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## Environmental and Nutritional Factors That Affect Growth and Metabolism of the Pneumococcal Serotype 2 Strain D39 and Its Nonencapsulated Derivative Strain R6

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## Supporting Information

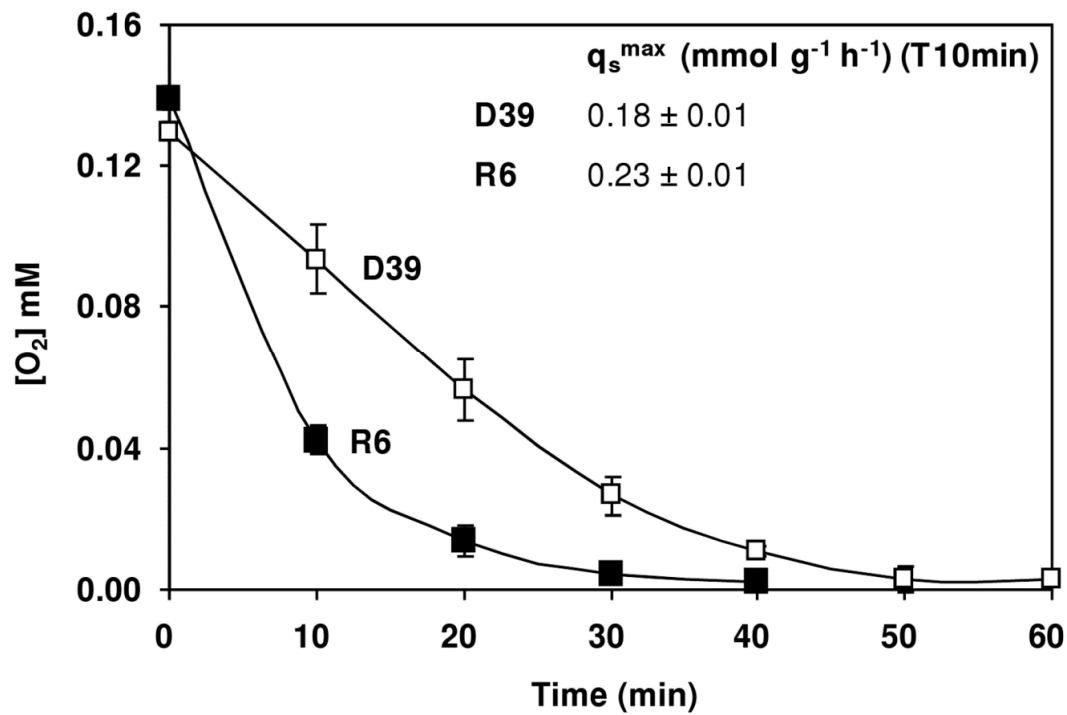


Figure S1.

**Kinetics of oxygen consumption of strains D39 and R6 grown under semi-aerobic conditions.** Strains D39 (□) and R6 (■) were grown under semi-aerobic conditions as in Fig. 2A. The oxygen consumption rates ( $q_s^{\max}$ ) are also shown. The plotted curves are averages of two independent experiments ± SD.

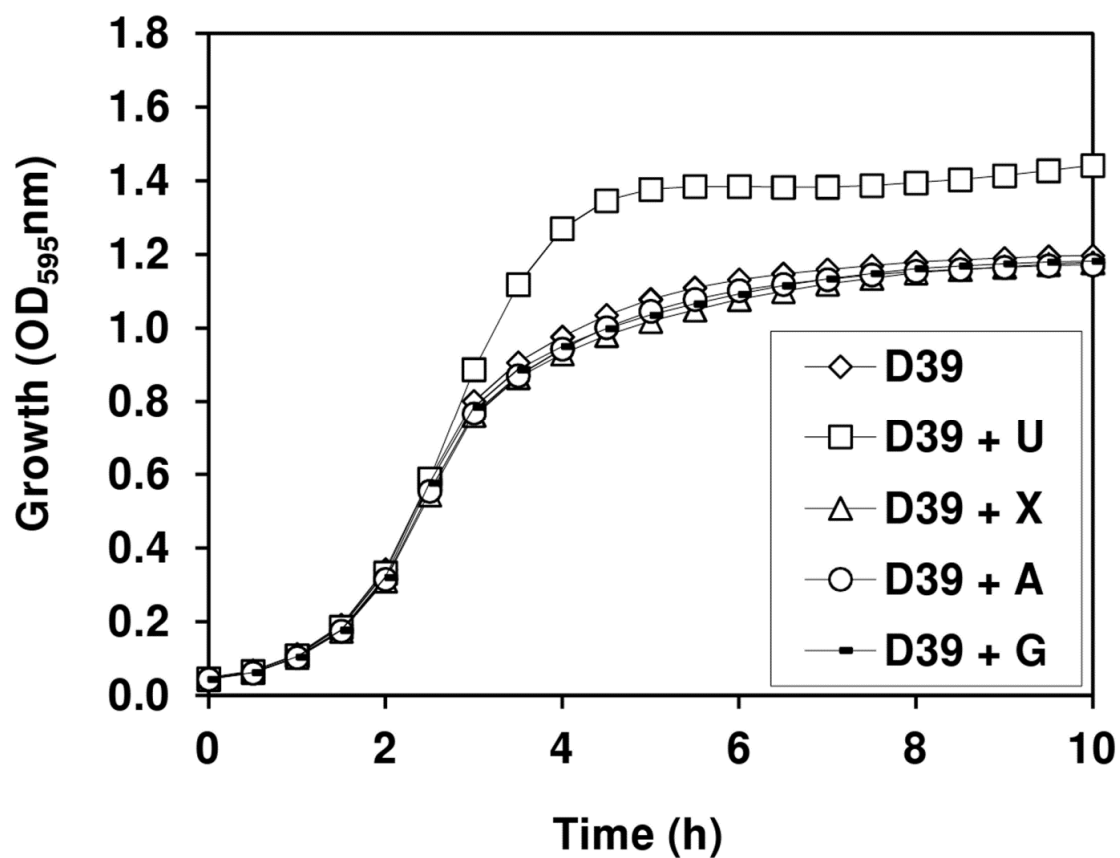
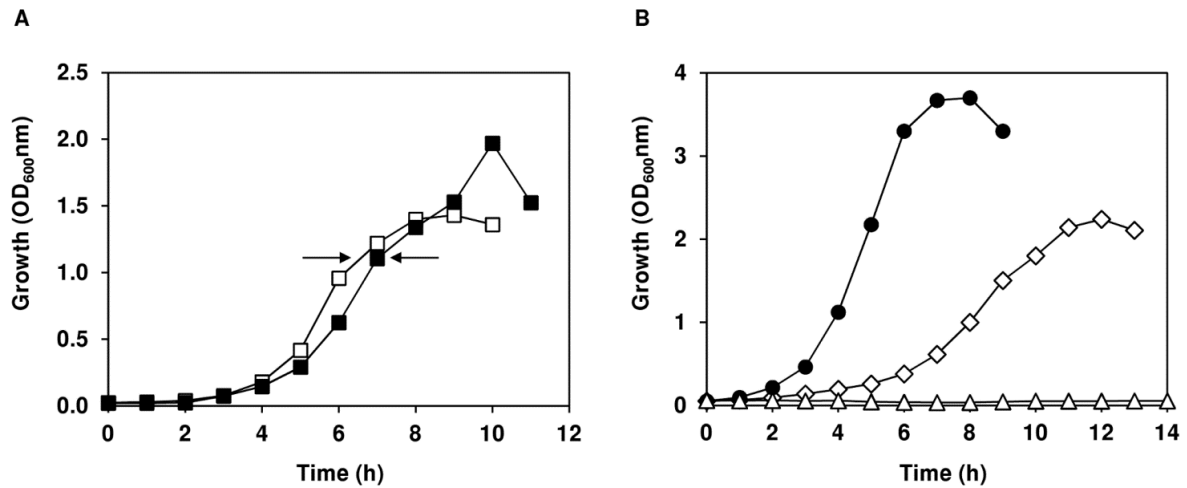


Figure S2.

**Effect on growth of increasing a single nucleobase.** Growth profile of strain D39 in CDM containing 0.25% (wt/vol) glucose with  $30 \text{ mg l}^{-1}$  of the specified nucleobase. Cultures were prepared in  $250 \mu\text{l}$  in 96-well microtiter plates and growth monitored at 595 nm and  $37^\circ\text{C}$ . Symbols: (◇), G, A, X, U  $10 \text{ mg l}^{-1}$  each; (□), G, A, X  $10 \text{ mg l}^{-1}$  each plus  $30 \text{ mg l}^{-1}$  U; (△), G, A, U  $10 \text{ mg l}^{-1}$  each plus  $30 \text{ mg l}^{-1}$  X; (○), G, X, U  $10 \text{ mg l}^{-1}$  each plus  $30 \text{ mg l}^{-1}$  A; (- - -), A, X, U  $10 \text{ mg l}^{-1}$  each plus  $30 \text{ mg l}^{-1}$  G. G = guanine; A = Adenine; X = Xanthine; U = Uracil.



**Figure S3.**

**Growth profiles of D39 and R6 precultures and R6 cultures started with precultures of different ages.** (A) Growth of precultures of strains D39 (□) and R6 (•) in CDM containing 60 mM glucose, without pH control (initial pH of 6.5), at 37 °C, under semi-aerobic conditions (B) Growth of strain R6 in CDM containing 60 mM glucose, under controlled conditions of pH (6.5), temperature (37 °C) and atmosphere (anaerobiosis), in a 2-l bioreactor. Symbols: (•), inoculation with a preculture in late-exponential phase (LExp, 6–7 hours of incubation at 37 °C, OD<sub>600</sub> = 0.8–1.0); (◊), inoculation with a preculture in early-stationary phase (EStat, 8–9 hours of incubation at 37 °C, OD<sub>600</sub> = 1.4–1.6); (△), inoculation with a preculture in late-stationary phase (LStat, 18 hours of incubation at 37 °C, OD<sub>600</sub> ~1).

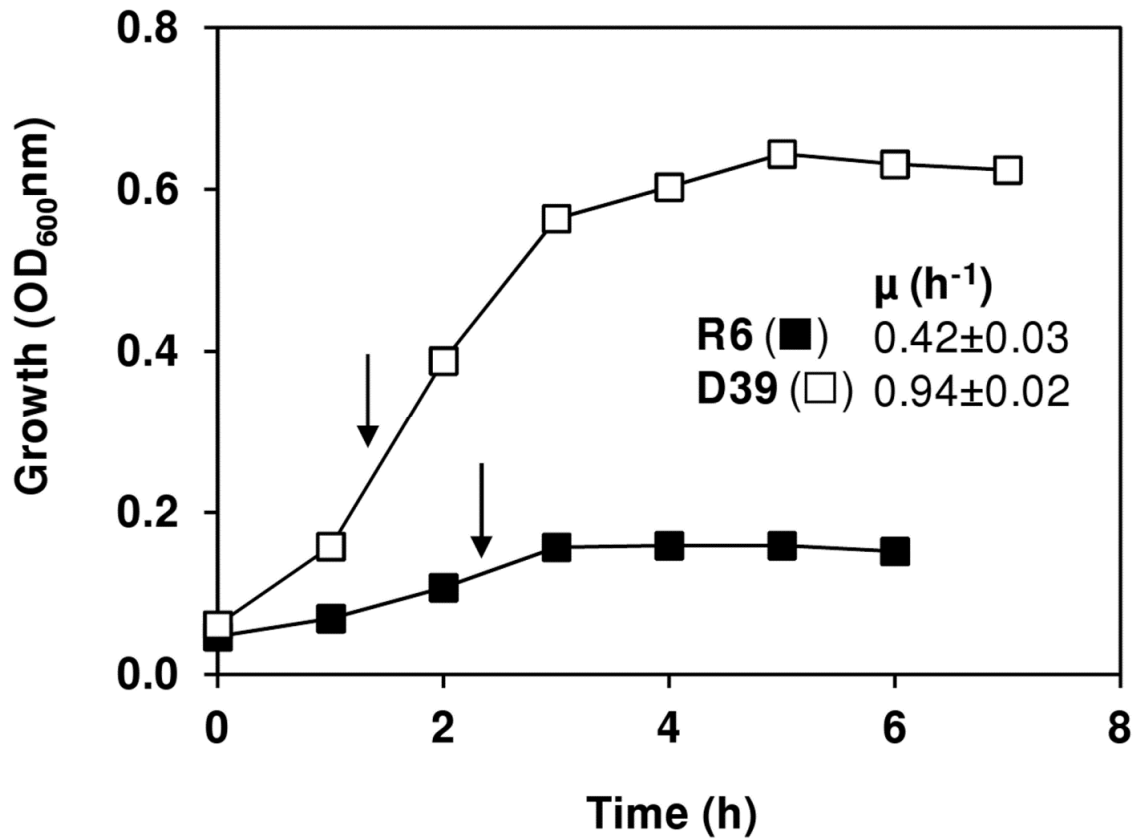


Figure S4.

**Growth profiles of cultures of strains D39 and R6 without pH control under aerobic conditions.**

Growth of strains D39 (□) and R6 (■) in CDM containing 60 mM glucose, without pH control (initial pH of 6.5), at 37°C, under aerobic conditions. The arrows indicate the time-points at which cells were harvested for measurement of NADH oxidase activities. The growth rate for each culture is also indicated and the values are averages  $\pm$  SD.

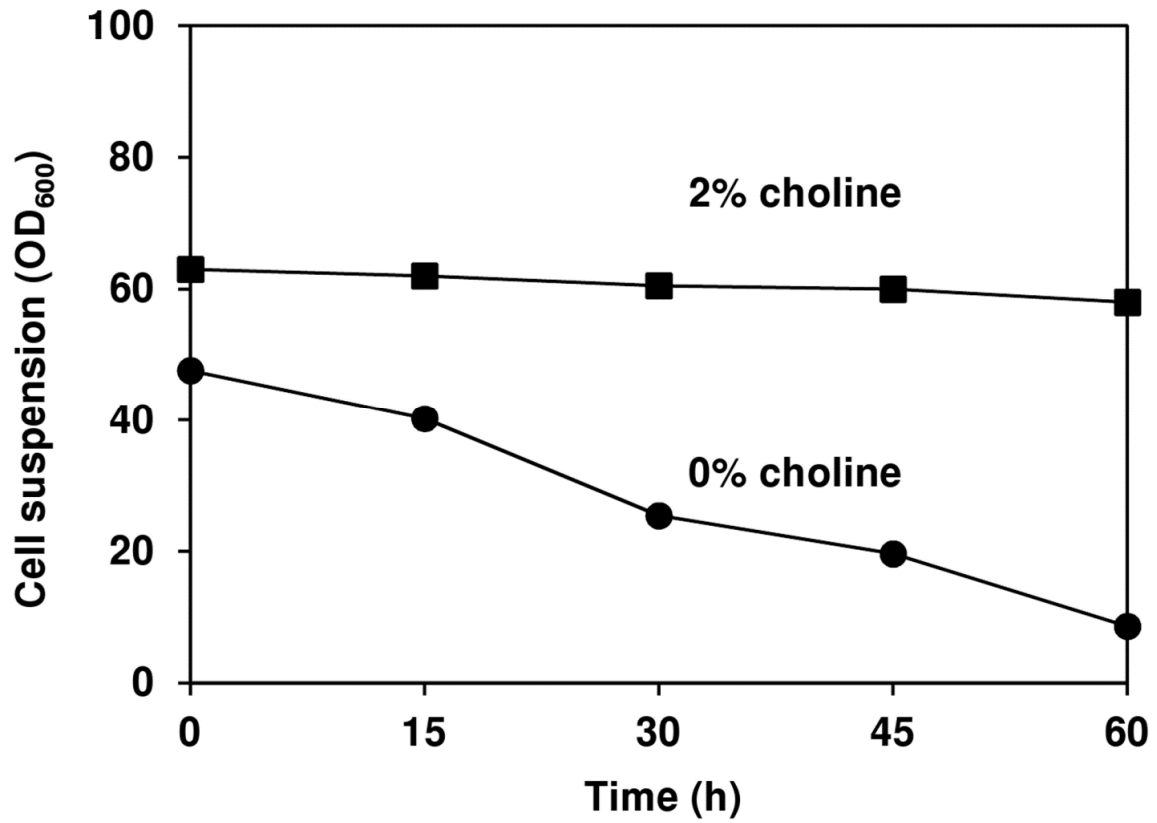


Figure S5.

**Pneumococcal lysis in resting cell suspensions.** Optical density variation during glucose (20 mM) metabolism of resting cells of strain R6, grown as for *in vivo* NMR, suspended in 50 mM  $KP_i$  with (▪) 2% or (•) 0% (wt/vol) choline.