Selection using light filters

Since the absorption spectra of different species of phototrophic bacteria vary considerably, it is possible to obtain direct isolation or enrichment by using selective light filters.

Since chlorophyll a does not absorb past 700 nm, using filters that pass only NIR radiation at wavelengths greater than 700 nm will exclude the cyanobacteria and all eukaryotic photosynthetic microbes. Using filters that transmit radiation only at wavelengths greater than 860 nm will exclude the green sulfur bacteria. Using filters that transmit radiation only at wavelengths greater than 920 nm will exclude all the Bchl a-containing phototrophic bacteria and will permit growth only of Bchl b-containing bacteria.

The least expensive type of "cut-off" filters for NIR transmission are the Wratten gelatin filters made by Eastman Kodak. The spectral characteristics of these filters are included in the table. Gelatin filters are fragile and must be protected from heat, intense light, moisture, and excessive drying.

It is possible to achieve very specific selection of a particular organism by using narrow band-pass interference filters which transmit only radiation of a particular wavelength and a few to several nm on either side of the central wavelength. Such filters can be chosen so that they transmit light that can only be used by a particular organism or group of organisms. For example 740 nm band-pass filters will only support growth of Bchl c-containing green sulfur or filamentous phototrophic bacteria, since no other organisms absorb light of this wavelength. While very useful, such narrow band-pass filters are difficult to construct and hence are very expensive. The coatings used on the filters are fragile and must be protected from excessive heat, cold, humidity, drying, intense light, and various solvents.

applications are included in the table.

FILTER	TYPE	WAVELENGTHS TRANSMITTED	%T	APPLICATION
88A	cut-off	730-950 nm	80-90% (790-950)	enrich. Bchl *a* organisms
87C	cut-off	800-950 nm	75-90% (890-950)	enrich. Bchl *a* organisms
87A	cut-off	900-1100 nm	50-65% (930-1100)	enrich. Bchl *b* organisms
87B	cut-off	850-1100 nm	25-90% (890-1100)	enrich. Bchl *b* organisms
IF 740	Narrow Band-Pass	740 nm $\pm$ 10	$\sim$ 50% at max λ	positive selection for Bchl *c* organisms
IF 1020	Narrow Band-Pass	1020 nm $\pm$ 10	$\sim$ 50% at max λ	positive selection for Bchl *b* organisms

1. Enrichment and Isolation of Phototrophic Prokaryotes

Objective -- to learn techniques of:

a) enrichment followed by isolation

b) direct isolation without enrichment

c) establishment, maintenance, and succession in microcosms

Activity

The rotation will involve making a variety of specific media to create selective culture conditions on the basis of variations in carbon, nitrogen, and sulfur sources; variations in vitamin requirements; variations in pH, salinity, and redox potential; the use of inhibitors. In addition, the specialized enrichment and selection technique of narrow band light transmission will be used in an attempt to isolate some previously non-cultured phototrophic bacteria from mats. A variety of sources of inocula will be used.

2. Spectrophotometric analysis of Phototrophic Prokaryotes from natural environments

Objective -- to learn techniques of:

a) extraction of photosynthetic pigments with organic solvents and analysis of complex spectra to determine groups of organisms present in natural environments

b) direct analysis of pigment/protein complexes without solvent extraction

c) use of VIS and NIR absorption spectroscopy in identification of phototrophic prokaryotes

Activity

Students will use both extraction and *in vivo* analyses on mat material collected from the salt marsh and other sources, including mats from Laguna

Mormona. Samples will be collected, extracted or suspended, and spectra recorded on the Cary 14 to compare the usefulness of results from extracted vs. non-extracted material. If time permits, the effect of storage of collected material for several days before analysis will be compared with the results of immediate analysis to determine the stability of pigment/protein complexes *in vivo*.