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Use of Prokaryotic Stress Promoters as Indicators of the Mechanisms of Chemical Toxicity

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ABSTRACT

Fifteen *Escherichia coli* strains and one *Salmonella typhimurium* strain, each containing a different stress promoter fused to a promoterless *lacZ* structural gene, were assembled on 96 well microtiter plates for use in a modified β-galactosidase protocol called the "Pro-Tox" assay. The microtiter plate format allowed the strains to be exposed simultaneously to a range of concentrations of xenobiotics. After incubation with the chemical, the cells were permeabilized and assayed for β-galactosidase activity. For any given chemical over a range of concentrations, a particular subset of the stress promoter::*lacZ* fusion genes in the various strains were induced in comparison to the unexposed controls. Using this method, large numbers of compounds can be screened for their ability to induce any of the promoter::*lacZ* fusions. Induction of a specific subset of promoter::*lacZ* fusions can reveal information on the mechanism of toxicity of certain compounds. As new prokaryotic damage-inducible genes are characterized, novel promoter::*lacZ* fusions may be included in the Pro-Tox assay.

INTRODUCTION

Every year the pharmaceutical, chemical, and consumer products industries produce thousands of new chemical compounds that require testing for genotoxic and cytotoxic effects. Currently, a few prokaryotic-based toxicity assays are available. These assays include the Microtox assay (Fort, 1992), the *Salmonella typhimurium*/microsome Ames assay (Ames et al., 1975; McCann et al., 1975), the SOS Chromotest (Quillardet and Hofnung, 1985), the SOS/*umu* test (Oda et al., 1985), and prokaryotic luminescence-based techniques utilizing fluorescence detection (*lux* genes) (Rattray et al., 1990; Burlage et al., 1992; Heitzer et al., 1992; Van Dyk et al., 1994). These assays are used for the detection of genotoxic and cytotoxic compounds. There is, however, a growing need for assays that can provide information on the mechanisms of toxicity of compounds. We describe here an assay system that is able to give an indication of such mechanism(s) of toxicity quickly and easily. The system consists of the *lacZ* structural gene fused to and under the control of a wide variety of *Escherichia coli* and *Salmonella typhimurium* stress gene promoters stably integrated into the *E. coli* chromosome.

Many *Escherichia coli* and *Salmonella* promoters have been described that respond to various stress factors and chemicals. Fusions of such promoters to the *lacZ* reporter gene have

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