

Background

in many systems most cells are near starvation, and are therefore inactive.
VBNC state - How can we tell who is an active player?

Methods that don't require cultivation

activity assays: CO₂, N, NO, etc. (Edwards, 1999)

Vital stains

FDA fluorescein diacetate – taken up by living cells and cleaved to fluorescent product.

Propidium Iodide, not taken up by cells with intact membranes; (see table Edwards 1999 for others)

Staining coupling with flow cytometry – cell sorting and counting

Molecular methods not requiring cultivation

Correlations: Fast growing cells have more RNA (esp. rRNA) and greater cell volume. Early step in growth initiation rRNA synthesis; because of stability of rRNA it may show past activity instead of present.

Quantification of rRNA via dot-blots

situ fluorescence (DeLong et al., 1989; Poulsen et al., 1993)

semi-quantitative PCR views (e.g., reverse transcription with DGGE)

FISH probing of unprocessed message (Licht et al. 1999)

Quantification of specific mRNAs? problems with extraction from soil: nucleases, inhibitors, adherence to clay (Mendum et al 1998)

Stable isotope analysis, natural abundance ¹⁵N plus Nif genes and morphology (heterocysts) - used to determine trophic interactions, MacGregor et al. 2001

Stable isotopes + labeling + PLFA (Pelz et al. 2001ab)

Stable isotopes + labeling with ¹³C + DNA or RNA isolation and Density Gradient Centrifugation - (Leuder et al 2004ab)

labeling community with ¹³CH₄OH to look at Methyloprotoph, incubate for 0, 6,9,13,42 days. Isolate DNA & RNA, density gradient centrifugation with CsCl (for DNA) and Cesium trifluoroacetate (for RNA)

lengthy labeling results in good incorporation, but transfer of label through food web.

Nucleotide analog (Bromodeoxyuridine) and immunocapture (Borneman 1999, Urbach et al. 1999)

Methods requiring cultivation & transformation

Reporter Gene methods (reviewed in Lindow, 1995)

Used to ask questions about when and where various genes are expressed
 Absolute amount of protein relates to expression, stability, turnover - so only relative changes are measured
 requires cultural manipulation/transformation – testing of strains for wt abilities

LacZ - β galactosidase activity

advantages: well worked out, easy to measure (X-gal, ONPG)
 Chemoluminescent substrates (MUG - Methylumbelliferyl B-D--
 galactopyranoside)

disadvantages: much native β galactosidase activity in communities

Gus

β glucuronidase activity, used in plant and culture studies
 disadvantages: much native β glucuronidase activity in communities

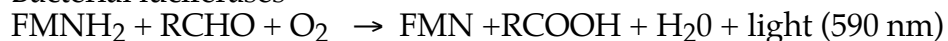
Ice nucleation (Lindow, 1995)

ice nucleation proteins - typically in outer membrane of gram + bacteria
 assay temperature of freezing (-2 to -10)
 warmest temp proportional to log of ice nuclei
 works well under "dirty conditions", single cells can cause nucleation, one of the most sensitive reporters

Amino acid and sugar availability along roots - (Jaeger et al., 1999)

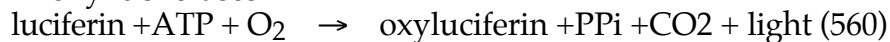
Bioluminescence (Lindow, 1995; Prosser et al., 1996)

Bacterial luciferases



can add entire operon (*lux* CDABE) or just *lux* AB and then add Aldehyde exogenously

Firefly luciferases



luciferin must be added exogenously

Requirements - O₂ requirement, metabolic activity

advantages: light emission linear over broad range, real time measurements possible, light doesn't accumulate or diffuse,

detection: scintillation counters, Luminometers – whole cells or extracted protein; for large numbers of cells or strong promoters: x-ray film, or CCDs - individual cells difficult, but not impossible

Problems: addition of substrates, quenching (in soils), need for O₂, response not linear if reporter cells are not metabolically active, if aldehyde is added its necessary to control amount carefully or lethal effects can occur

uses : general metabolic activity (soil), sensing O₂ (Rhizobium nodules), naphthalene (nahG-fusion) (Heitzer et al., 1994), mercury (mer-fusion)

GFP (Molin & Givskov, 1999)

no metabolic requirement (but O₂ required), highly sensitive (single cell), normally very stable, can be assayed *in vivo*
unstable form used to assay ribosomal synthesis (Andersen et al., 1998; *Sternberg et al., 1999)

Some problems with expression and/ visualization in fungi (“quelling” and “quenching”)

Use in biosensing (Joyner DC, Lindow SE. 2000)

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