

Bacterial Vitality Detected by a Novel Fluorogenic Redox Dye Using Flow Cytometry

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Abstract

Sensitive detection of cell viability is important for bacterial work in bioindustrial and environmental studies. While plating efficiency and membrane integrity are commonly used for viability assessment, measurements of cellular metabolic activity may provide more sensitive and direct assays for vitality and viability. We report a novel fluorogenic redox indicator that yields green fluorescence when modified by the bacterial reductases, many of which are located in the electron transport system (ETS). This reagent was evaluated with both gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*) bacteria using inhibitors reported to act on different components of the ETS: rotenone, antimycin A, sodium azide, and CCCP. Responses were measured by flow cytometry using 488 nm excitation with green and red emission. The new redox dye gave fluorescence signals in all bacteria tested, although intensity was brighter in gram-positive organisms, and intensities were decreased to varying extents by the different ETS inhibitors. Staining was rapid, detected within ten minutes, and required no special treatment of the bacterial cells. When compared with the tetrazolium salt CTC, another redox indicator, fluorescence responses differed between dyes and organisms in response to inhibitors, suggesting that the novel dye may interact with different ETS components. The redox dye intensity correlated with the detection of vital bacteria as measured by both esterase activity (carboxyfluorescein diacetate) and by membrane integrity (propidium iodide). Results indicate that this new redox dye, named RedoxSensor™ Green reagent, provides a novel measurement of bacterial vitality, and may provide a tool to dissect the function of ETS components.

Vitality Spectrum

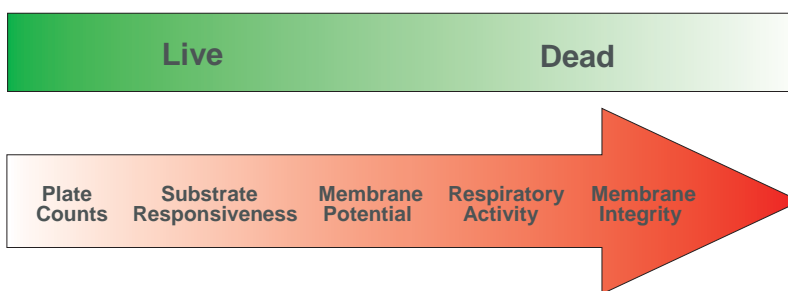


Figure 1. The range of viability and vitality parameters suggests that viability may be more of a continuum than a discrete cut-off point.

Method of Analysis

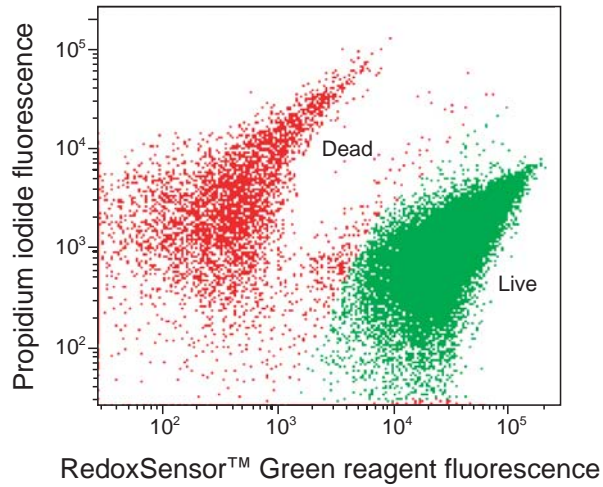


Figure 2. A mixture of healthy and alcohol-killed *S. aureus* cells was stained with 100 nM RedoxSensor™ Green reagent and 20 μM propidium iodide for 10 minutes at room temperature. Dual-color plot analysis of the sample using the BD™ LSR II system, gated on the bacteria population with appropriate compensation, shows both the vital (live) and membrane-compromised (dead) cell populations.

Time Course of Staining

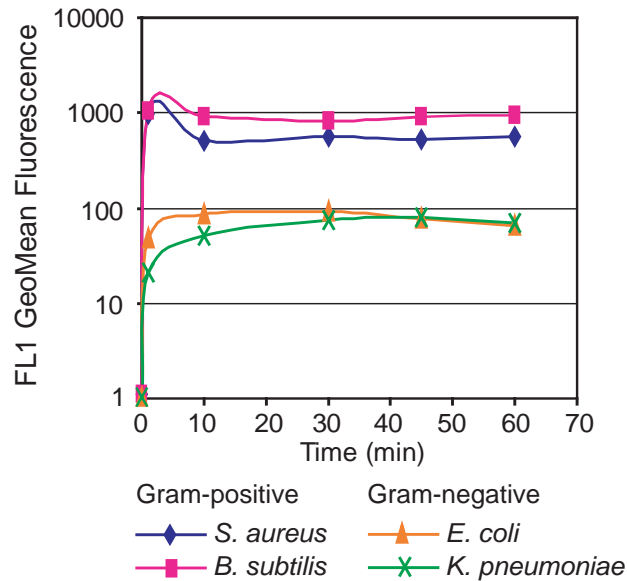


Figure 3. Various gram-positive and gram-negative species stained with 100 nM RedoxSensor™ Green reagent in PBS at room temperature and sampled at several time points up to 60 minutes. Samples were analyzed on a BD FACSCalibur™ flow cytometer using 488 nm excitation and collected in the green channel (530BP). Fluorescence signals stabilized after 10 minutes and remained steady over the 60 minute time course. RedoxSensor™ Green reagent stained both gram-positive and gram-negative organisms, although overall staining intensity was greater in gram-positive bacteria.

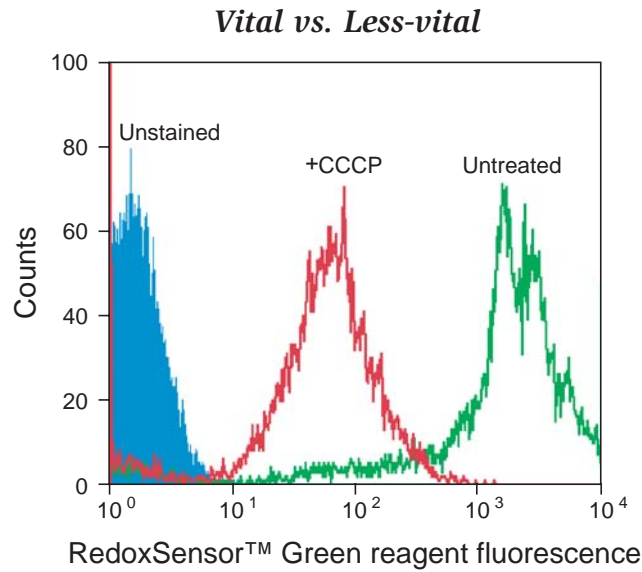


Figure 4. *S. aureus* were treated with 100 nM RedoxSensor™ Green reagent in the absence or presence of 10 μ M carbonyl cyanide 3-chlorophenyl hydrazone (CCCP). CCCP is a protonophore which destroys the membrane potential required for ETS function. Data was obtained on the BD FACSCalibur™ system gating on live bacteria by scatter and by fluorescence. Untreated cells show more intense green fluorescence than CCCP-treated cells. For comparison, the histogram corresponding to unstained *S. aureus* cells is shown on the far left.

Electron Transport System and Inhibitors

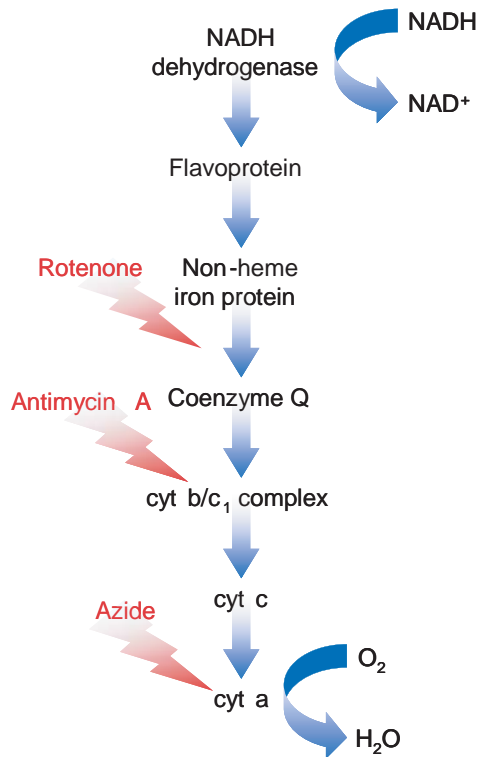


Figure 5. Diagram representing a generic electron transport system (ETS) and the points at which ETS inhibitors affect respiration.

Vitality Indicator Comparison

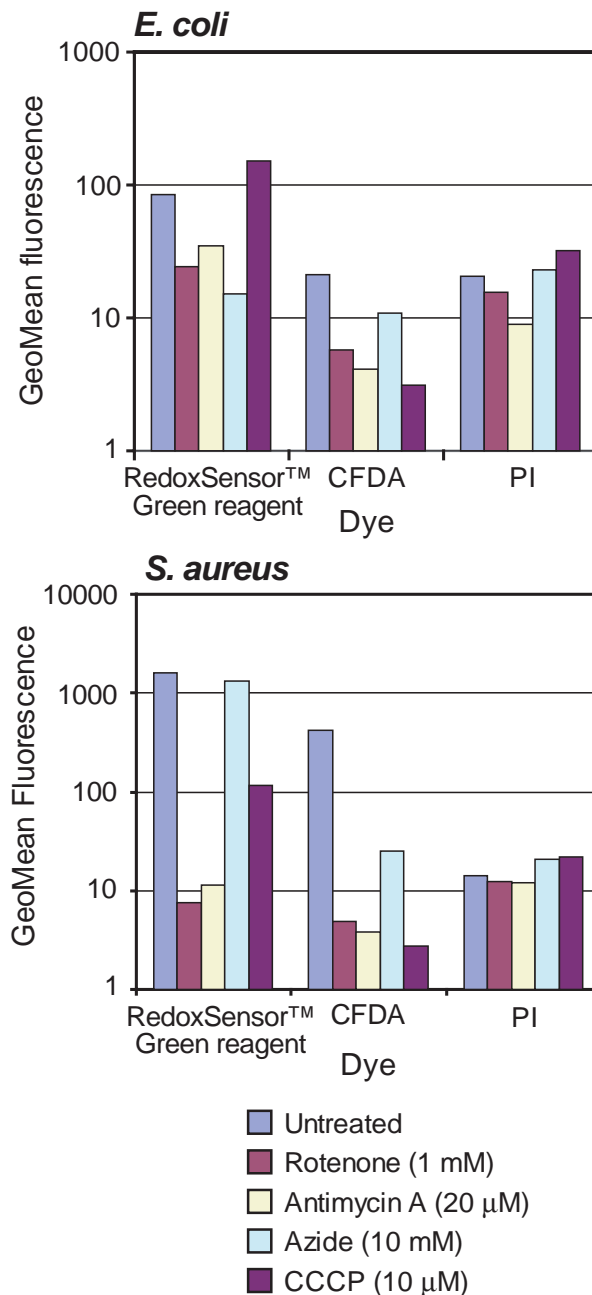


Figure 6. *S. aureus* and *E. coli* were treated with various ETS inhibitors. Samples were removed and stained in 0.85% NaCl + 10 mM glucose with 1 μM RedoxSensor™ Green reagent, or 50 μM CFDA, or 20 μM PI for 30-60 minutes at room temperature. Unwashed samples were analyzed on a BD FACScan™ system using 488 nm excitation and fluorescence signals collected in the green channel (530BP) for RedoxSensor™ Green reagent and CFDA or red channel (> 670LP) for PI. If reduction takes place before the inhibitor point-of-action, then the signal is expected to be the same or higher than that of the untreated control. If reduction occurs after the inhibitor point-of-action, then the signal is expected to be lower than that of the untreated control. None of the inhibitors altered membrane permeability while all affected esterase activity (CFDA). The two bacteria species responded differently to azide and CCCP.

Electron Transport Chain Inhibitor Effects

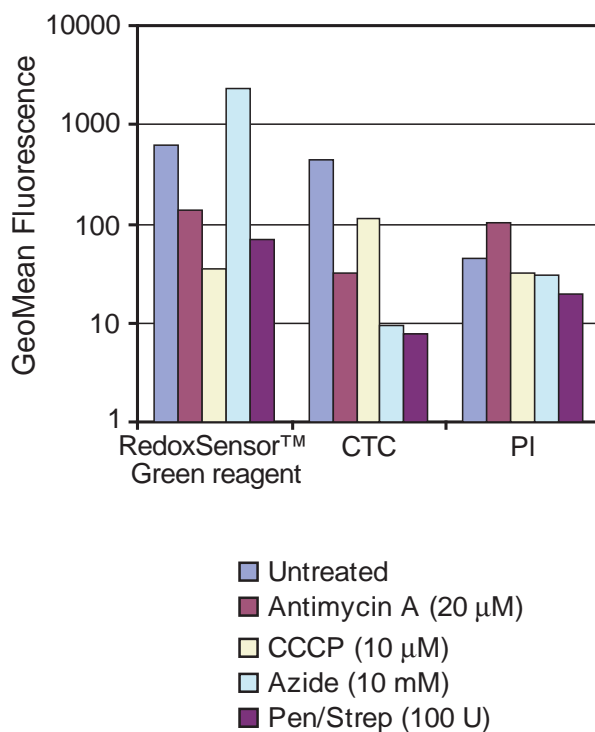


Figure 7. *S. aureus* were treated with various ETS inhibitors and antibiotics. Samples were removed and stained in 0.85% NaCl + 10 mM glucose with 1 mM RedoxSensor™ Green reagent, or 5 mM CTC, or 20 mM PI for 60 minutes at 37°C. Unwashed samples were analyzed on a BD FACScan™ system using 488 nm excitation and fluorescence signals collected in the green (530BP) channel or red channel (650LP) for CTC and PI. Expected responses are explained in Figure 6. The two redox-sensing dyes behaved differently relative to azide and possibly CCCP, suggesting that they interact with different parts of the ETS.

Summary

Redox Sensor™ Green reagent is a novel dye that:

- produces fluorescence signal when modified by bacterial reductases and is affected in species-specific ways by various ETS inhibitors.
- is modified rapidly (10 minutes) by both gram-positive and gram-negative organisms, and the fluorescence signal is stable for at least 60 minutes.
- is detectable using 488 nm excitation and a green emission filter, a standard configuration for most flow cytometers.
- is a new reagent for measuring bacterial vitality.