The growth of the soil bacterium Pseudomonas putida KT2440 on glycerol as the sole carbon source is characterized by a prolonged lag phase, not observed with other carbon substrates. We examined the bacterial growth in glycerol cultures while monitoring the metabolic activity of individual cells. Fluorescence microscopy and flow cytometry, as well as the analysis of the temporal start of growth in single-cell cultures, revealed that adoption of a glycerol-metabolizing regime was not the result of a gradual change in the whole population but rather reflected a time-dependent bimodal switch between metabolically inactive (i.e., nongrowing) and fully active (i.e., growing) bacteria. A transcriptional ϕ (glpD-gfp) fusion (a proxy of the glycerol-3-phosphate [G3P] dehydrogenase activity) linked the macroscopic phenotype to the expression of the glp genes. Either deleting glpR (encoding the G3P-responsive transcriptional repressor that controls the expression of the glpFKRD gene cluster) or altering G3P formation (by overexpressing glpK, encoding glycerol kinase) abolished the bimodal glpD expression. These manipulations eliminated the stochastic growth start by shortening the otherwise long lag phase. Provision of glpR in trans restored the phenotypes lost in the ΔglpR mutant. The prolonged nongrowth regime of P. putida on glycerol could thus be traced to the regulatory device controlling the transcription of the glp genes. Since the physiological agonist of GlpR is G3P, the arrangement of metabolic and regulatory components at this checkpoint merges a positive feedback loop with a nonlinear transcriptional response, a layout fostering the observed time-dependent shift between two alternative physiological states.