very useful approach to environmental remediation and will be an area of increasing research activity.

MARINE ECOLOGY AND BIOLOGICAL OCEANOGRAPHY

Application of Molecular Techniques to the Study of Marine Mammals, Fish and Invertebrates

The study of marine mammals, fish, and invertebrates has traditionally been regarded as belonging in the realm of biological oceanography. Application of modern techniques of molecular biology to the study of these organisms is included here in recognition of its being an important part of marine biotechnology. Molecular methods that have application in the identification of species and the detection of genetic variation within species include protein-based methods, e.g., immunological methods, the study of isozymes, and nucleic acid-based methods, such as DNA hybridization and restriction analysis, and rRNA (rRNA) analysis.

The application of molecular and biotechnological techniques to the study of large marine ecosystems was recently reviewed by Powers (1993) and the use of molecular techniques in the study of fish and invertebrate populations was discussed in detail (vide supra). Therefore, only a brief outline is provided in this section, along with several of the examples also included in the review by Powers (1993).

Separation of proteins by electrophoresis, followed by specific histological or immunological staining of particular proteins, has been widely used to detect variation in homologous proteins. These patterns of variation can be used to distinguish between morphologically similar species and to measure hybridization between species. For example, isozyme analysis was used to distinguish two sympatric species of Hawaiian bonefish that were morphologically identical (Shaklee and Tamaru, 1981).

Management of commercially important species relies on identification of stocks of organisms and the study of isozymes has been widely used in this regard. Isozyme analysis has revealed some cases
of tissue-specific expression of particular isozymes, which may be related to metabolic requirements of those tissues (Powers, 1993). There have been some attempts to correlate isozyme changes to changes in physical environmental parameters, such as temperature and salinity. For example, the gene frequencies of the heart-type lactate dehydrogenase locus from the fish *Fundulus heteroclitus* was found to vary in different populations of the fish located along the east coast of North America (Place and Powers, 1978). These allelic isozymes were found to be structurally and functionally different [reviewed by Powers (1990) and Powers et al. (1991)].

A sensitive method for measuring genetic variability is the use of restriction endonuclease digestion of mitochondrial and chloroplast DNA. Mitochondrial DNA (mtDNA) is an extensively studied cytoplasmic DNA element that is relatively easy to purify and characterize. Since mtDNA is maternally inherited, changes in mtDNA can be used to trace matriarchal lineages and distinguish between populations. Restriction analysis of several populations of *F. heteroclitus* revealed intergradation in these populations (Gonzalez-Villasenor and Powers, 1990). Size variation in mtDNA restriction patterns was used to discern discrete stocks of striped bass along the east coast of the U.S. within the Chesapeake Bay, and along the Gulf coast (Chapman, 1987; Chapman and Brown, 1990). Similarly, the geographic distribution of mtDNA haplotypes in humpback whales revealed differences between populations in the Atlantic and Pacific Oceans, as well as differences within each population (Baker et al., 1990).

Isozyme and mtDNA analysis have usually given confirmatory results. For example, it was confirmed by electrophoretic analysis of 31 proteins and analysis of mtDNA that morphologically similar hake found along the coast of southern Africa comprised two sympatric species, *Merluccius capensis* and *Merluccius paradoxus* (Grant et al., 1987; Becker et al., 1988). Furthermore, in other cases, mtDNA studies have answered questions about relatedness that could not be resolved by isozyme studies. For example, the Atlantic eel of the genus *Anguilla*, which migrates over vast regions of Europe and America, was considered to be a separate species from the European
eel, on the basis of some isozyme studies, while other investigators concluded that small gene frequency differences between populations indicated only partial reproductive isolation (Powers, 1993). This was resolved by mtDNA studies that indicated no genetic divergence among eels along the coast of North America but found that European eels were significantly different from those along the North American coast (Avise et al., 1986).

Chloroplast DNA (cpDNA) has been used in some studies of marine algae and phytoplankton. cpDNA is significantly larger than mtDNA, 120-200 kb, compared to 14.5 to 19.5 kb in higher eukaryotes (Watson et al., 1987). Relationships among red algal species and populations were studied using analysis of restriction patterns by Goff and Coleman (1988) and an evaluation of kelp cpDNA was performed by Fain et al. (1988). Strain improvement, employing biotechnology, for algae in aquaculture (see page 15) relies on fundamental research of the biology and genetics of these marine plants. As new molecular genetic information is accumulated, the mtDNA studies are likely to become increasingly important for the aquaculture industry.

Sequence analysis of ribosomal RNA (rRNA), widely used in taxonomic and evolutionary studies of microorganisms (see below), has also proved useful for studies of eukaryotes. For example, nucleotide sequences from two teleostean fish species, *F. heteralos* and *Schastobius alvii*, from the spiny dogfish, *Squalus acanthias*, and the prickly shark, *Icthourophus cokeri*, were used to elucidate their molecular phylogeny (Bernardi et al., 1992). Comparison with sequences of the coelacanth, *Latimeria chalumnae*, the frog *Xenopus laevis*, and humans was done by maximum parsimony analysis. A single phylogenetic tree was obtained that was in agreement with the expected phylogeny. Another example is the use of restriction fragment length polymorphism (Rowan and Powers, 1991) and rRNA sequences (Rowan and Powers, 1992) to investigate unicellular algae that occur as endosymbionts in many different invertebrate species. Closely related algal zooxanthellae were found to be symbionts in distantly related hosts, indicating a flexible evolutionary relation between algal symbionts and their animal hosts.
Application of Molecular Techniques to Marine Microbial Ecology

Conventional microbiological methods that rely on culturing of microorganisms have very limited application in the study of marine microbial ecology, since it is estimated that the great majority of bacteria in picoplankton communities cannot be cultured, an observation made by marine microbiologists more than thirty years ago. Generally, less than one percent of bacterial cells observed by direct microscopy can be recovered on laboratory media (Jannasch and Corwin, 1959; Kogure et al., 1979; Ferguson et al., 1984). It can be shown by microautoradiography that a considerably greater proportion of the bacterial community retains metabolic activity, even though they cannot be cultured (Meyer-Reil, 1978). Bacteria that cannot be cultured may belong to known bacterial groups that can be grown on laboratory media but may have entered a viable but nonculturable state (Roszak and Colwell, 1987). Alternatively, some of these bacteria may belong to groups not previously isolated (Giovannoni et al., 1990).

Application of molecular techniques is essential in understanding species composition, variability and metabolic activity of natural marine bacterial communities. A relatively rapid and simple method that does not necessarily identify individual species, but that gives general information about the variability of species composition, is rRNA-DNA hybridization (Lee and Fuhrman, 1990). This technique gives an indication of the proportion of identical or very closely related bacterial strains present in a given assemblage of bacteria and in other assemblages sampled at different times or locations. This technique can be useful in the selection of the most frequently occurring type of sample composition for more detailed and time-consuming individual species composition analysis (Lee and Fuhrman, 1990), such as by using 16S rRNA sequencing. Another hybridization technique which may have application in marine microbial ecology is reverse sample genome probing, in which total DNA extracted from samples is labeled with a radiisotope and hybridized to relevant bacterial species "standards" arrayed on hy-
bridization membranes (Voordouw et al., 1991). This procedure can be useful in the identification of bacteria.

A powerful molecular technique in the analysis of microbial communities is 16S rRNA sequence analysis (Pace, 1986), in which sequences derived from bacteria in natural samples are compared to known sequences by phylogenetic analysis. The 16S rRNA sequences can be selectively retrieved by cloning and sequencing cDNA synthesized from the 16S rRNA molecule; cDNA synthesis is primed with a synthetic oligonucleotide complementary to a universally conserved region in the 16S rRNA molecule (Weller and Ward, 1989). Another approach is the cloning and sequencing (or direct sequencing) of 16S rRNA genes amplified by PCR (Ward et al., 1992). Analysis of 16S rRNA sequences, for example, revealed that a novel microbial group, the SAR 11 cluster, is a significant component of the oligotrophic bacterial community in the Sargasso Sea (Giovannoni et al., 1990). This discovery and the subsequent identification of two novel eubacterial lineages in samples of bacteria from the Sargasso Sea provide support for the conclusion that a majority of planktonic bacteria are probably new, previously unrecognized species (Britschgi and Giovannoni, 1991). Sequences that were not closely related to any known rRNA sequences from cultivated organisms were obtained from north central Pacific Ocean surface water samples. One of these rRNA sequences was nearly identical to those from some Sargasso Sea bacteria, suggesting a global distribution of these newly discovered bacteria (Schmidt et al., 1991). The presence of broadly diverse microbial assemblages containing many phylogenetically undescribed groups was indicated by the results of Fuhrman et al. (1993) who used 16S rRNA sequencing to compare samples taken in the western California current of the Pacific Ocean and in the Atlantic Ocean near Bermuda. In the 61 clones sequenced, no more than two occurrences of the same sequence were found in a given sample, although identical sequences were found between samples four times, two of which were between oceans.

A profound discovery, resulting from phylogenetic characterization of microorganisms based on 16S rRNA sequencing, was the existence of two primary groupings of microorganisms, the archae-
bacteria and the eubacteria (Woese, 1987). These groupings have
since been termed the domains Archaea and Bacteria (Woese et al.,
1990). Archaea are generally considered to be microorganisms present
in extreme environments, i.e., in the oceans primarily found in or
near the hydrothermal vent environments. However, 16S rRNA
analysis of samples of planktonic bacteria has indicated that archaea
may also be part of the planktonic assemblage of microorganisms in
coastal and oceanic waters. Fuhrman et al. (1992) found sequences
from a previously undescribed archaeal group in oligotrophic oceanic
water, and archaea from this group and from a second group, related
to the methanogens, were found to be present in coastal surface
water samples collected near Woods Hole, MA and Santa Barbara,
CA (DeLong, 1992).

In some cases, use of PCR to recover rRNA genes from natural
communities can result in differential amplification of different
rRNA genes. In particular, rRNAs of extremely thermophilic ar-
chaea from hydrothermal vent communities appear to be difficult to
amplify (Reysenbach et al., 1992). Therefore, the interpretation of
these data needs to be done carefully, i.e., with respect to extrapola-
tion to other sites and other regions. However, the examples de-
scribed above show that analysis of 16S rRNA sequences has clearly
had a major impact on understanding the diversity of microorgan-
isms in marine environments and will continue to provide exciting
new findings well into the next decade.

Sequencing of rRNA molecules isolated from strains of marine
cultures has been important in determining taxonomic relationships. For example, in addition to the 16S rRNA,
the SS rRNA sequences of Shewanella, Vibrio, and Alteromonas strains
has permitted better definition of the species comprising these gen-
era (Ortiz-Conde et al., 1989; Muir et al., 1990), which are impor-
tant in the marine environment. Recently a large, morphologically
peculiar microorganism, Epulopiscium fishelsoni, found in the intestinal
tract of a surgeonfish, Acanthurus nigrofuscus, was confirmed to be a
bacterium by rRNA sequence analysis (Angert et al., 1993). This
microorganism was previously considered to be a protist because of
its large size and is the largest known bacterium to date, with indi-
vidual cells reported to be larger than 600 μm by 80 μm, ca. one hundred fold larger than other bacteria.

Fluorescent-labelled oligonucleotide probes based on rRNA sequences can be used for phylogenetic identification of single cells (Distel et al., 1988) by fluorescent microscopy or microautoradiography of natural samples, an approach used for detection of marine nanoplanckton protists by Lim et al., (1993) and for localization of a bacterial symbiont in tissue sections of the shipworm, Lyrodus pedicellatus, by Distel et al. (1991).

Phylogenetic characterization of bacterial symbionts of marine invertebrates and fish, many of which cannot be propagated in pure culture, has been possible by using 16S rRNA sequence analysis. Sulfur-oxidizing bacterial endosymbionts in three invertebrates from deep-sea hydrothermal vents and three invertebrates from shallow coastal marine environments were found to be species-specific. Furthermore, the symbionts were concluded to be unique to their respective hosts (Distel et al., 1988). The gill symbiont of a marine bivalve, Thyasira flexuosa was identified by 16S rRNA sequencing and shown to be closely related to known symbionts of lucinid clams (Distel and Wood, 1992). Previously, it was reported that the T. flexuosa symbiont was identified as Thiohaella thyasiris TG-2 (Wood and Kelly, 1989). However, the bacterium, isolated in pure culture from gill tissue homogenates, was likely to have been on the gill surface and not an authentic intracellular symbiont of T. flexuosa (Distel and Wood, 1992). Bioluminescent symbionts of flashlight fish (family Anomalopidae) and deep-sea anglerfish (suborder Ceratioidei), extracellular parasites that cannot be cultured by conventional techniques, were recently shown by phylogenetic analysis of 16S rRNA sequences not to be previously described luminous bacterial species but, instead, new groups related to Vibrio species (Haygood and Distel, 1993).

In addition to elucidating phylogenetic relationships and the identification of microorganisms, molecular techniques are useful for the study of metabolic processes of microorganisms in the environment. Variations in the rRNA content of the marine bacterial species Vibrio alginolyticus and Vibrio furnissii, during starvation-sur-
vival and recovery, was assessed by measurement of the amount of hybridization to 16S rRNA probes. Mechanisms controlling starvation-survival in these marine bacteria were found to be linked to the physiological state at the onset of starvation (Kramer and Singleton, 1992).

A probe for a portion of the 23S rRNA gene of Pseudomonas was used to investigate the ratio between growth rate and nucleic acid content in this marine denitrifying bacterium (Kerkhof and Ward, 1993). Identification of particular genes in extracted nucleic acids from environmental samples can provide information about the presence of bacteria with the potential to carry out specific functions. PCR amplification of naphthalene-catabolic genes from sediment samples indicated the presence of bacteria carrying naphthalene deoxygenase genes (Herrick et al., 1993).

An exciting development is the detection of gene expression in natural populations of microorganisms by mRNA analysis. Pichard and Paul (1991) used this approach to detect expression of the gene (rbcL) encoding the large subunit of ribulose 1,5-bisphosphate carboxylase/oxygenase (RUBISCO) in natural phytoplankton populations. RUBISCO is a key enzyme in photosynthesis and rbcL expression was greatest during the day and least at night, as would have been expected. Expression of the catechol-2,3-dioxygenase gene (xylE) on plasmid pLV1013 in a marine vibrio was detectable in the environment and gene expression was expressed, relative to gene dose, in this study (Pichard and Paul, 1993). Measurement of regulation of gene expression at the transcriptional level in marine microorganisms (which complements measurement of enzyme levels) will be increasingly important in the understanding of activities of indigenous populations and microorganisms released into the marine environment for bioremediation purposes.

Gene exchange between microorganisms in the marine environment is currently of interest because of the importance of assessing the probability of gene exchange with genetically engineered microorganisms that may be released into the marine or estuarine environment (see Chapter 5). Gene transfer has been demonstrated in microcosms containing marine water and sediment (Pichard and
Paul, 1991) and a marine vibrio was shown to develop competence, i.e., ability to take up DNA, under a wide range of conditions, similar to those found in tropical and subtropical estuaries (Frischer et al., 1993). It has been shown that bacterial cells maintain plasmids even after entry into the viable but nonculturable state (Byrd and Colwell, 1990; Byrd et al., 1992). Retention of plasmids by bacteria, after release into the marine environment, is significant relative to introduction of genetically engineered microorganisms into the aquatic environment. Molecular techniques, in addition to conventional culturing methods, will be needed to examine gene exchange in natural communities of marine microorganisms.

The recent discovery of large numbers of viruses in marine natural waters is proving to be important for understanding microbial ecology and gene transfer in the environment. Abundances of $10^3$ to $10^4$ viruses ml$^{-1}$ have been reported in marine waters (Bergh et al., 1989; Proctor and Fuhrman, 1990), estuarine waters (Wommack et al., 1992) and in a coral reef environment (Frischer et al., 1993). There are indications that bacteriophages may be important in marine bacterial mortality (Proctor and Fuhrman, 1990; Heldal and Bratbak, 1991) and that viruses may infect phytoplankton, reducing primary productivity (Suttle et al., 1991). As in other aspects of marine biotechnology, molecular techniques, such as detection of viruses and prophage by gene probing (Ogunseitan et al., 1992) are already proving useful in assessing the ecological role of viruses. It is clear that, although marine viruses have been studied for several decades (Spencer, 1955; Spencer, 1960; Chen et al., 1966; Baross et al., 1978; Torella and Morita, 1979), the recent reports of virus abundance have stimulated new research. The widespread distribution of viruses in the marine and estuarine environment indicates that viruses are likely to play an important role in marine microbial ecology.

An important technique for molecular studies in marine microbial ecology is the extraction of nucleic acids from water and sediment samples. These procedures are also important for detection of pathogens in the environment by gene probing (see page 54). Extraction of DNA from natural planktonic microorganisms can be achieved by filtration, followed by standard lysis procedures
(Fuhrman, 1988; Somerville et al., 1989). Extraction of intact miRNA requires the use of RNase inhibitors and satisfactory results have been obtained using this approach (Pichard and Paul, 1991, 1993). Recovery of nucleic acids from sediment, compared to seawater, is much more difficult and frequently a high content of humic substances can inhibit PCR reactions. Several methods recently have been described which involve extracting DNA in a sufficiently pure form that it can be used as a substrate for PCR reactions, without inhibition of the reaction (Pillai et al., 1991; Tsai and Olson, 1992a; Tsai and Olson, 1992b; Young et al., 1993), making possible the sensitive detection of pathogens and other bacteria of interest.

Advances in marine microbial ecology are likely to be greatly accelerated by application of molecular techniques, in combination with conventional approaches, where appropriate. This is well exemplified by results of recent studies of the impact on benthic microbial ecology of disposal of sewage and sewage sludge into the deep sea. Results of enumeration of Clostridium perfringens spores by conventional plating were used to show that sewage dumped at the Deep Water Municipal Sewage Disposal Site (ca. 100 miles off the coast of New Jersey in water depths of 2,600 m) had contaminated the benthic environment (Hill et al., 1993). Application of conventional techniques, such as enumeration of bacteria by microscopy and plate counts, combined with molecular methods for community DNA hybridization (Lee and Fuhrman, 1990), yielded data showing that contamination of the benthic environment had induced changes in the indigenous benthic microbial assemblages (Hill et al., 1993b; Takizawa et al., 1993). These studies advance our understanding of pollution in the marine environment.

**Marine Ecology and Human Health**

The quality of estuarine and marine water used for recreation and seafood harvesting has traditionally been assessed by enumeration of coliforms and fecal coliforms, rather than by direct detection of pathogenic bacteria and viruses. Direct detection of pathogens has not been adopted as a standard method in the U.S. (American Public Health Association, 1989) because the practical conclusion, before
the advent of molecular genetics methods, was that standard bacteriological culturing methods for direct detection of pathogens were expensive, time-consuming, and not efficient. In addition, direct detection of pathogens by standard microbiological methods is of questionable value. Exposure of bacterial pathogens to the aquatic environment reduces efficiency of recovery when standard culture methods are employed (Bissonnette et al., 1975). Several important bacterial pathogens, including *Vibrio cholerae* (Colwell et al., 1985), *Salmonella* species (Roszk et al., 1984), and *Campylobacter jejuni* (Rollins and Colwell, 1986) have been shown to enter a viable but nonculturable state (Roszk and Colwell, 1987) in the environment, in which they are no longer culturable by conventional techniques but may retain pathogenicity (Colwell et al., 1985; Colwell et al., 1990). Molecular approaches have made it possible now to monitor water quality by direct and reliable detection of pathogenic bacteria, even cells in the viable but nonculturable state.

Molecular methods for detection of bacterial pathogens in the aquatic environment that have been published to date include DNA probes (Knight et al., 1990) and PCR (Way et al., 1993) for detection of *Salmonella* species and PCR and fluorescent antibody methods for detection of *Shigella dysenteriae* Type I (Islam et al., 1993) and *Vibrio cholerae* (Brayton and Colwell, 1987; Huq et al., 1990). *Escherichia coli* and enteric pathogens (*Salmonella* and *Shigella* species) have been detected in environmental samples with sufficient specificity and sensitivity for monitoring water quality by PCR amplification of the *lamB* gene (Bej et al., 1990), and PCR amplification of the *uid* gene that codes for β-glucuronidase was used to detect *Escherichia coli* and *Shigella* species (Bej et al., 1991). Detection of virulence factors in *E. coli* isolates from water samples was achieved using seven different DNA probes (Martins et al., 1992). PCR amplification of a region of the enterotoxin gene in enterotoxigenic *E. coli* and *V. cholerae* was found to be suitable for detection of these organisms (Knight et al., 1991). Other examples include a biotype-specific probe that reliably differentiates between the El Tor and Classical biotypes of the *Vibrio cholerae* 01 serogroup (Alm and Manning, 1990) and a monoclonal antibody-based test that allows rapid and
sensitive detection of *V. cholerae* 01 in contaminated water (Colwell et al., 1992). Enteroviruses, which pose a public health risk in the environment and can be transmitted via contaminated water, were detected in groundwater by PCR. This assay is applicable for detection of enteroviruses in other environmental samples (Abbaspour et al., 1993).

Contamination of shellfish, because shellfish are filter feeders and, therefore, concentrate bacterial and viral pathogens, can present a severe public health risk. To assess the risk, molecular techniques have been developed for detection of several important shellfish-related pathogens, one of which is *Vibrio vulnificus*, a human pathogen, associated with oysters, that can cause septicemia after ingestion of raw oysters. *V. vulnificus* septicemia has a mortality rate of greater than 50% (Morris and Black, 1985). A DNA probe with excellent specificity and sensitivity for *Vibrio vulnificus* was developed from a fragment of the cytolysin gene of this organism (Morris et al., 1987). The hemolysin-cytolysin structural gene *vvhA* was subsequently sequenced (Yamamoto et al., 1990) and an alkaline phosphatase-labeled oligonucleotide probe based on this gene was used for rapid isolation and enumeration of *V. vulnificus* without the need for enrichment or selective media (Wright et al., 1993). A fluorescent-labeled oligonucleotide probe has proven useful for enumeration of *V. vulnificus* (Heidelberg, 1993). Detection of another important shellfish pathogen, *Vibrio parahaemolyticus*, in artificially contaminated oysters, was accomplished using an oligonucleotide probe specific to the thermostable direct hemolysin (*tdh*) gene of the organism (Lee et al., 1992a).

Molecular techniques are now available for detection of many important pathogens in water and shellfish samples. The use of direct detection methods can no longer be ignored. They are important for assuring public health and food safety, and will supplant culture methods in the very near future.

**Use of Biosensors and Remote Sensing**

There is growing interest in the use of biosensors and remote (usually satellite-based) sensing of the marine environment. Biosen-
sensors may offer sensitive methods for detection of low levels of nutrients or toxic compounds. Furthermore, remote sensing allows detection of large-scale changes in such parameters as water temperature, wind-induced mixing, and phytoplankton biomass. This capability will be greatly expanded by the NASA Sea-viewing Wide Field-of-view Sensor (SeaWiFS) and the Earth Observing Satellite (EOS) (Hooker and Esaia, 1993; Hooker et al., 1993) and remote sensing should be considered by biologists as a useful tool for environmental research and monitoring.

Development of biosensors is dependant on the coupling of biological material, such as enzymes, antibodies, or whole cells, to a transducing element which converts the biological signal of interest to some form of electronic readout. An example is the use of mid-ultraviolet range spectrophotometry (MUUVS) to detect the redox state of bacterial cells immobilized in a gel membrane. Toxins flowing over the membrane cause changes in redox state, detectable by MUUVS (Bains, 1992).

Biosensors will be particularly useful in detection of toxic compounds and may allow development of true toxicity sensors that would interact with any compound that is toxic; this could be followed by conventional chemical approaches that identify the specific toxic compound (Bains, 1992). One assay system of this type, which may be useful for biosensor development, measures luminescence of *Photobacterium phosphoreum*. Light generation by luciferase in this bacterium is dependant on a high level of ATP, which is not maintained in the presence of toxins that affect the intracellular ATP pool (Bulich et al., 1990).

Biosensors are also under development for detection of specific substances, i.e., development of optical and electrochemical enzyme-based methods for detection of very low concentrations of DNA (Downs et al., 1988). A diversity of biological elements has been incorporated into biosensors (Hendry et al., 1990). It is clear that sophisticated approaches to environmental sensing will have a significant impact on the ability to gather information about marine ecosystems. As satellite technology becomes more sophisticated and the molecular biology of biosensing systems is better understood,
this rather futuristic area of marine biotechnology will revolutionize data gathering in the marine environment.

**SUMMARY**

In 1983, marine biotechnology was first recognized as an area of biotechnology of great potential (Colwell, 1983). In the decade that has since passed, rather astonishing progress has been made. More than 1,000 publications describing new compounds, natural products, discoveries of the molecular genetics of fish and shellfish growth, metabolism, and reproduction, and expansion of marine biotechnology research on bioremediation, biofouling, and related aspects have appeared in literature. Where only a few pioneers toiled in their laboratories, there are now major centers of marine biotechnology research and development in Bergen and Tromsø, Norway, Kamaichi and Shimizu, Japan, and in Baltimore, MD, Monterey, CA, and elsewhere in the U.S. and across the globe.

Promising new antibiotics, anti-cancer therapeutic agents, improved aquaculture stocks, marine polysaccharides as food additives, and potential new energy sources from the sea, driven by molecular genetic control of marine biological systems, are in the discovery, post-discovery, scale-up, and/or production stages. There is no doubt but that there has already been, and will continue to be, major successes, both intellectual and commercial, in marine biotechnology.

Areas of marine biotechnology in the more-or-less expansive stage are bioremediation and marine biodiversity inventories and assessments. Yet to be fully exploited is the nucleic acid fingerprinting for global marine biological stock assessments and monitoring, although this application is moving rapidly. More futuristic are the applications of bio-signaling and biosensor technology, especially in global change/global monitoring, where the role of microorganisms in weather regulation and weather processes is only vaguely suspected and poorly understood, the latter especially the case for biogeochemical cycling. The notion of a "microbial loop" and its role in global ocean processes has only relatively recently been enunciated (Azam et al., 1983; Ducklow et al., 1986). This aspect of marine biotechnology is very much in the future, perhaps 10-15 years down
the road, although some investigators are currently pursuing biosensors/bioimaging and its applications (Bains, 1992). Similarly, the use of microalgae for addressing global warming is being considered by Miyachi and colleagues (Kodama et al., 1993).

During the 1970s and early 1980s, ecosystem modeling was very much in vogue, later falling into lesser prominence, mainly because the software (and in some cases, the hardware) was not sufficiently sophisticated, nor were the data complete enough to pass the "reality checks." With the extraordinary power of computing and the volume of data now able to be collected, as well as historical data available in data banks, ecosystem modeling will provide a powerful means of interpreting microbial ecosystems of the world oceans.

Marine biotechnology is a long-term investment technology. There are some immediate commercial successes, notably in the food industry, but as the Japanese government has decided, the view should be with an 8-10 year investment strategy. The U.S. Congress has now passed a marine biotechnology initiative that will provide up to $20 million for marine biotechnology research and development. Industry, over the past decade, has begun to move into marine biotechnology. The development of this aspect of marine biotechnology, industrial marine biotechnology, is detailed in the next chapter.

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A REPORT ON THE U.S., JAPAN, AUSTRALIA, AND NORWAY • 65


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