Biotechnological Applications of Photosynthetic Bacteria

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Abstract

Oxygenic and anoxygenic photosynthetic bacteria have been used for many years, and with much success, as model systems to study the molecular events of photosynthesis which occur in higher plants. Recently, attention has also become focussed on the biotechnological importance of these organisms in their own right, since they often produce useful materials from readily available resources (water and sunlight). Due to the high degree of biodiversity which exists among photosynthetic bacteria, renewed efforts have been undertaken to isolate new species or strains capable of producing novel substances or of being used in new environmental processes. This manuscript describes recent progress in establishing a program of genetic engineering in new species of biotechnologically important purple bacteria. These studies have been initiated to allow the improvement and analysis of desirable functions. Namely, hydrogen photoproduction in a marine *Rhodobacter* sp and antbiotic synthesis in a marine *Chromatium* sp. Expression vectors have been constructed from *Rhodobacter* specific replicons in addition to the use of broad host range plasmids. These results are discussed in view of parallel studies which have been carried out for marine cyanobacteria. In addition, a review of recent examples of the biotechnological utilization of photosynthetic bacteria is presented.

Introduction

The aim of biotechnology in general is to supply products and services which are based on the materials produced by cultured microorganisms or cells. In recent years, the use of organisms from marine habitats has received much attention and the new field of 'Marine Biotechnology' has developed. The products and services which marine biotechnology can provide may be divided into six main categories: (1) Waste recycling and pollution control (Environmental Biotechnology), (2) Energy production (3) Biomaterials and their modification, (4) Heavy chemicals including animal feedstocks and solvents, (5) Fine chemicals including pharmaceuticals and agrochemicals, and (6) Extraction of minerals (metals or oil recovery).The use of solar power as an energy source is increasing in importance due to environmental considerations and to the political and economic factors associated with using conventional fossil fuels. Conventional agriculture and forestry, essentially the only technologies to use photosynthesis, are very inefficient, storing only

0.2% of the total incident radiation [1]. However, by using photosynthetic microorganisms combined with the use of photobioreactors up to 18% of incident energy can be stored [2]. The use of photobioreactors for the primary production of biomass can readily overcome the limitations of conventional agriculture. Recently, highly efficient photobioreactors which employ light diffusing optical fibers have been developed for the production of glutamate from marine cyanobacteria [3,4,46]. Such reactors are ideal candidates for scale up to the manufacture of a more diverse array of new products.

I Photosynthetic Bacteria

Although an extremely well studied group of microbes, the biotechnological applications of photosynthetic bacteria have remained relatively limited to such processes as hydrogen production and waste water treatment. In addition, until recently the majority of studies on marine photosynthetic bacteria in particular, have been confined to the characterization of new marine species. It is surprising that despite the powerful molecular genetic tools which exist for the manipulation of freshwater nonsulfur bacteria such as *Rhodobacter capsulatus* and *Rb.sphaeroides* [5,31] very few reports of biotechnological applications involving recombinant strains have appeared [6]. A gene transfer system for marine photosynthetic bacteria was first reported for a hydrogen producing marine *Rhodobacter* [7]. In this work it was shown that many marine photosynthetic bacteria contained 2-6 endogenous plasmids which were less than 15 kb in size. This is unusual since all of the plasmids detected so far in freshwater bacteria are greater than 40 kb in size [32-37]. A comparison of plasmid sizes so far reported is shown in Table 1.

Table 1 Comparison of plasmids from marine and freshwater photosynthetic bacteria.

Species	Habitat	Size kb	Reference
Rb sphaeroides	Freshwater	42-110	33 34 37
Rb capsulatus	Freshwater	116, 148	35
R. rubrum	Freshwater	55	36
Ectothiorhodospira sp.	Halophilic	13.2	47
Rhodobacter sp	Marine	3 - 16	7

Compared to the freshwater photosynthetic bacteria, recombinant DNA techniques for marine species are not as advanced despite recent improvements [8,30]. A small plasmid, isolated from a marine *Rhodobacter* sp. was used to construct a series of shuttle vectors which contained a *Rhodobacter* specific replicon. The resulting vectors had greater stability in the absence of antibiotics and an increased copy number when compared with commonly used broad host range plasmids [8]. The most useful of these vectors which were used to successfully express chloramphenicol transferase in a marine *Rhodobacter* sp are shown in Fig. 1. The *Rhodobacter* specific replicon, which was presumably responsible for the increased stability of the hybrid plasmids was localised and its nucleotide sequence determined [30].

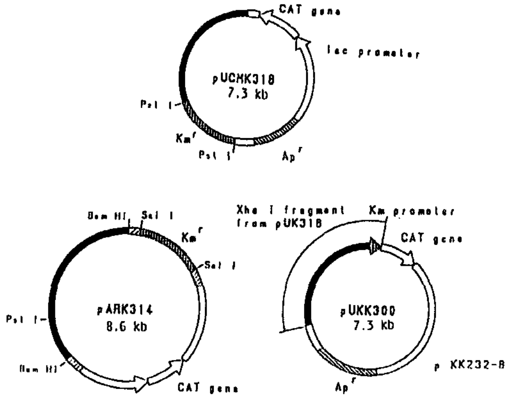
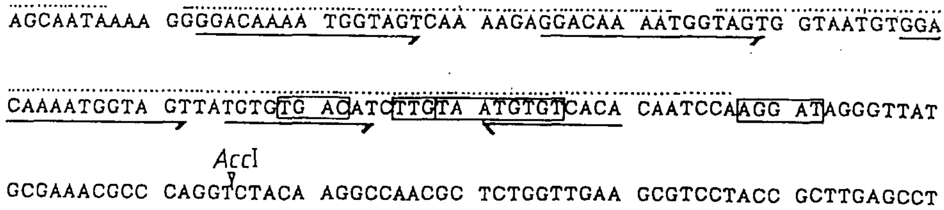


Figure 1. Restriction maps of marine *Rhodobacter* shuttle vectors [8] using pRD31 DNA (solid line) promoters are indicated by an arrow.

Analysis of this sequence revealed features characteristic of a replication origin, including A:T rich direct repeats and a putative promoter which showed homology to the bacteriophage P1 *rep*A promoter (Fig. 2). A model for the mechanism of regulation of plasmid regulation is also shown in Figure 3.



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AGCAATAAAA GGGGACAAAA TGGTAGTCAA AAGAGGACAA AATGGTAGTG GTAATGTGGA

CAAAATGGTA GTTATCTGTG ACATCTTGTA ATGTGTCACA CAATCCAAGG ATAGGGTTAT

GCGAAACGCC CAGGTCTACA AGGCCAACGC TCTGGTTGAA GCGTCCTACC GCTTGAGCCT
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Figure 2. Nucleotide sequence of the replication region of the marine *Rhodobacter* plasmid pRD31 showing A:T rich (dotted) direct repeats (arrows). The boxed region represents a putative promoter with homology to the P1 phage *rep*A promoter [30]

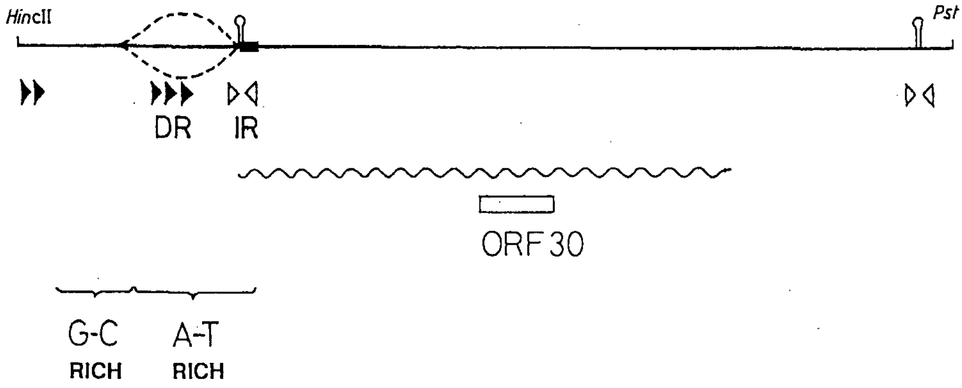

Figure 3. A model of pRD31 replication. Filled arrows represent direct repeats (DR), open arrows represent inverted repeats (IR). Possible hairpin loops are drawn (not to scale). The putative promoter is indicated by a filled box and the possible transcript pattern and open reading frame ORF 30 are indicated. The most likely region of replication initiation is indicated by opening of the double helix, drawn as dotted lines.

We have recently observed the production of antimicrobial compounds from a marine sulfur photosynthetic bacterium *Chromatium* sp.. These substances have a broad antimicrobial spectrum being antagonistic towards both Gram negative and Gram positive bacteria as well as mold and yeasts (Table 2). These findings represent the first example of the purification of an antimicrobial compound from a photosynthetic bacterium. High density culture of this species has also been obtained in a photobioreactor and the structure of the active compounds determined. A gene transfer system for marine *Chromatium* sp. has also recently been established (unpublished work).

Table 2 Antimicrobial spectrum of substances extracted from *Chromatium purpuratum* NKPB031704

TEST ORGANISMS	ANTIMICROBIAL ACTIVITY	
	Yellow fraction	Red fraction
Mucor racemosus	-	++
Candida albicans	±	++
Saccharomyces sake	±	++
Bacillus subtilis	++	+++
Staphylococcus aureus	++	+++
Micrococcus luteus	++	+++
Escherichia coli	±	++
Pyricularia oryzae	++	+++
Candida utilis	±	++
Trichophyton asteroides	±	++

+++ Inhibition zone > 25 mm; + > 10 mm; + > 5 mm; ± 1-2 mm
No effect

In addition to this work, a number of studies have recently been carried out which specifically utilize photosynthetic bacteria. Table 3 summarizes some of these applications and distinguishes between purification of novel materials from biomass and the applications of molecular biology. Of note is the current interest in the photosynthetic production of polyhydroxy alkanoates (PHA) from *Rhodospirillum rubrum* and *Rb.sphaeroides*. PHAs are used for the manufacture of biodegradable plastics (see 29 for a recent review). No reports have yet been published describing the secretion of foreign proteins from a photosynthetic bacterium. This appears to be a area where further research would be very rewarding. Apart from 2 exceptions the expression of foreign proteins has been confined to the functional analysis of the photosynthetic apparatus and RUBISCO.

Table 3 'NEW MATERIALS' FROM PHOTOSYNTHETIC BACTERIA

Material	Description / Use	Reference
ENZYMES	Restriction enzymes eg *RsaI* and *RsrII* from *Rb. sphaeroides*	14,15
	Proteases	16,17
	Polynucleotide phosphorylase (interferon production)	18
	6 APA ases (production of penicillin derivatives)	19
PIGMENTS	Porphyrins (anti tumour therapy)	20-23
ANTIBIOTIC	Broad spectrum antibiotics from marine *Chromatium* sp.	24
LIPO POLYSACCHARIDES	Production of diphosphoryl a from lipopolysaccharides (prevention of endotoxin shock during gram negative infection.)	25,26
PHA	*Rb. sphaeroides* 60 - 70 % dry weight	27
	Rps. Rubrum 45 % dry weight	9
	(Production of biodegradable plastics)	29
Molecular biological applications		
FOREIGN GENE EXPRESSION	Cellulase	6
	Fish growth hormone	39
	Light harvesting complex	40
	RUBISCO	48
FOREIGN PROTEIN SECRETION	New vectors	-
	Copy number mutants	-
PROTEIN ENGINEERING	Reaction centers	28
	Light harvesting complexes	31,38, 41,42
	Energy storage devices	-
	Herbicide biosensors	49
" OPERON ENGINEERING "	Novel photosynthetic complexes	38,40
	Improved pigment production (carotenoid biosynthesis)	-
	Metabolism control	-

Biodiversity

An additional advantage of using marine bacteria for biotechnological applications is the high degree of biodiversity which exists in marine environments. Recently two completely new phylogenetic groups of bacteria were discovered after PCR amplification of 16S RNA genes from natural populations of marine picoplankton [13]. One group was related to the oxygenic phototrophs while the other was phylogenetically related to the α-proteobacteria.We have also found during the course of our studies that the physiological diversity of marine anoxygenic photosynthetic bacteria isolated from coastal and open waters from the Pacific and the Sea of Japan is greater than previously reported. One marine *Rhodobacter* strain NKPB0021 was found to be sensitive to oxygen, being unable to grow aerobically in the dark. In addition, strain 0021 was found to be sensitive to sulfide since growth was not possible at concentrations of over 0.7mM sulfide. This isolate appears to be a new marine *Rhodobacter*.

Environmental Biotechnology

Recent environmental concern has prompted a renewed interest in the photoproduction of molecular hydrogen by autotrophic bacteria as a clean energy source. In addition, the production of PHAs has become increasingly important for the manufacture of biodegradable plastics. As well as specific pollution problems associated with contamination of local environments, anxiety over the increasing global carbon dioxide levels has been increasing. The probable connection between carbon dioxide increase and global warming [50] has prompted major initiatives in both Japan and the United States to confront the problem of excessive industrial production of carbon dioxide. Many of these programs involve the conversion of waste CO_2 into valuable biomaterials using photosynthetic microorganisms [46]. It may also be possible to remove sulfur compounds from fossil fuels or combustion gases using photosynthetic sulphur bacteria [51]. By absorbing sulfur compounds and producing biomass using light energy for the production of high value compounds such as antibiotics, purple sulfur bacteria can used for environmentally beneficial as well as economically attractive processes. The potential of *Chromatium* sp and *Chlorobium* sp for the desulfurization of pyritic coal has been demonstrated in a preliminary report [51] which described removal rates as high as 86 %. By screening purple sulfur bacteria from high temperature ecosystems, such as hot springs, industrial bioprocessing of for example, power station flue gas, could be optimised. The release of CO_2 as mentioned above is also a great worry. However CO_2 removal may be more efficiently carried out by cyanobacteria. The CO_2 removal ability of these procaryotes makes them ideal candidates for use in industrial CO_2 removal processes and also in the more specialized field of carbon dioxide recycling in space environments (see below)

II Cyanobacteria

The development of recombinant DNA methods for marine cyanobacteria has also occurred less rapidly than for freshwater species. Genetic transformation procedures are now available for marine *Synechococcus* sp. [10] using shuttle vectors constructed from endogenous cryptic plasmids. The strain for which these techniques were developed , *Synechococcus* sp. NKBG 042902, has been found to contain compounds which stimulate somatic embryogenesis of carrot [11]. In addition, extracts of this cyanobacteria greatly enhance germination frequency when added to artificial seeds encapsulating carrot somatic embryos . Marine cyanobacteria have also been used for the production of glutamate and γ- linoleic acid [12]. Table 4 shows some recent applications of marine cyanobacteria in particular. More detailed coverage of the biotechnology of freshwater cyanobacteria may be found elsewhere [52].

Table 4 SOME BIOTECHNOLOGICAL APPLICATIONS OF MARINE CYANOBACTERIA

Application	Example	Reference
" TRADITIONAL USES"		
HYDROGEN PRODUCTION		
SPIRULINA	HEALTH FOOD	52
	PHYCOCYANIN (IMMUNODIAGNOSTICS)	
	SCP FOR ANIMAL AND FISH FOOD	
	γ LINOLENIC ACID	12
" NEW BIO MATERIALS "		
BIOACTIVE METABOLITES	*LYNGBYA* SP. O-METHYL ACID (G +ve ANTIBIOTIC)	53
	RIVULARIA SP ANTI INFLAMMATORY DRUGS	
	HAPLOSIPHON SP POTENT ANTIBIOTIC	
	ANTICANCER AGENTS (TUMOUR PROMOTERS)	
	TOXINS (NEUROBIOLOGICAL RESEARCH)	
PLANT GROWTH REGULATORS	PROMOTION OF SOMATIC EMBRYO-GENESIS AND PLANTLET FORMATION USING EXTRACTS FROM MARINE BACTERIA.	11
AMINO ACIDS	EG. GLUTAMATE SECRETION, AND PHOTO-PRODUCTION OF OTHER NITROGENOUS COMPOUNDS SUCH AS AMMONIA	3 52
U.V. ABSORBING COMPOUNDS	USED IN COSMETICS / SKIN CARE	54

In addition to the production of useful substances photosynthetic biomass production by cyanobacteria also results in fixation of carbon dioxide. Thus the manufacture of new materials by marine photobiotechnology may also be beneficial to the earth's environment and to more specialized closed systems.

Space Biotechnology

A surprisingly large amount of information has been published on the behavior of single cells and microorganisms in space, see [43] for a recent review and introduction. Of biotechnological interest are the findings that *E.coli* has increased resistance to antibiotics and that conjugative gene transfer efficiencies increase in space [44]. In addition to this research, much current effort is being directed towards the technology required for bioregenerative life support systems using photosynthetic microorganisms. Such systems, also known as CELSS (Controlled Ecological Life Support Systems) are currently being developed by NASA (National Aeronautics and Space Administration of the United States) and by ESA (European Space Agency) for future use on the space station 'Freedom'. Although plants are expected to be the major food source in such systems, cyanobacteria will be more efficient with regard to oxygen production, carbon dioxide removal and the nitrogen cycle. In this respect, the recently described light diffusing optical fiber (LDOF) biosolar reactor technology should help provide good light distribution in the high density cultures required for these purposes [3,46]. The use of highly efficient photobioreactors together with the genetic engineering and screening of naturally occurring strains will help to establish processes suitable for recycling wastes not only in space, where such advanced processes become economical, but for future use on earth where such initiatives will eventually become more necessary.

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