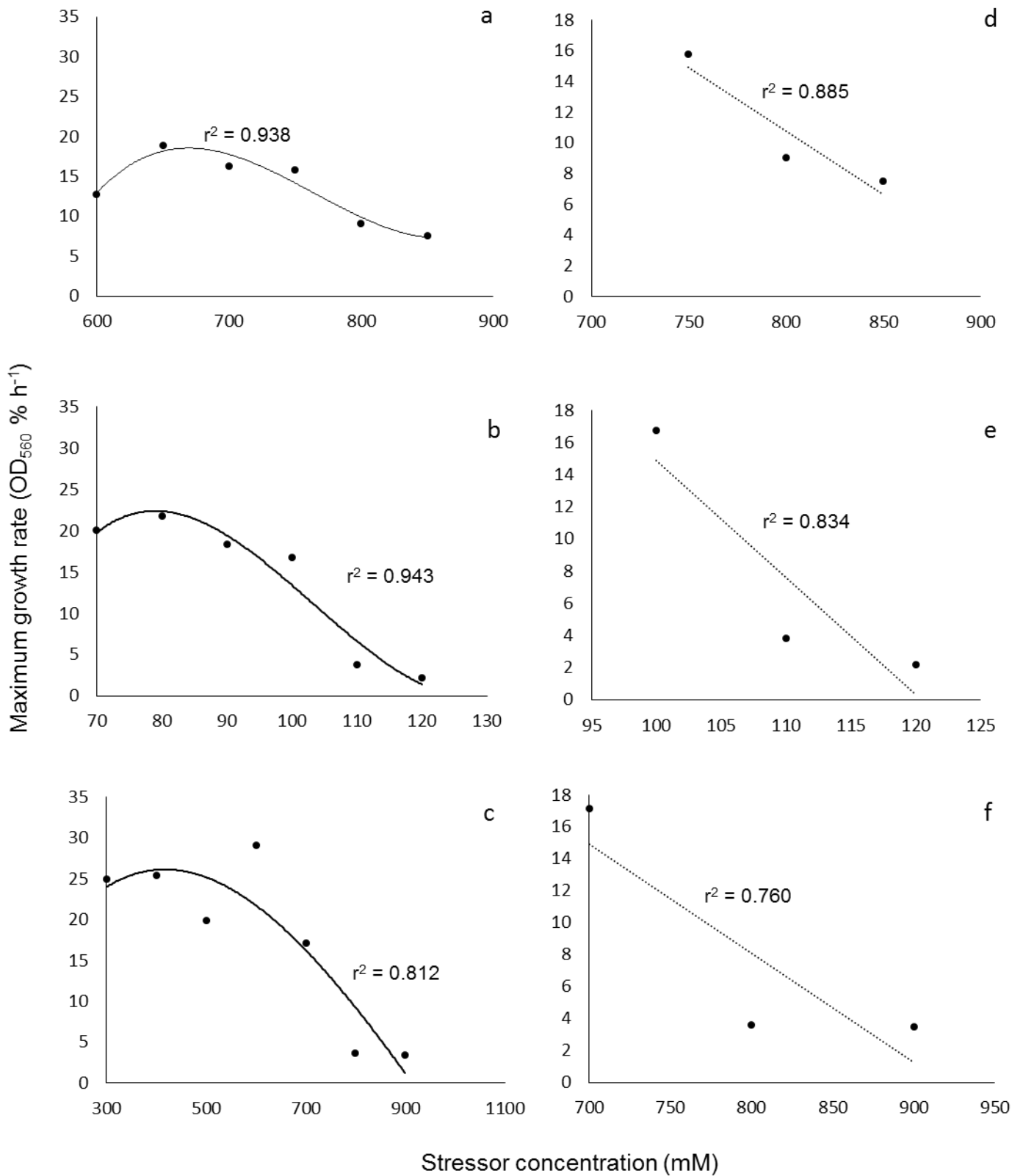


Figure. S6

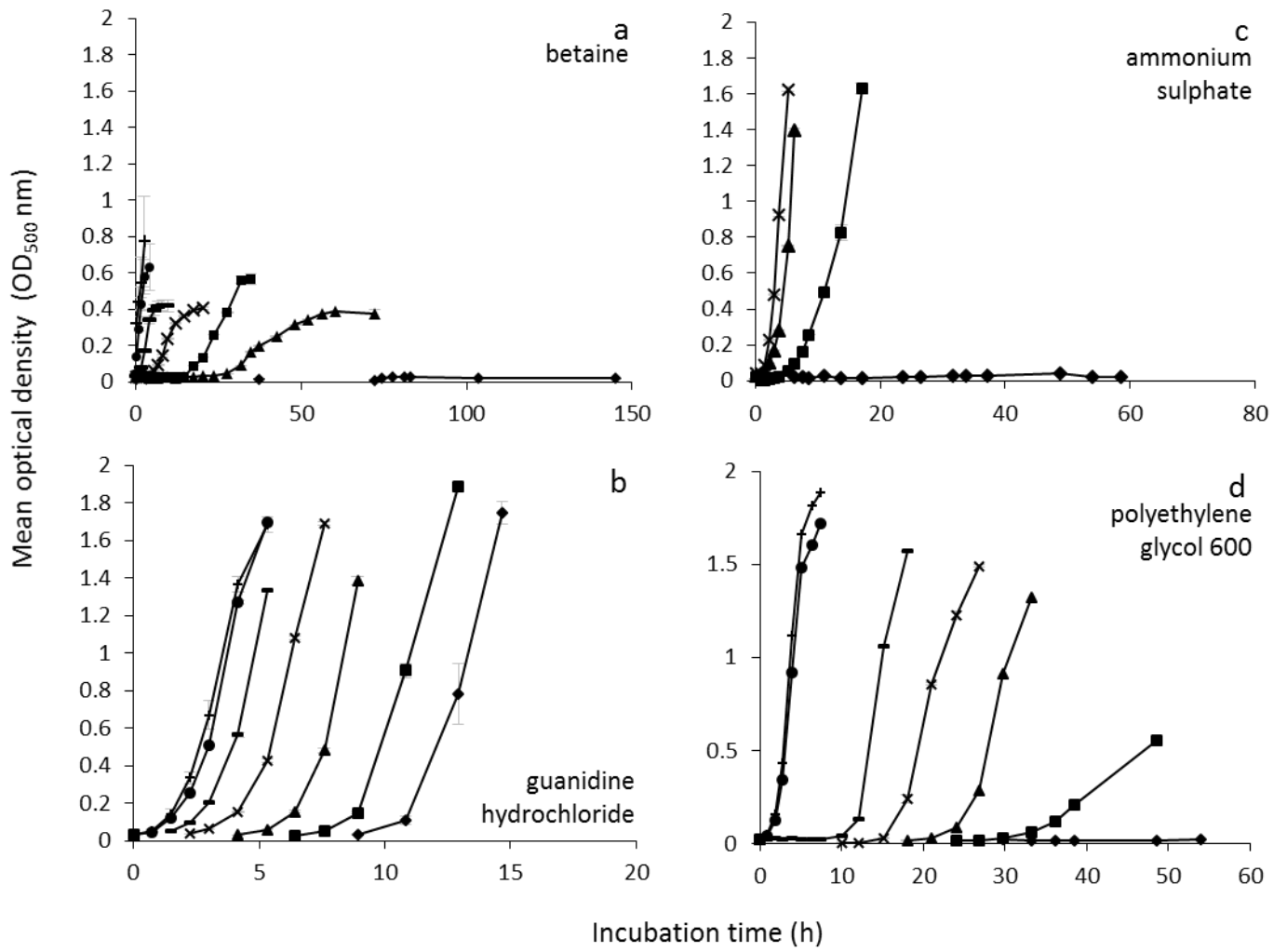


Figure. S7

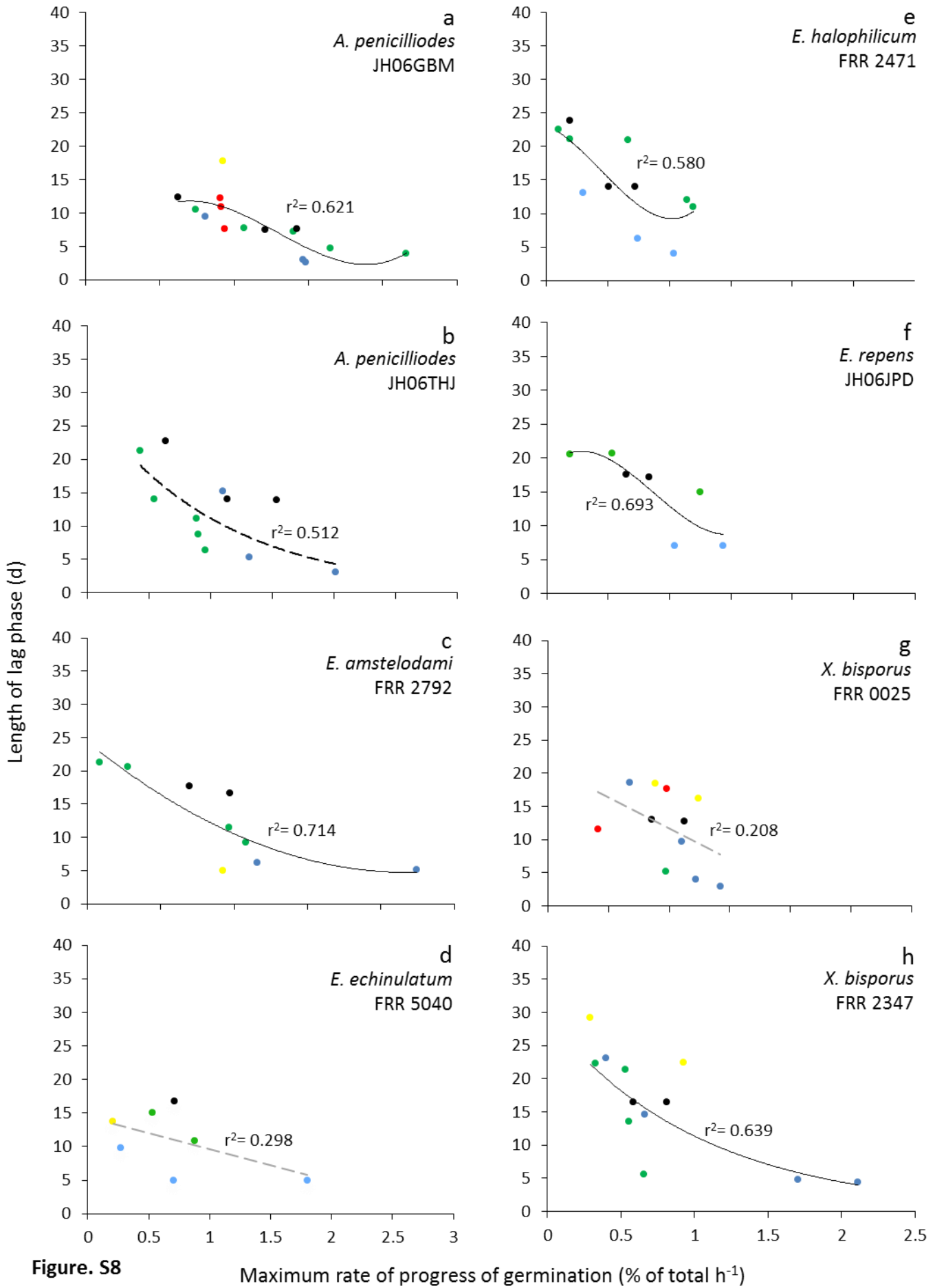


Figure. S8

Maximum rate of progress of germination (% of total h^{-1})

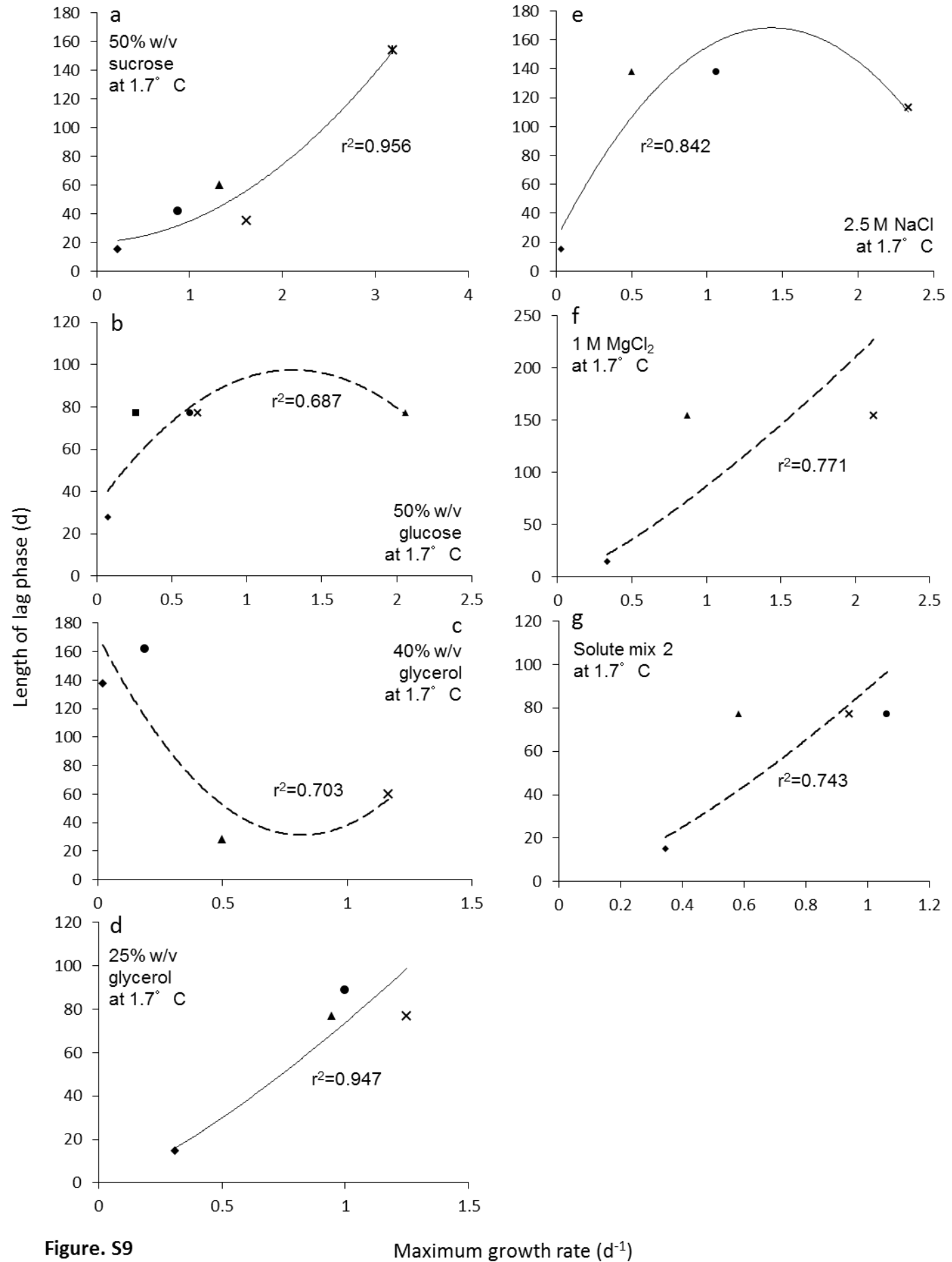


Figure. S9

Maximum growth rate (d^{-1})

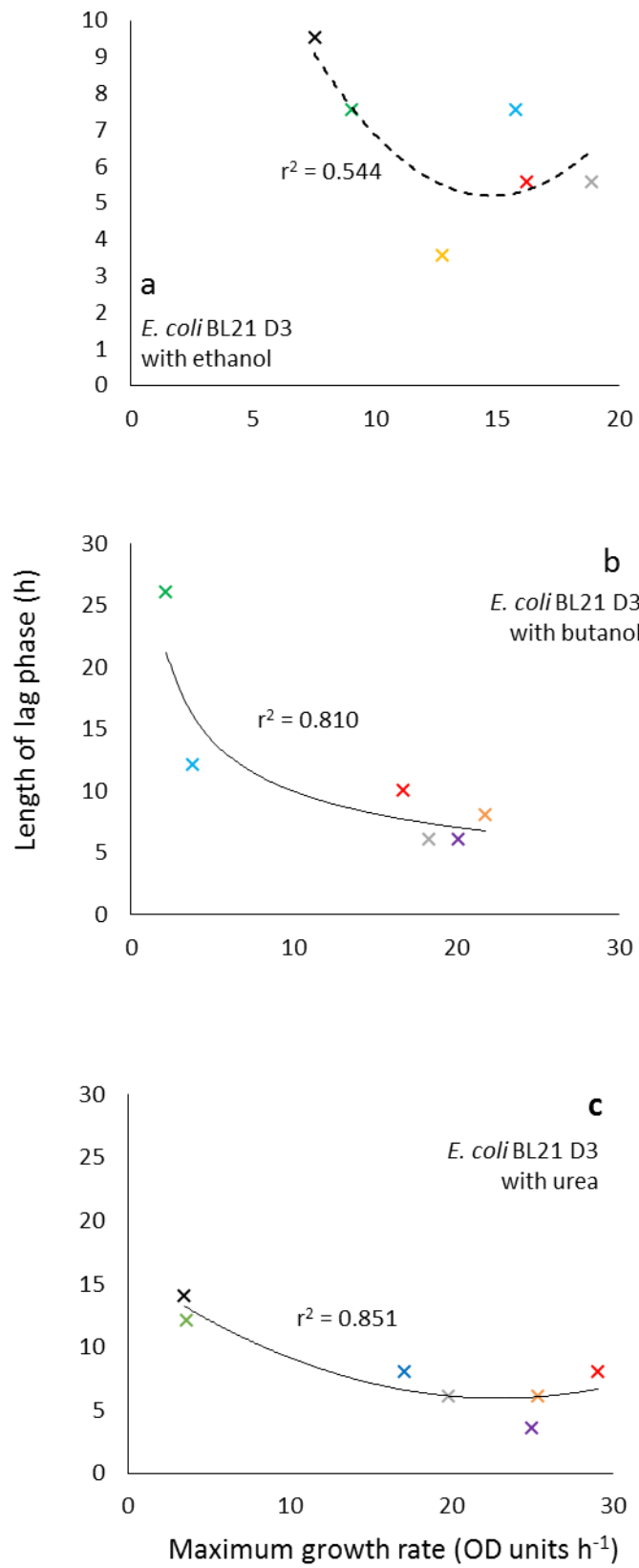


Figure. S10

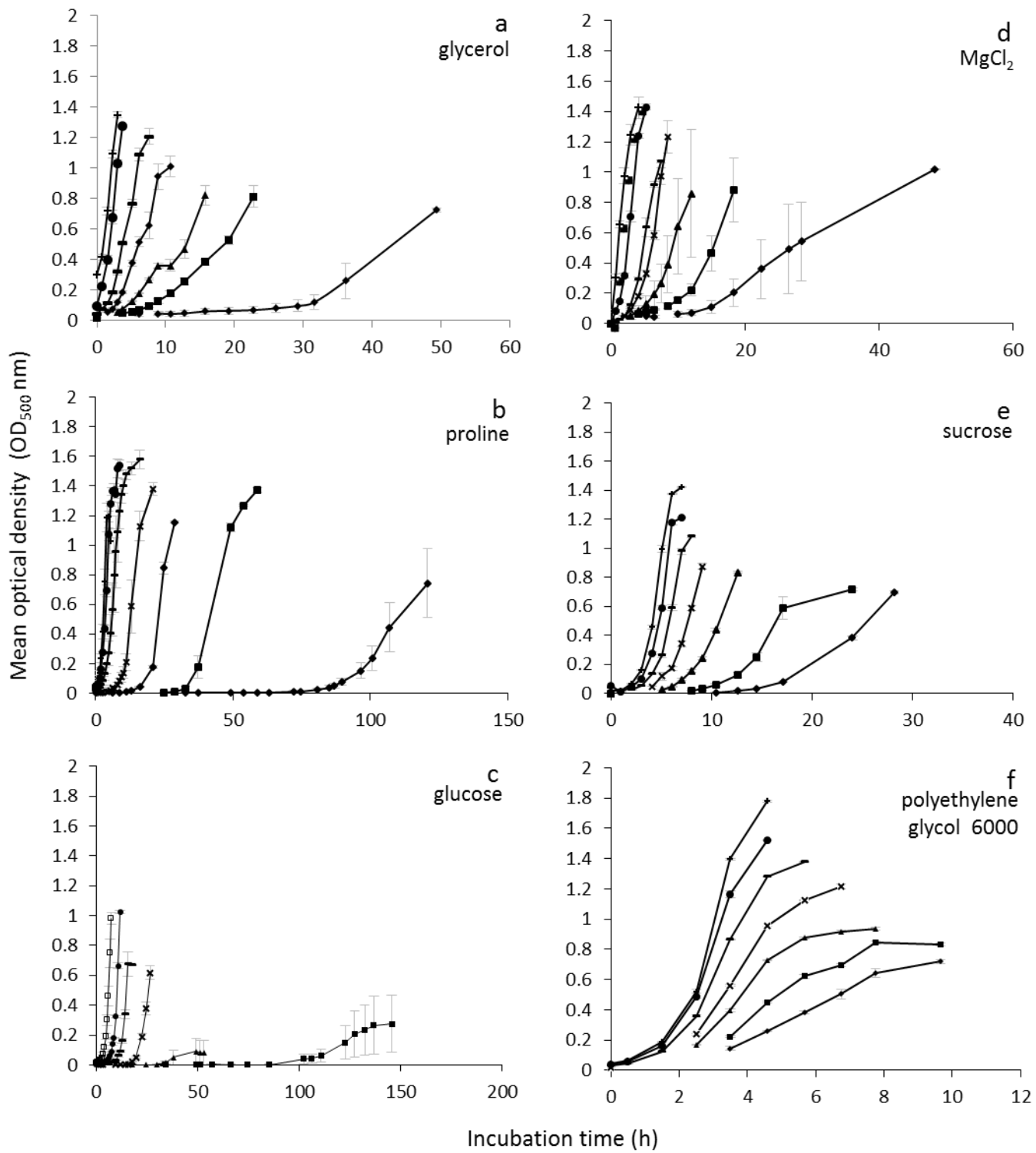


Figure. S11

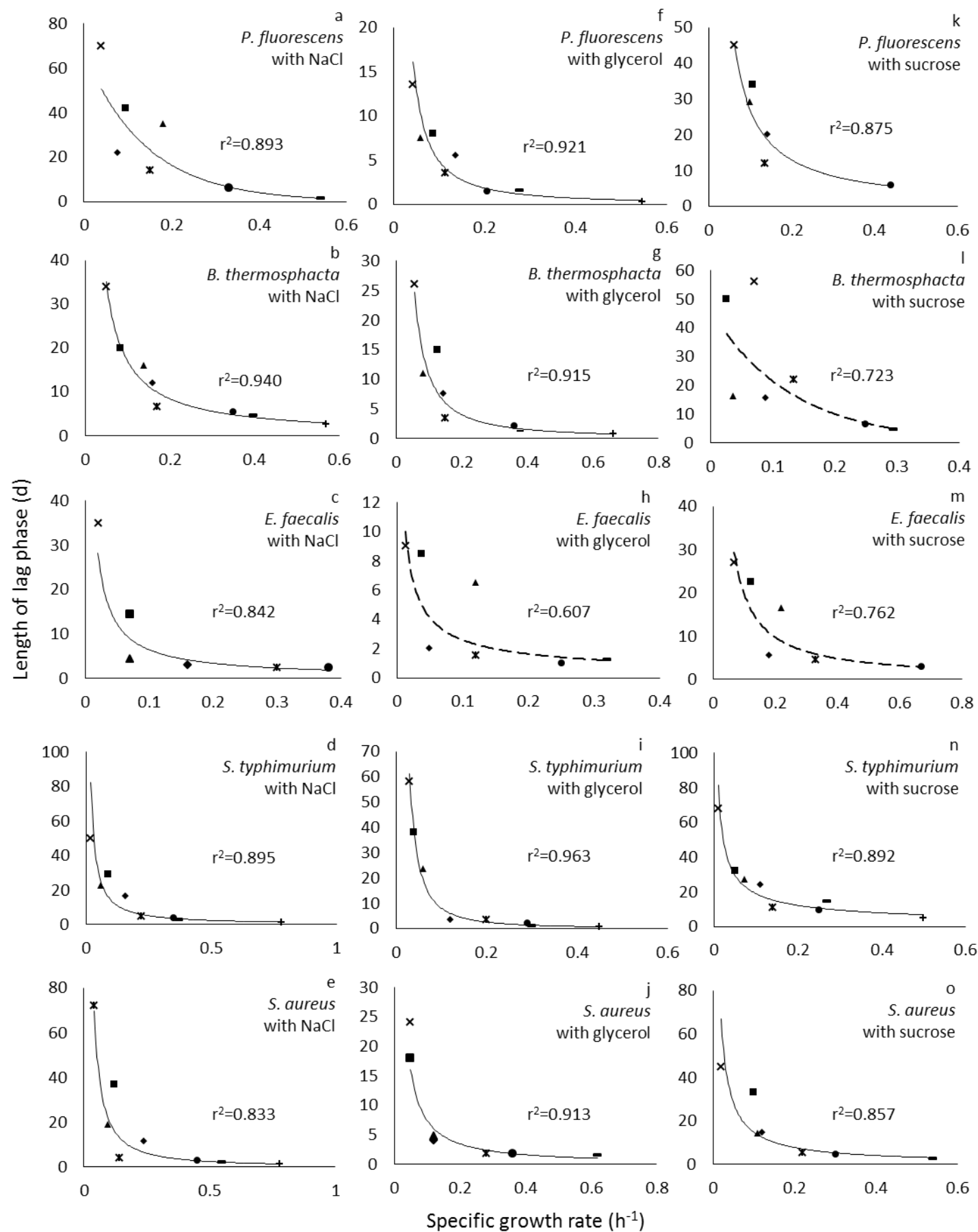


Figure. S12

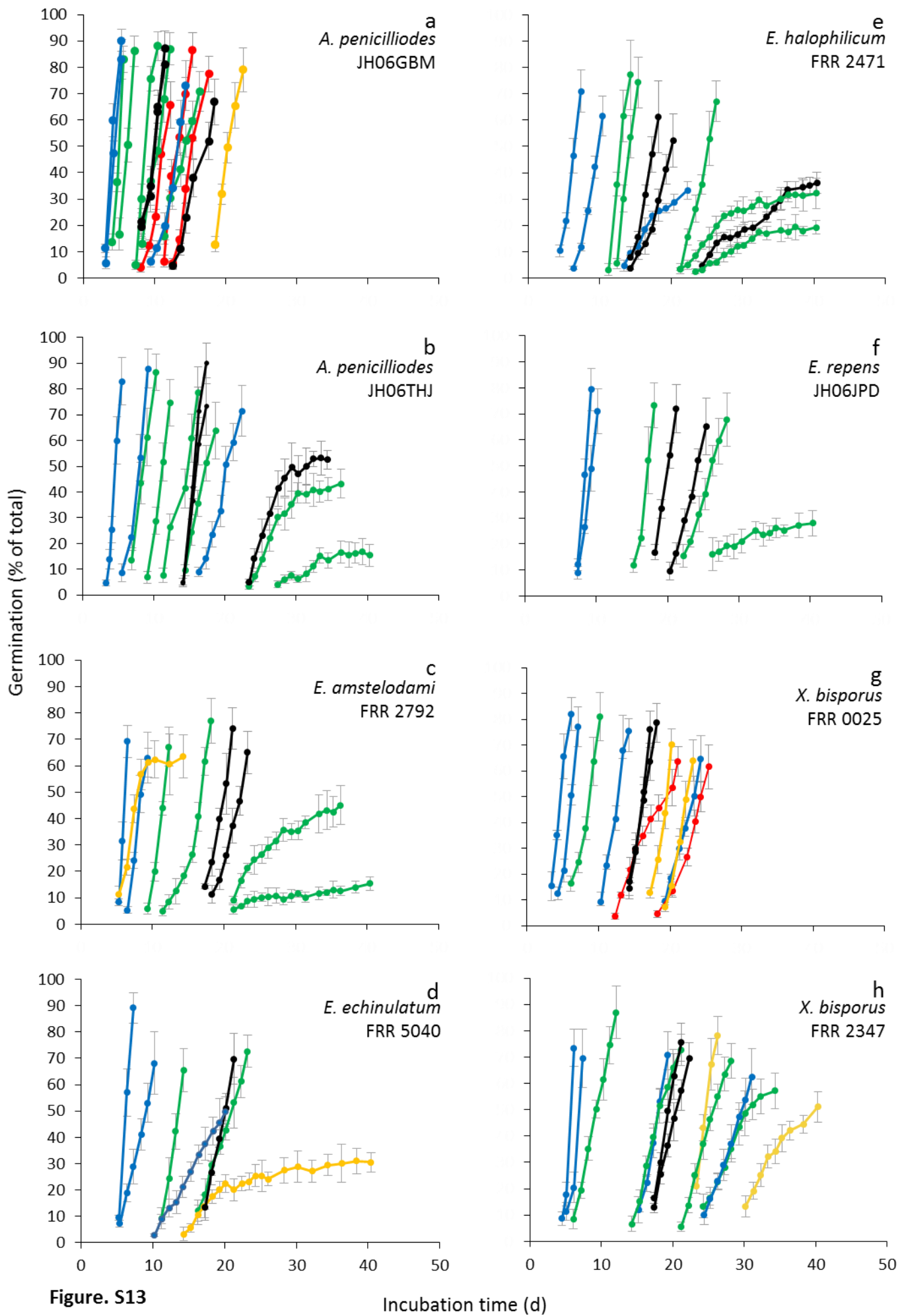


Figure. S13

Incubation time (d)

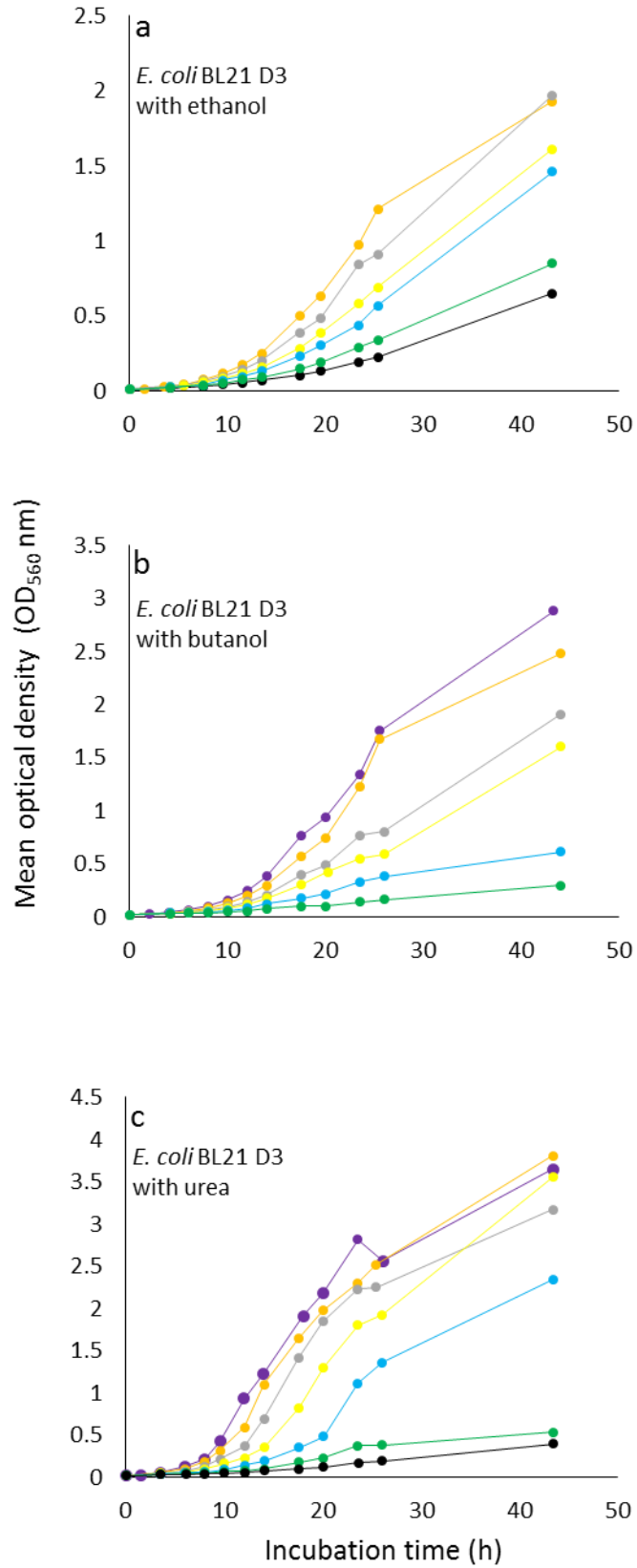


Figure. S14