Cytochromes in *Beggiatoa alba*

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Abstract. A strain of *Beggiatoa alba*, B18LD, was investigated for the presence of cytochromes by running difference absorption spectra on cell-free extracts using dithionite, KCN, and Na₂S. Cytochrome spectra with major peaks at 418-421 nm, 522 nm, and 551-554 nm were recorded with heterotrophic cells, sulfide-oxidizing cells, and cells being shifted from heterotrophic to the sulfide-oxidizing conditions. Using Na₂S, peaks or shoulders were also observed at 424-428 nm, along with some widening of the 522-nm and 552-nm peaks in the heterotrophic cells and the heterotrophic cells being shifted to sulfide-oxidizing conditions. This is the first evidence indicating the presence of cytochromes in *Beggiatoa*.

*Beggiatoa* is a microaerophilic, filamentous, gliding bacterium that has been shown to deposit sulfur into extracytoplasmic granules upon exposure to hydrogen sulfide [14]. It has further been shown that the beggiatoas can grow well under defined heterotrophic growth conditions, i.e., with acetate and asparagine [11], or under sulfide-oxidizing growth conditions, i.e., Na₂S, CO₂, and minute amounts of acetate [7,14]. Whether the sulfide oxidation process in *Beggiatoa* is an energy-yielding reaction is not yet known [2,3,12,14].

Burton and Morita [2] reported that *Beggiatoa leptomitiformis* produced hydrogen peroxide during metabolism and lacked characteristic cytochrome spectra. They theorized that a flavin-linked system, similar to that found in the lactobacilli, might be present. Carr et al. [5] found ubiquinone 8 and traces of a naphthoquinone in a Pringsheim *Beggiatoa* strain, and Callies reported that a strain of *Beggiatoa* contained ubiquinones [4]. Nicotinamide adenine dinucleotide (NAD) oxidoreductase is also present in *Beggiatoa* [3].

Here we report, for the first time, the presence of cytochromes in a strain of *Beggiatoa alba* as determined by reduced-minus-oxidized difference absorption spectra. The cytochrome spectra were observed when the organism was grown under either heterotrophic or sulfide-oxidizing growth conditions.

We believe that this finding sheds new light on the electron transport and possibly on the energy metabolism of the beggiatoas.

Materials and Methods

*Beggiatoa alba* strain B18LD was isolated from an enrichment culture from Lacassine, Louisiana, USA, by techniques formerly described [14]. The *Beggiatoa* strain was grown at 30°C on a rotary shaker for 24 h in a liquid culture medium containing Pringsheim basal salts [14], 0.05% (wt/vol) sodium acetate, 0.05% (wt/vol) asparagine, and 0.005% (wt/vol) NaHCO₃ (heterotrophic growth conditions), or in a medium containing the basal salts. 0.03% Na₂S, 0.01% sodium acetate, 0.005% NaHCO₃ (sulfide-oxidizing growth conditions). The trichomes were harvested by centrifugation at 10,000 × g (4°C) for 15 min, washed with basal salts, and repelleted. They were then resuspended in 10 mM K₂HPO₄-KH₂PO₄ buffer, pH 7.3, and were broken by 90 s of sonication (4°C). The extracts were centrifuged twice at 70,000 × g for 30 min (4°C) and the supernatants were combined. The pellets were resuspended in 10 mM K₂HPO₄-KH₂PO₄ buffer, pH 7.3. The “heterotrophic converted” trichomes were first grown in heterotrophic medium as above and then pelleted, washed, and resuspended for 1 h in sterile sulfide medium. These trichomes were harvested, broken, and fractionated as above.

The samples for spectrophotometric analysis were placed into 1-ml glass cuvettes and shaken by hand to oxidize the cytochromes. Oxidized-minus-oxidized control spectra were made, and then the experimental samples were treated by the addition of a few crystals of dithionite. KCN, or Na₂S·9H₂O. Reduced-minus-oxidized spectra were immediately run. Spectra were made using a Perkin-Elmer-Hitachi spectrophotometer (model 200) from 380-680 nm using 0.1A, 0.05A, and 0.02A scale settings.

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Results
Characteristic cytochrome spectra were not observed using the 70,000-x-g pellets.

Figs. 1, 2, and 3 show the various reduced-minus-oxidized absorption spectra of the 70,000-x-g supernatants from each of the three described growth conditions after treatment with Na₂S, dithionite, or KCN. Strong peaks at 418–421 nm were observed in all three treatments and growth conditions, along with somewhat weaker and wider bands at 521–526 nm and 551–557 nm. The exception to these was the occurrence of a peak at 424 nm in the heterotrophic and 426–428 nm in the heterotrophic sulfide-oxidizing converted trichomes treated with Na₂S. Broader peaks occurred at the 521 nm and 552 nm regions when the samples from all three growth conditions were treated with Na₂S.

Discussion
We have observed that although the beggiatoa are colorless and pigmentless microorganisms, pellets of trichomes were always slightly pink, even after several washings. This led us to believe that possibly a c-type cytochrome might be present in the trichomes.

It has been observed that five groups of beggiatoas were sensitive to 0.05% KCN and that two of five groups were sensitive to 0.05% (wt/vol) NaN₃ [14]. Because these sensitivities were similar to those of other flexibacteria [6], it was noted that an electron transport system might indeed be present, although negative results were obtained using the colorimetric cytochrome oxidase test [14]. Carr et al. [5] found that the endogenous oxygen uptake rate by a Beggiatoa strain was very high as compared with several cyanobacteria and that it was further increased by the addition of glucose or acetate. Scotten and Stokes [13] also showed oxygen uptake by Beggiatoa that was increased upon the addition of several citric acid cycle intermediates and acetate but not by glucose.