Genetic analysis of stress responses in soil bacteria for enhanced bioremediation of mixed contaminants

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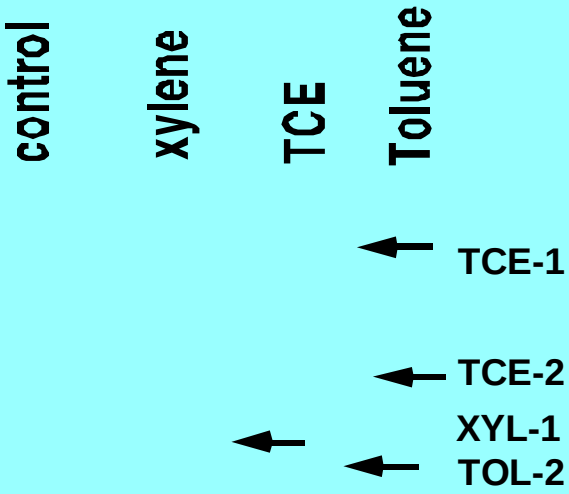
Introduction

Bioremediation is, in some cases, a cost-effective approach to site clean-up. Many bacteria have been isolated and are capable of degrading various environmental pollutants. However, in natural environment or contaminated sites, the number of such bacteria is usually too scanty to have an impact on the bioremediation process. To enhance the bioremediation process, nutrient have been used to stimulate the growth of such indigenous bacteria. To fully understand the potential of biostimulation, we have analyzed the competitive growth of a few subsurface bacteria under limiting nutrient supply. We found that in contrast to large dose of nutrient supply, limiting nutrient stimulation may have a harmful effect on the relative abundance of biodegradative bacteria.

To realize the full potential of bioremediation, an understanding of microbial community and individual bacterial responses to the stresses encountered at contaminated sites is needed. Knowledge about genetic responses of soil and subsurface bacteria to environmental stresses, which include low nutrients, and pollutants, will allow extrapolation of basic principles to field applications either using indigenous bacteria or genetically-engineered microorganisms. Defining bacterial responses to those stresses presents an opportunity for improving bioremediation strategies, both with indigenous populations and genetically-engineered microbes, and should contribute to environmental management and restoration actions that would reduce the cost and time required to achieve OEM's clean up goals.

Solvent stress



TCE-1: mannosyltransferase (mtfB)

TCE-2: modification methylase, putative

Xyl-1: glycosyltransferase

Tol-2: glycosyltransferase

Figure 1. Identification of solvent inducible genes by RNA fingerprinting.

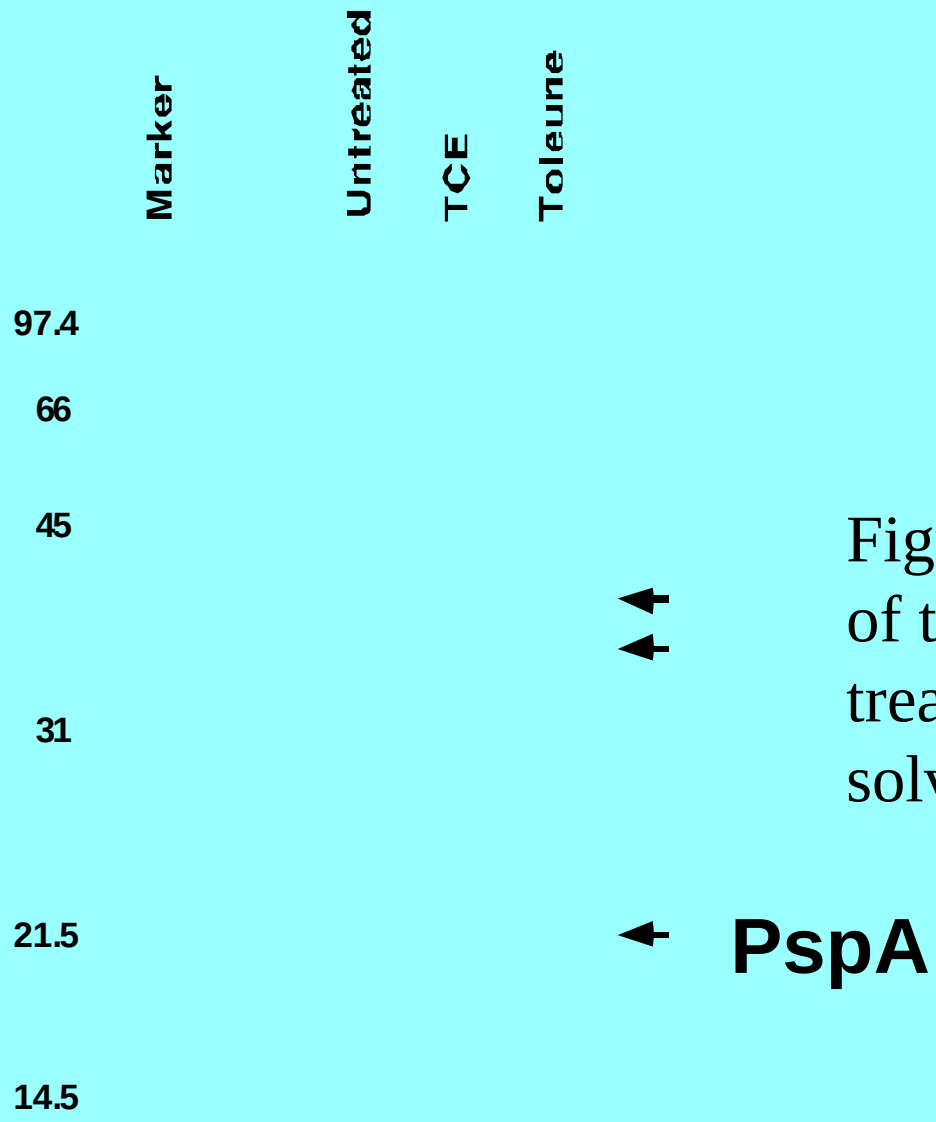


Figure 2. SDS-PAGE analysis of total proteins from solvent treated cells. Arrows indicates solvent inducible proteins.

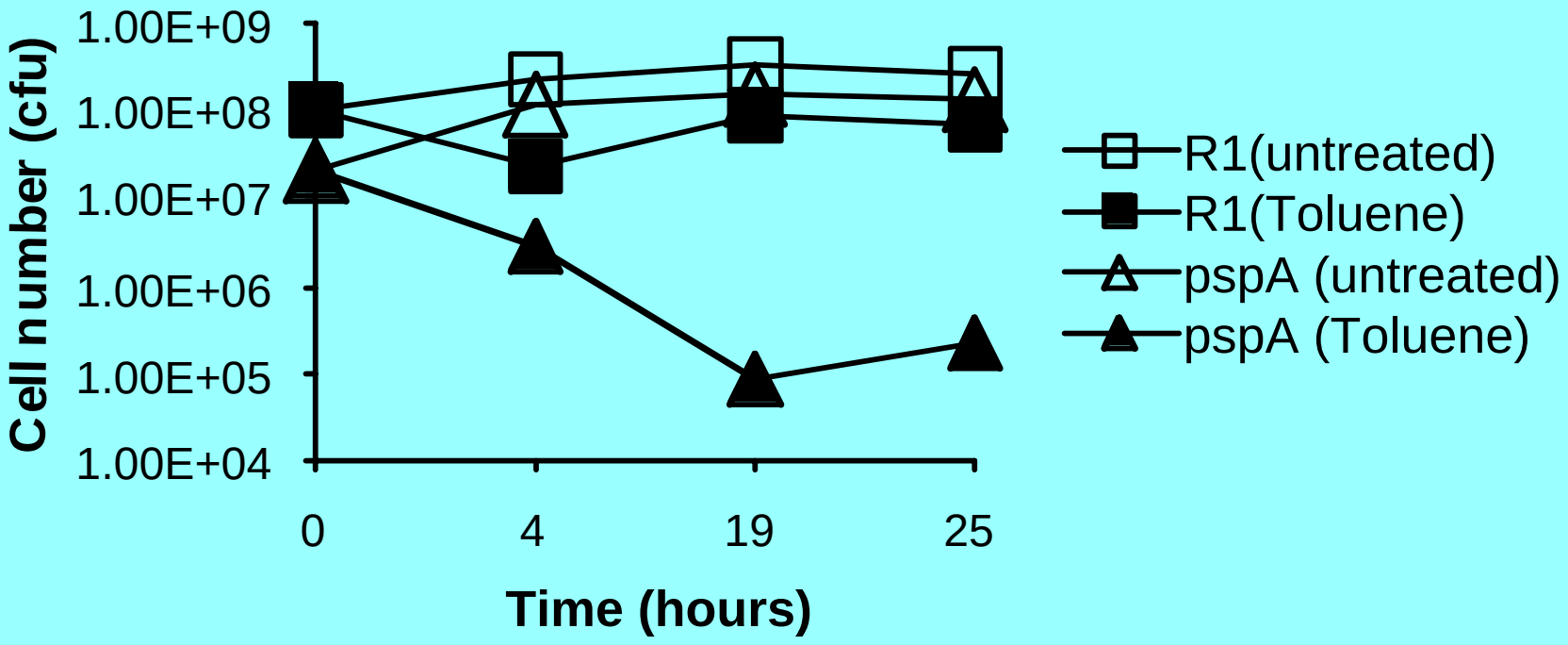
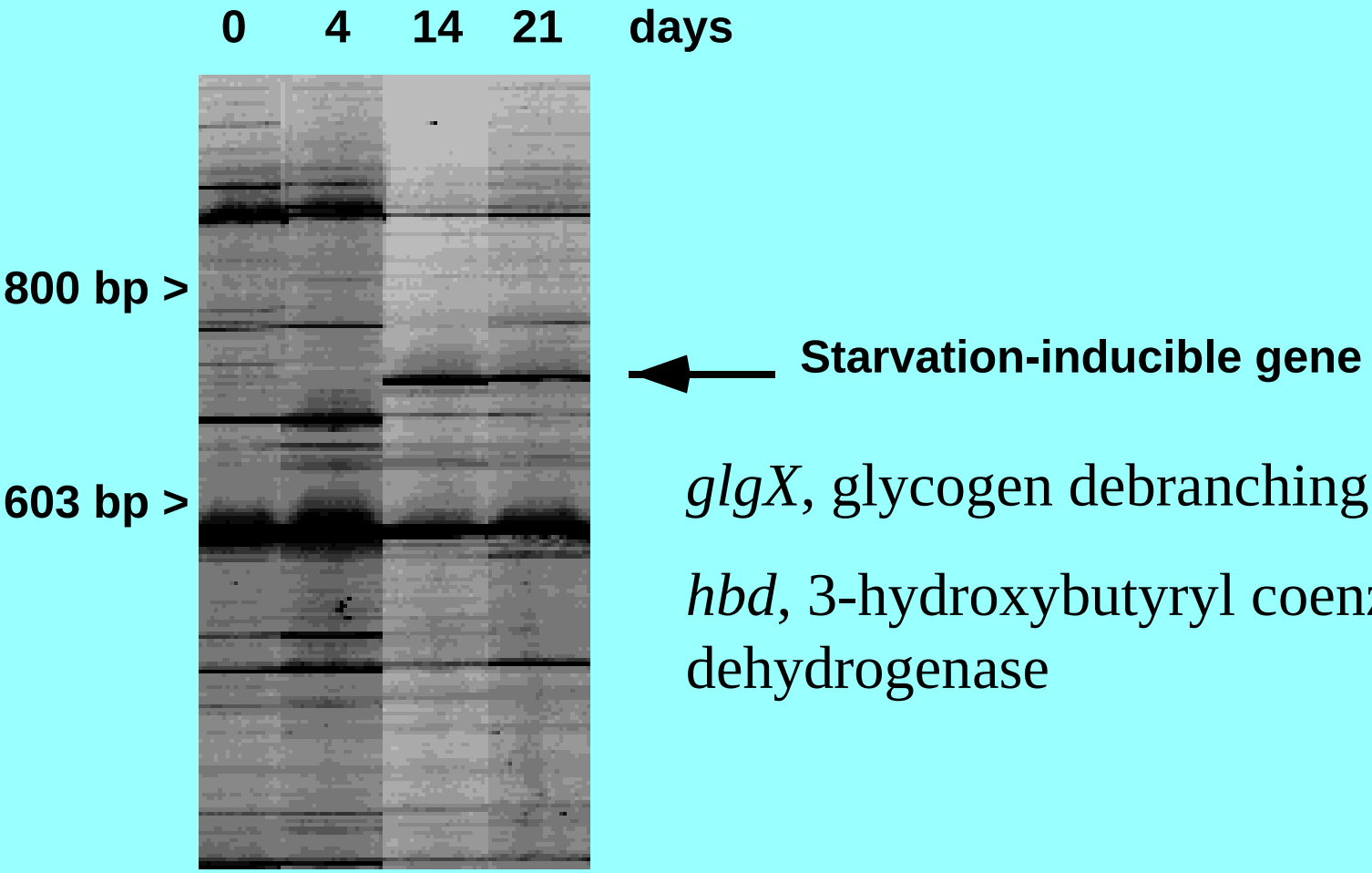


Figure 4. Solvent Sensitivities of *pspA* mutant and the wild-type strain R1.

Starvation stress



glgX, glycogen debranching enzyme

hbd, 3-hydroxybutyryl coenzyme A dehydrogenase

Figure 3. Identification of starvation-inducible genes by RNA fingerprinting analysis.

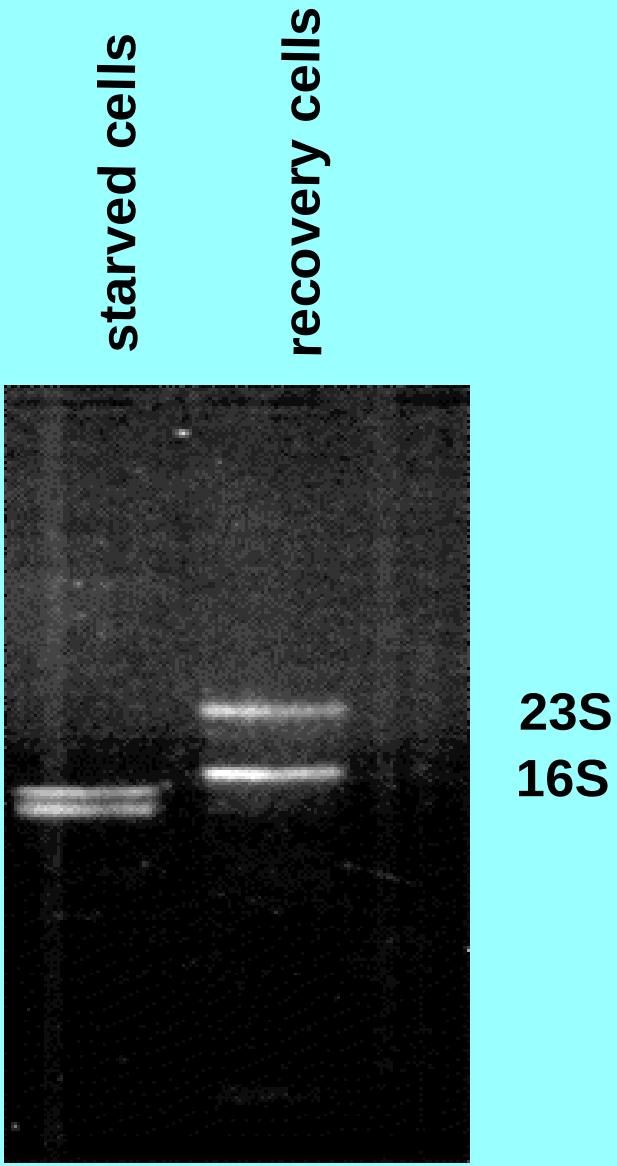


Figure 8. Analysis of rRNA from starved culture.

Oxidative and ionizing stress

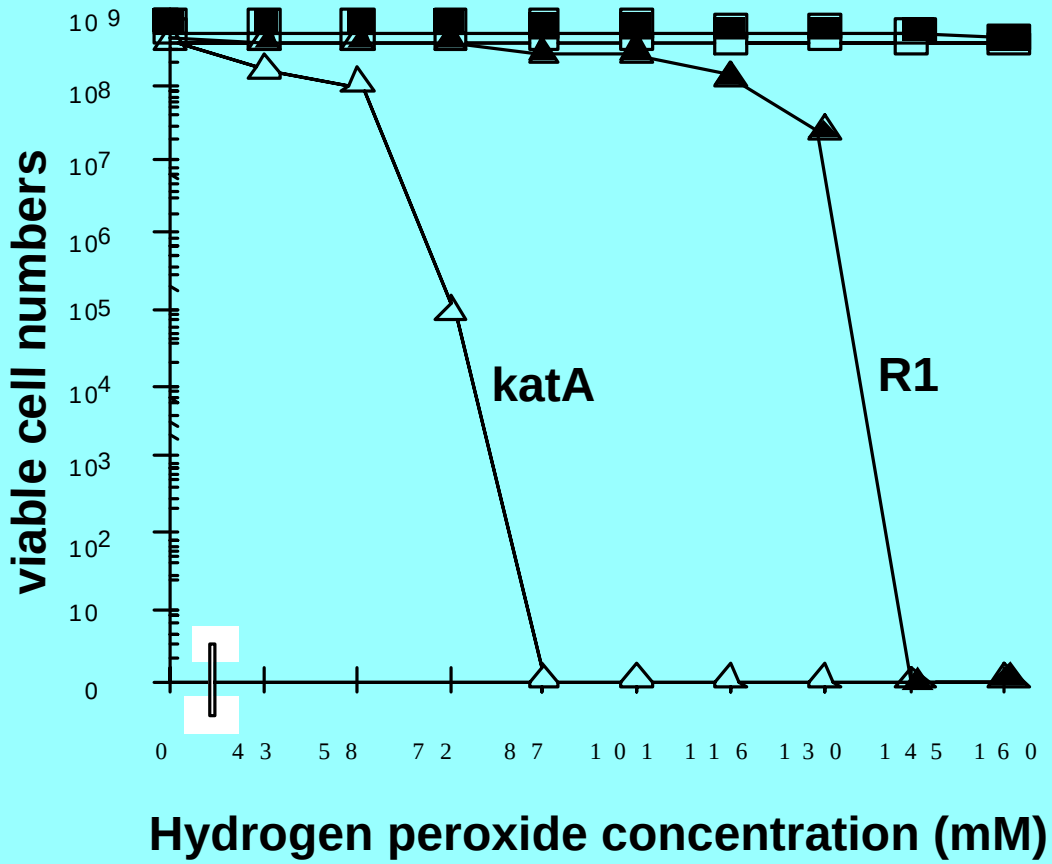


Figure 5. Hydrogen peroxide sensitivity of wildtype R1 and *katA* strains. Δ, treated cell; , untreated control.

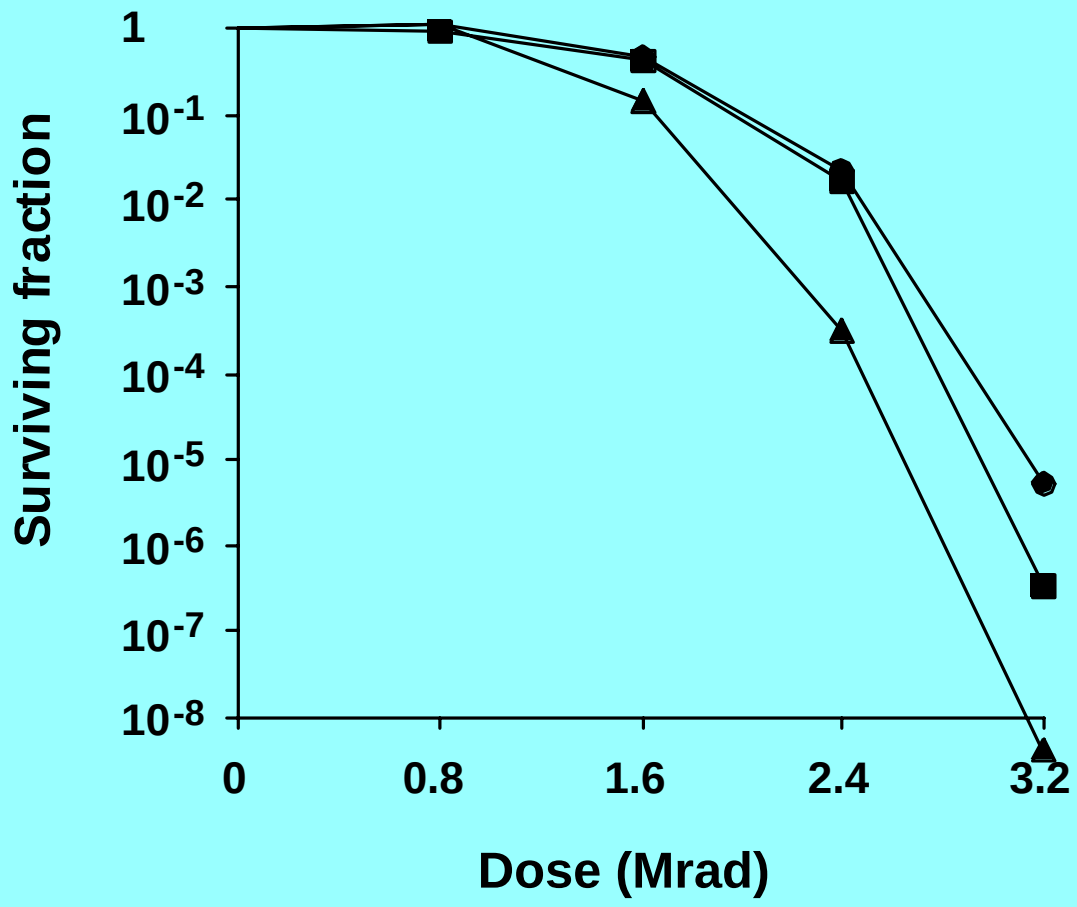


Figure 6. Radiation sensitivities of *katA* and *sodA* mutants. ●, wild-type; ■, katA; ▲, sodA. (Markillie et al., 1999)

Recovery from starvation

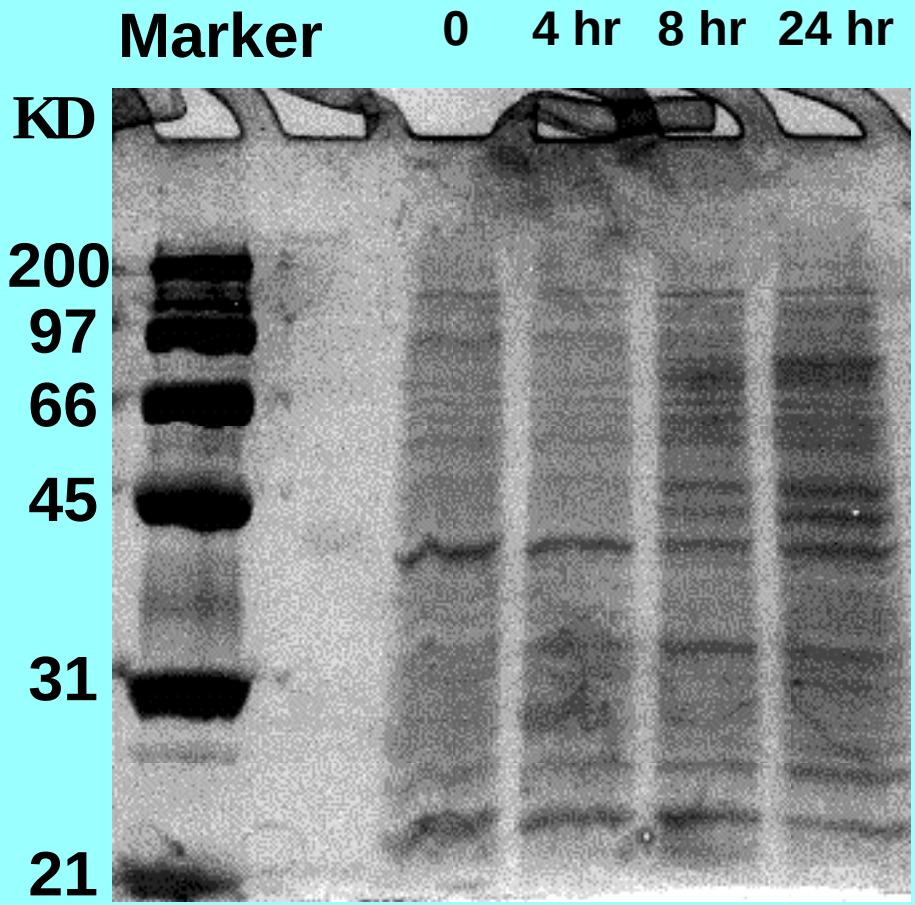


Figure 7. Analysis of total proteins of resuscitated starved *Sphingomonas aromaticivorans* F199 cultures.

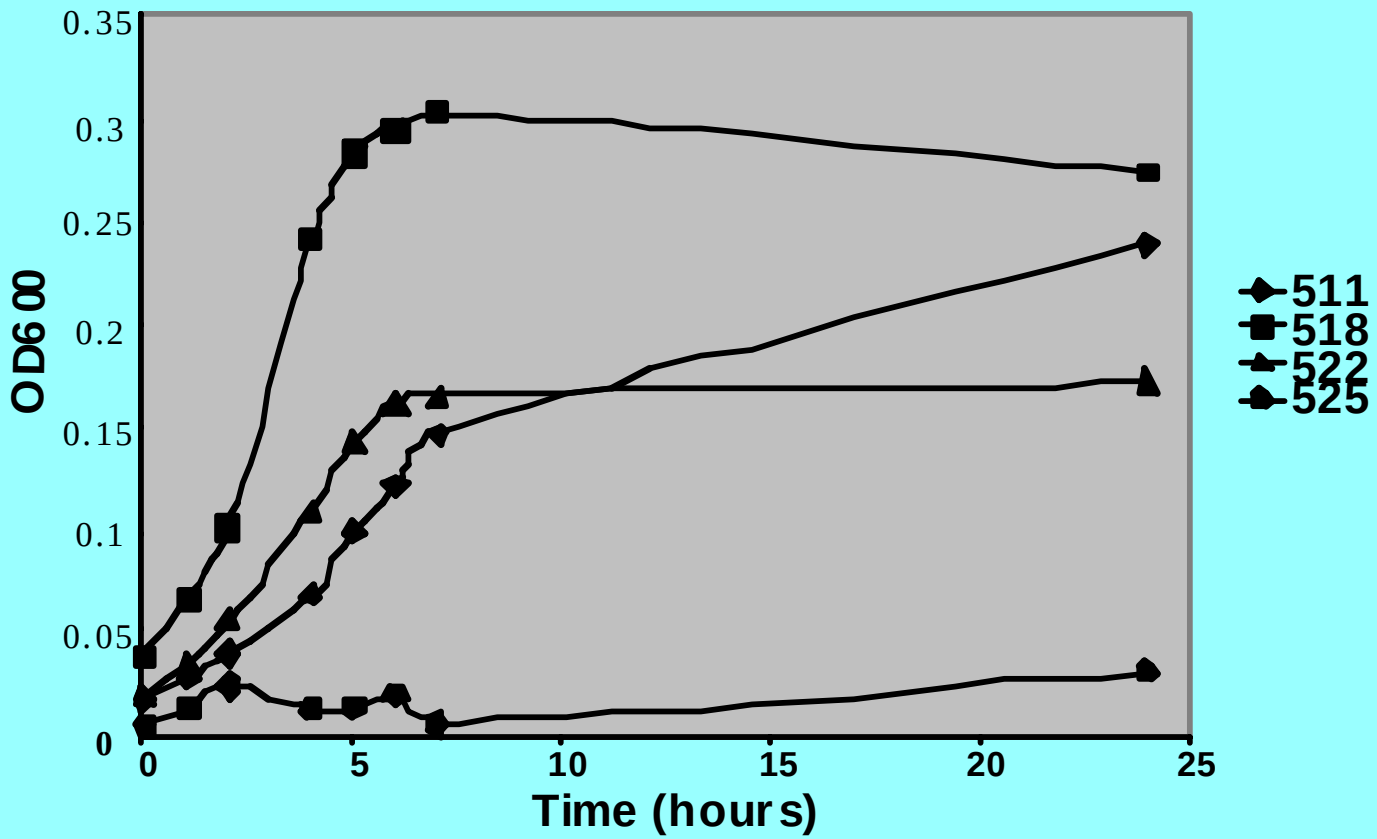


Figure 10. Growth Curves of subsurface bacteria in 1% PTYG (low nutrient).

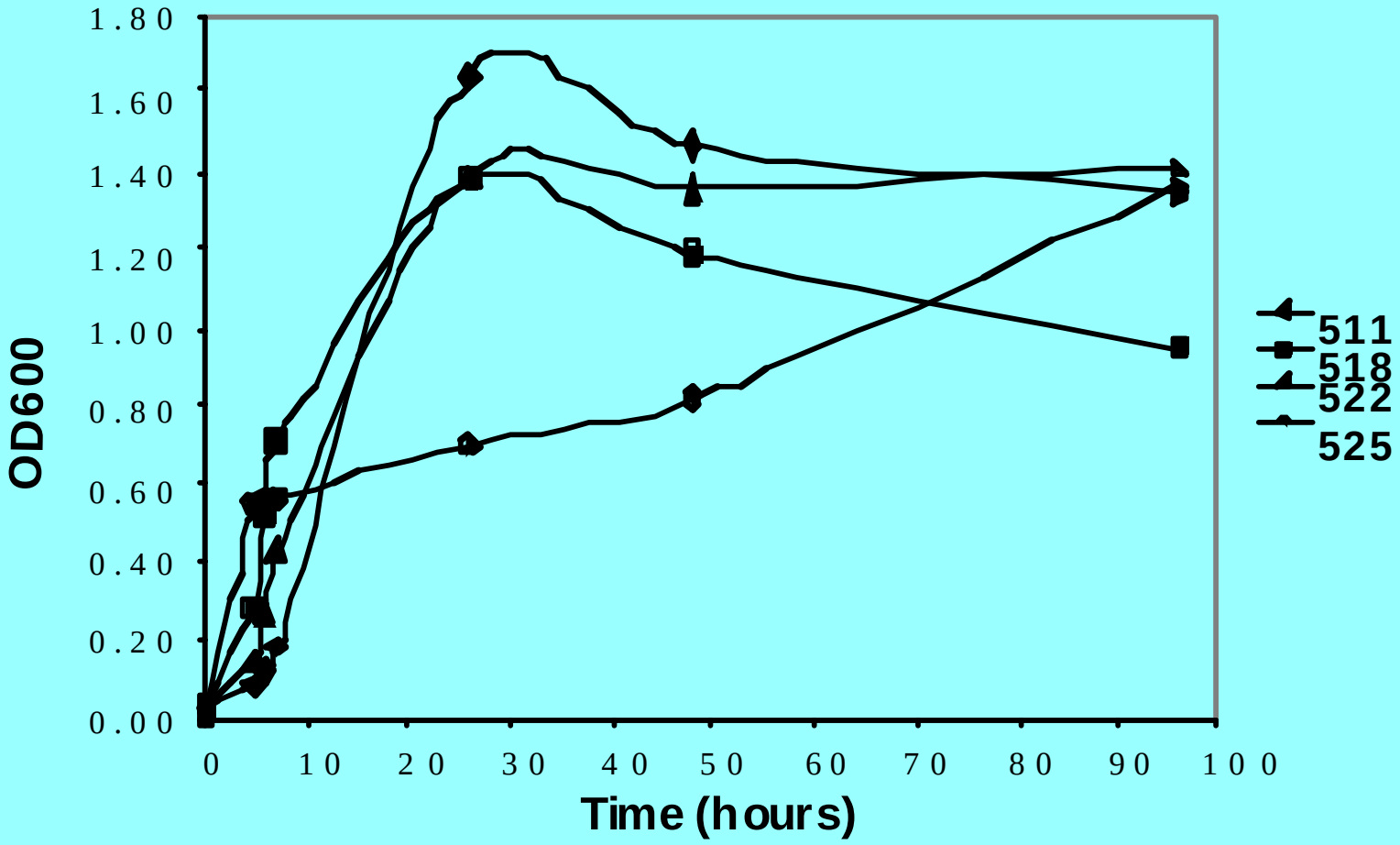


Figure 11. Growth curves of subsurface bacteria in 10% PTYG (high nutrient).

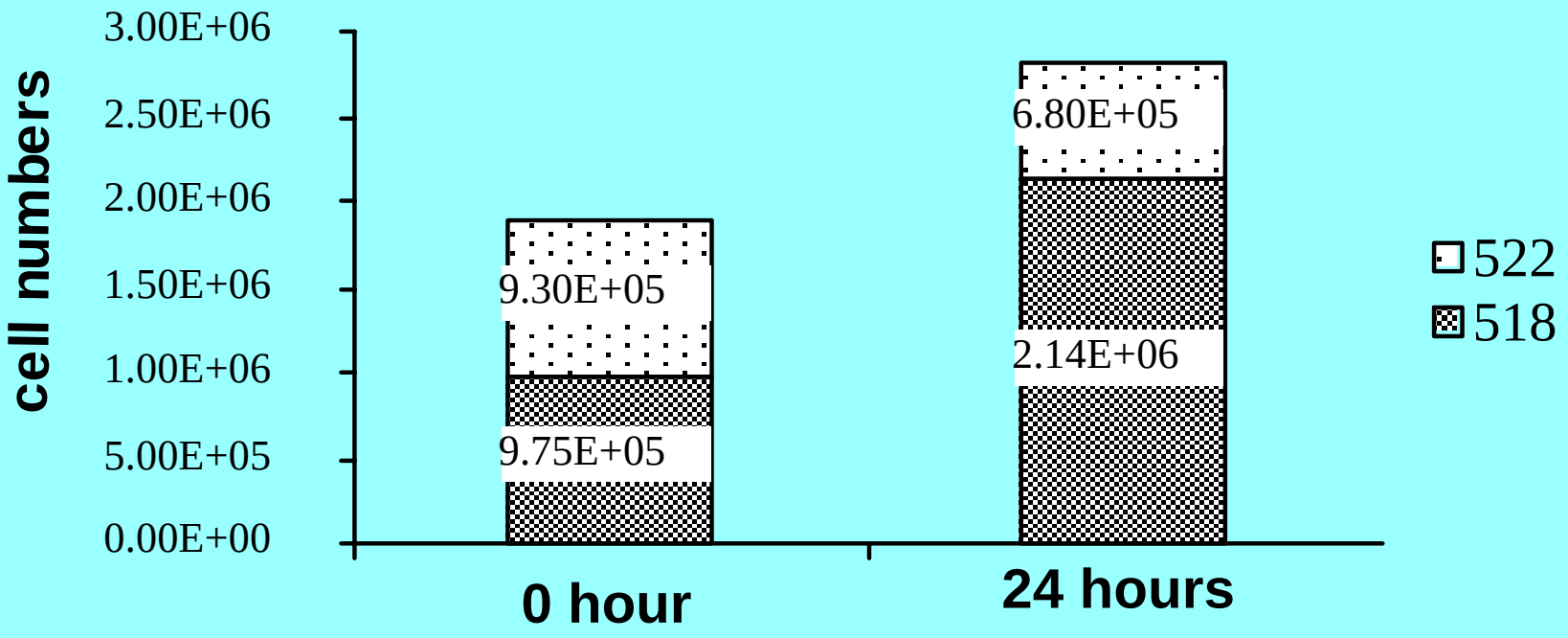


Figure 12. Relative ratio of B518 and B522 cocultures after starvation for 24 Hours

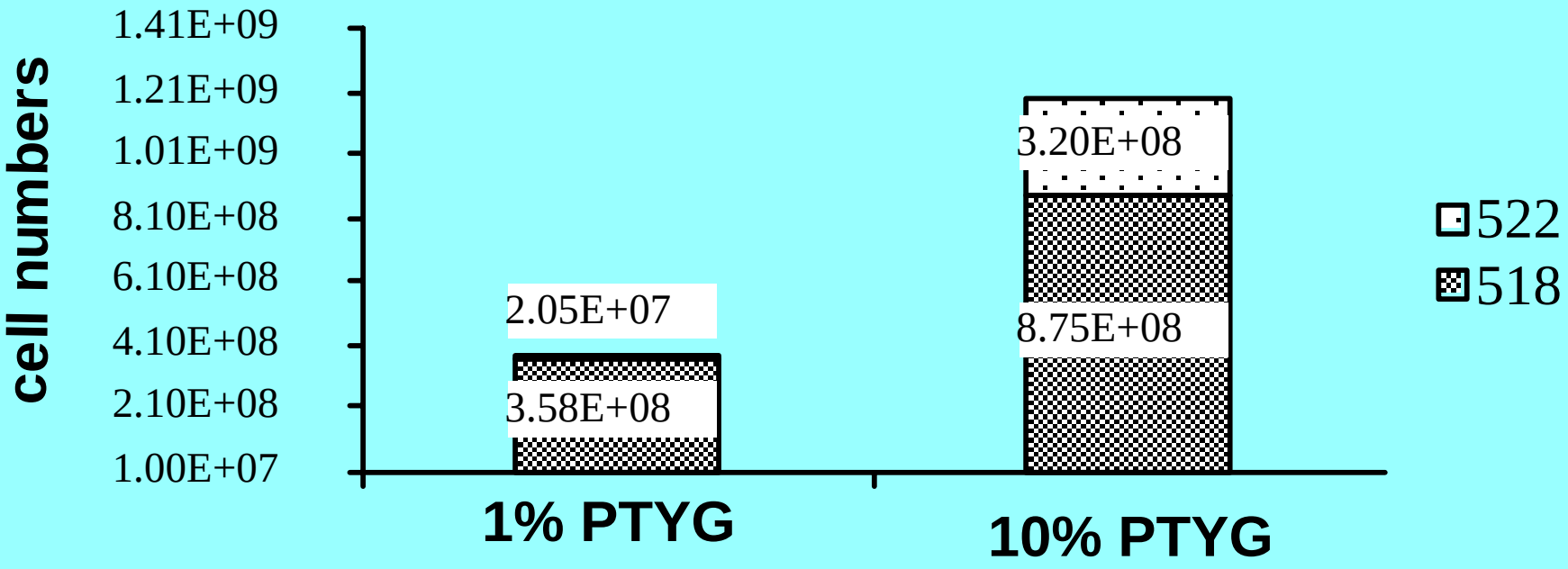


Figure 13. Effect of nutrient supplies on starved B518 and B522 cocultures

Summary

Genetic analysis of stress responses suggests:

•*Deinococcus radiodurans* use reserved glycogen or lipid during starvation.

•*Deinococcus radiodurans* has an increase in lipopolysaccharide synthesis and PspA protein amount during solvent stress.

•The 23S rRNA of *Sphingomonas aromaticivorans* undergoes defined fragmentation during starvation.

•The strength of nutrient supply affect the relative growth of individual bacterium in a mixed culture.

Potential application in bioremediation

• Use of stress inducible genes to monitor the bioremediation process.

•Design 23S rRNA probes to monitor biostimulation

•Use of stress inducible genes to direct the expression of biodegradative genes cloned from other bacteria into *Deinococcus radiodurans* R1.

•Design nutrient composition to stimulate indigenous subsurface bacteria capable of degradation of wastes.