Title:
Accurate and efficient method for monitoring microbial growth of low-cell number bacterial populations

Authors:
Timothée Fuselier, Angel Rain-Franco, Jakob Pernthaler

Institution(s):
Limnological Station, Department of Plant and Microbial Biology, University of Zurich

Abstract (300 words maximum): :
Bacterial abundances in natural freshwater habitats are in the range of a few million cells per mL. Laboratory experiments mimicking such realistic cell densities cannot rely on traditional laboratory techniques, such as quantification of absorbance at ~ 600 nm. While flow cytometry (FC) would be an accurate method for this purpose, it is time-consuming and does not allow for the continuous live monitoring of cultures over time. Spectrofluorometric assays using fluorogenic stains such as DAPI, HOECHST or Resazurin have previously been used to monitor cell viability, cell abundance and metabolic activity of aerobic bacteria but only over short-term periods. We used isolates from Lake Zurich grown in an oligotrophic artificial lake water medium to compare bacterial enumeration by FC and spectrofluorometric assays. We monitored cell abundances and fluorescence signal over 10 days. There was a positive correlation between cell abundances determined by FC and metabolic activity monitored with Resazurin ($R2 > 0.75$, $P\text{-value} < 0.05$), whereas no such correlation was found for DAPI and HOECHST. Interestingly, the ratio between cell numbers and metabolic activity varied even between closely related isolates. In a subsequent experiment, we monitored bacterial cell growth and Resazurin-based metabolic activity with and without vitamin supplementation. The addition of vitamins increased total cell numbers determined by FC but not the Resazurin-based metabolic activity. Our results suggest that the ratio between cell abundance and metabolic activity is highly affected by vitamin limitation. Resazurin proved to be suitable for live monitoring of the growth and metabolic state of low-density microbial populations over time in response to specific experimental conditions, however strain-specific calibrations need to be considered for estimating cell numbers from metabolic activity.