Ultraviolet Light Mutagenesis of Rhodobacter sphaeroides

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ABSTRACT

Rhodobacter sphaeroides is a Gram-negative, purple photosynthetic bacterium that uses many alcohols as a carbon and energy source. In order to metabolize alcohols, they must be broken down by enzymes known as alcohol dehydrogenases (ADHs). Researchers have previously isolated and studied transposon-induced mutants affected in *pqq* genes. These mutants are unable to synthesize the cofactor pyrroquinoline quinone (PQQ) and are deficient in butanol and methanol metabolism. The purpose of this study is to isolate ultraviolet light-induced and transposon-induced mutants resistant to 3-butyn-1-ol (a suicide substrate for ADH enzymes). The broad goal of this study is to identify genes involved in alcohol metabolism. A survival curve of *R. sphaeroides* for UV light mutagenesis was performed to determine the optimal exposure for mutagenesis (99-99.9% kill). UV mutagenesis was confirmed by an increase in aberrantly pigmented colonies relative to non-mutagenized controls. To date, thirty-five mutants resistant to 3-butyn-1-ol have been isolated. Ten mutants were UV-induced, five were transposon-induced, and twenty were spontaneous. These mutants will be complemented with *R. sphaeroides* DNA in a mobilizable cosmid bank to identify sequences involved in the process of alcohol metabolism.

INTRODUCTION

R. sphaeroides belongs to the diverse group of purple, nonsulfur photosynthetic bacteria. This bacterium can grow with or without oxygen. It also has the ability to utilize a wide variety of carbon and energy sources, including some alcohols (7). In order to metabolize alcohols, bacteria use alcohol dehydrogenase (ADH) enzymes to oxidize alcohols into compounds that can be readily used by the cell. *R. sphaeroides* can grow on butanol, methanol, and isobutanol (5), so we hypothesize that it contains at least one ADH. In other organisms cofactors such as NAD+ or pyrroquinoline quinone (PQQ) are required for function of many ADHs (6).

To gain an understanding of the function of a particular enzyme, it is often useful to generate mutants that do not possess this function. Mutations can be induced using chemicals, radiation, or mobile genetic elements (e.g., Transposons). In this project *R. sphaeroides* was mutated using both ultraviolet light and a transposon. The goal of this study is to isolate and characterize UV light-induced and transposon-induced mutants to better alcohol metabolism in *R. sphaeroides*. To screen for these mutants, cells were plated on suicide substrates for

ADH enzymes. These alcohol analogs are converted into toxic products by ADHs. Therefore, mutants which do not have a functional ADH survive when grown on suicide substrates.

MATERIALS AND METHODS

Bacterial strains, media, and growth conditions. Strains used in this study are described in Table 1. *R. sphaeroides* strains were grown in Sistrom's (SIS) minimal medium at 32°C (9). *Escherichia coli* strains were grown in Luria-Bertani (LB) medium at 37°C. Tetracycline (Tc) was added to LB media at a concentration of 5 μg/ml when culturing *E. coli* strains harboring cosmids containing *R. sphaeroides* DNA. *R. sphaeroides* strains complemented with cosmid DNA were grown on SIS + 3-butyn-1-ol (0.4% v/v) + Tc (1 μg/ml) and SIS + Tc (1 μg/ml). Cell density was measured turbidometrically with a Klett Summerson colorimeter equipped with a red (660 nm) filter. One Klett unit (KU) is equal to 1 x 10^7 cells/ml.

TABLE 1. Bacterial strains, plasmids, and cosmids used in this study.

Strain or plasmid	Description	Source
R. sphaeroides		
2.4.1.	Wild-type	Lab strain
E. coli		
S17-1A	Cosmid-harboring strain, conjugable with *R. sphaeroides*	8
Plasmids		
pLA2917	Tc^r, cosmid parent plasmid	1
Cosmids		
cos 77	Tc^r *R. sphaeroides* DNA in pLA2917	3
cos 473	Tc^r *R. sphaeroides* DNA in pLA2917	3
cos 726	Tc^r *R. sphaeroides* DNA in pLA2917	3
cos 747	Tc^r *R. sphaeroides* DNA in pLA2917	3

Survival curve. Optimum mutagenesis (99% to 99.9% kill) was determined by performing a survival curve. Thirty milliliters of log phase culture was centrifuged at 3619 x *g* for 5 min at 5°C. The cell pellet was resuspended in 2 ml of sterile 0.1 M $MgSO_4$ and this cell suspension was added to 5 ml of sterile 0.1 M $MgSO_4$ until a density of 100 Klett units/ml was reached. Five ml of this suspension was then transferred to 40 ml of sterile 0.1 M $MgSO_4$ in a sterile petri dish with a stir bar. The remainder of this procedure was done in the dark to prevent photoreactivation (an enzymatic reaction in which visible light contributes to the elimination of some of the DNA damage introduced by UV light) (2). The cell suspension was placed on a magnetic stir plate 10 cm from a UV light (Compact 4-Watt Mineralight " & Blak-Ray " Lamp) that was warmed for 20 min. Cells were stirred throughout the irradiation period. One milliliter of culture was removed at each chosen time point and diluted serially in 1X SIS

broth. Finally, 0.1 ml of each dilution was spread onto SIS plates and incubated at 32°C. Countable plates for each time exposure were identified, the number of viable cells/ml was calculated, and the results were plotted on semi-log paper.

UV light mutagenesis. At five minutes of UV light exposure, 1 ml of the irradiated cell suspension was added to 50 ml of SIS medium and incubated for 24 to 36 h in a foil-wrapped flask to prevent photoreactivation. The cells were then diluted, plated onto SIS to give 30-300 colonies/plate, and incubated at 32°C for approximately three days. The colonies were then replica plated to SIS + 3-butyn-1-ol (suicide substrate) plates. Colonies that could not metabolize 3-butyn-1-ol were stored in 10% glycerol (v/v) at -80°C for further study.

Complementation assays. To determine if genes affected in the mutants were previously identified, mutants were complemented with *R. sphaeroides* DNA in a mobilizable cosmid bank. One and a half milliliters of each saturated *E. coli* donor strain was harvested for 20 s at 14,000 rpm in a microcentrifuge and the supernatants were discarded. On top of the cell pellet, 600 µl of the mutant *R. sphaeroides* recipient at a cell density of approximately 150 KU/ml was harvested as above and the supernatant was again discarded. The cells were resuspended in 150 µl of SIS broth by gentle vortexing. From this suspension 100 µl was removed and spread onto an LB plate and incubated for 1 hour at 32°C to allow conjugal transfer to occur. Cells were then replica plated onto SIS + Tc plates to select for transconjugants. After approximately three days of growth between 40 and 50 colonies were patched onto SIS + Tc + 3-butyn-1-ol and also onto SIS + Tc (4).

RESULTS AND DISCUSSION

Survival curves of *R. sphaeroides*, produced by UV light mutagenesis, indicated that 99-99.9% of the bacterial cells were killed between 4 min 30 s and 5 min 6 s. UV mutagenesis was then confirmed by an increase in aberrantly pigmented colonies relative to non-mutagenized controls (Table 2).

TABLE 2. The number of aberrantly pigmented colonies in mutagnized versus non-mutagenized R. sphaeroides cultures. [a]

Trial	Number of color mutants in a non-mutagenized culture	Number of color mutants in a mutagenized culture
1	0	43
2	2	55

[a] Approximately 2000 colonies were screened for color mutations in each separate culture

To date, thirty-five mutants resistant to 3-butyn-1-ol have been isolated (Table 3). Ten mutants were UV induced, five were transposon-induced, and twenty were spontaneous. The variation in mutant phenotypes indicates that mutations occurred at various loci.

TABLE 3. Rhodobacter sphaeroides mutants isolated in this study.

Mutants	Mode of mutagenesis	Phenotype(s)	Cosmids [a]
LAP1	UV[b]	stable	726
LAP2	UV	stable	—[c]
LAP3	UV	leaky	77
LAP6	Tn[d]	leaky	—
LAP9	Tn	stable	726
LAP10	spont[e]	color, stable	—
LAP11	Tn	stable	—
LAP16	Tn	stable	726
LAP17	Tn	stable	—
LAP22	spont	stable	747
LAP23	spont	stable	—
LAP24	spont	stable	—
LAP25	spont	stable	—
LAP27	spont	stable	77
LAP29	spont	stable	—
Colonel Mustard	spont	stable, color	—
LAP30	UV	unstable	—
LAP31	UV	stable	—
LAP32	UV	stable	—
LAP33	UV	color, unstable	—
LAP34	UV	leaky	—
LAP35	spont	color, leaky	—
LAP36	spont	stable	—
LAP37	UV	stable	—
LAP38	UV	leaky	—
LAP39	spont	color, stable	—
LAP40	spont	stable	—
LAP42	spont	stable	—
LAP43	spont	unstable	—
LAP44	spont	unstable	—
LAP45	spont	leaky	—
LAP46	spont	leaky	—
LAP47	spont	color, leaky	—
LAP48	spont	leaky	—

[a] Refers to the cosmids that were capable of restoring alcohol metabolism in the mutants

[b] UV = Ultraviolet light-induced mutants

[c] – to date, none of the cosmids used have restored alcohol metabolism

[d] Tn = Transposon-induced mutants

[e] spont = spontaneous mutants

Complementation of the mutants with *R. sphaeroides* DNA in a mobilizable cosmid bank is currently in progress. Six mutants have been shown to be affected in genes that have been previously studied. The complementation of the other mutants with the same cosmids has resulted in no effect. Therefore, it is possible that some of these mutants will lead to the discovery of additional genes involved in alcohol metabolism.

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