Video Article

Ecotoxicological Method with Marine Bacteria *Vibrio anguillarum* to Evaluate the Acute Toxicity of Environmental Contaminants

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Abstract

Bacteria are an important component of the ecosystem, and microbial community alterations can have a significant effect on biogeochemical cycling and food webs. Toxicity testing based on microorganisms are widely used because they are relatively quick, reproducible, cheap, and are not associated with ethical issues. Here, we describe an ecotoxicological method to evaluate the biological response of the marine bacterium *Vibrio anguillarum.* This method assesses the acute toxicity of chemical compounds, including new contaminants such as nanoparticles, as well as environmental samples. The endpoint is the reduction of bacterial culturability (*i.e.,* the capability to replicate and form colonies) due to exposure to a toxicant. This reduction can be generally referred to as mortality. The test allows for the determination of the LC_{50}, the concentration that causes a 50% decrease of bacteria actively replicating and forming colonies, after a 6-h exposure. The culturable bacteria are counted in terms of colony forming units (CFU), and the "mortality" is evaluated and compared to the control. In this work, the toxicity of copper sulphate ($CuSO_4$) was evaluated. A clear dose-response relationship was observed, with a mean LC_{50} of 1.13 mg/L, after three independent tests. This protocol, compared to existing methods with microorganisms, is applicable in a wider range of salinity and has no limitations for colored/turbid samples. It uses saline solution as the exposure medium, avoiding any possible interferences of growth medium with the investigated contaminants. The LC_{50} calculation facilitates comparisons with other bioassays commonly applied to ecotoxicological assessments of the marine environment.

Video Link

The video component of this article can be found at https://www.jove.com/video/55211/

Introduction

Ecotoxicological bioassays evaluate the toxicity of chemicals or environmental samples with standard biological models, integrating the effects of physical, chemical, and biological stressors on ecosystems. Due to the complexity of ecosystems, ecotoxicological risk assessments must consider a battery of bioassays that involve organisms from different trophic levels. Toxicity assays on laboratory animals may be expensive, time consuming, and ethically questionable. The drive to limit animal testing and develop alternative approaches (*e.g.,* on bacteria and non-vertebrate animals) is now a pivotal issue, as reported in the framework of the current European legislation, including the EU Animal Protection Directive, the 7th Amendment to the EU Cosmetics Directive, and REACH.

Crustaceans, fish, and algae are largely used for toxicity measurements in the marine environment[1]. Bacteria are an important component of the ecosystem, and alterations to microbial communities can have significant effects on biogeochemical cycling and other critical ecosystem services. Toxicity testing based on microorganisms are gaining popularity because they are relatively quick, reproducible, and cheap and do not raise ethical issues[2]. The aim of this work is to describe an ecotoxicological protocol to evaluate the response of the marine bacterium *Vibrio anguillarum* (*Listonella anguillarum,* Vibrionaceae) when exposed to environmental contaminants.

V. anguillarum is a Gram-negative, short, curve-shaped rod bacterium (0.5×1.5 µm) with a polar flagellum. Typically found in brackish or salt water, it is halotolerant, with an optimal salinity of about 20 and an optimal temperature between 25 and 30 °C[3]. It has been chosen as an organism model due to its ubiquity and its important ecological roles in oceans worldwide[4]. Some serotypes of *V. anguillarum* are known to cause vibriosis in a variety of marine or brackish fish species[5,6]. For this, some steps of the experiment require standard microbiological practices, but no special safety equipment or precautions are needed. The proposed toxicity testing protocol uses the bacterial culturability (*i.e.,* the capability to replicate and form colonies) as the endpoint and allows for the determination of the LC_{50}, the concentration that causes a 50% reduction of bacteria actively replicating and forming colonies, after a 6-h exposure. In *Vibrio*, as in other microbes, this reduction, which we generally indicate as mortality, can partially be due to individuals in the viable but non-culturable (VBNC) phase[7]. In this study, we applied this method to measure the toxic effects of copper sulphate ($CuSO_4$), a reference toxicant.

