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Marine biotechnology.

REPORT TO UNITED NATIONS

INDUSTRIAL DEVELOPMENT ORGANIZATION (UNIDO)

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SUMMARY

A review of the field of marine biotechnology was undertaken to assess major developments which appear to be most promising. It is concluded that marine pharmaceuticals, genetic engineering of marine and estuarine animals and plants for food production, and marine specialty chemicals offer both immediate and long term reward for island and riparian nations.

In the short term, practical results are anticipated in improving stocks of fish and shellfish for aquaculture and in developing compounds of pharmaceutical value from marine sources for human and domestic animal application. Also anticipated to be of immediate benefit are vaccines produced by genetic engineering methods for prevention and control of diseases of fish and shellfish in aquaculture.

Marine biotechnology holds the greatest promise for developing countries where fish and shellfish are a major source of food, as well as an industrial commodity. Furthermore, developing countries possessing abundant marine and estuarine natural resources are ideally suited for exploration of marine biotechnology, since access to unusual and/or novel marine life permits direct utilization of new and/or unique products by genetically engineering and ensures continuous production in the laboratory and can eliminate fluctuations in production caused by weather and climate.

Island and riparian countries should explore the potentialities of marine biotechnology that are unique to their regions. Existing marine laboratories should be linked with molecular genetic laboratories, if any exist within the country. If sufficient financial resources are available, investment in a genetic engineering laboratory, with sufficient staff to accomplish the necessary work for genetic engineering of marine systems, should be established. If financial constraints limit development, linkage of the marine facility with a molecular genetic/genetic-engineering laboratory in a developed country can be a cost-effective mechanism for establishing a marine biotechnology center.

Workshops, seminar programs, and short-course training for molecular biologists to become familiar with the workings of marine systems can also provide a means of technology transfer, i.e., to abbreviate the route to establishment of a marine biotechnology capability.

Direct linkage of a marine field station with molecular genetic facilities and staff can be done to develop a marine biotechnology capability. However, for technology transfer to industry, further, direct linkage to marine industries of the country will ensure results of marine biotechnology research being made available to relevant and interested industries.

A draft plan for development of marine biotechnology appropriate for UNIDO to employ, or request, as a basis for providing advisory services to island or riparian countries wishing to draft national programs in marine biotechnology or to establish national biotechnology research centers is provided.

INTRODUCTION

Genetic engineering, i.e., applied molecular biology represents a giant, technological "leap" forward, a major saltation in the structural development of modern civilization. All institutions are being affected, not the least of which is the influence of this new technology on the role of universities in society. As with the invention of the steam engine and splitting of the atom and concurrent with the advent of the computer, a revolutionizing of science and society is underway.

Aquaculture

Genetic engineering applied to the production of fish, mollusks, and crustaceans, either in natural environments or hatchery systems, although at a somewhat rudimentary stage, offers unique promise. *In vitro* manipulations, such as cloning, cell fusion, production of chimeras, and other recombinant DNA techniques applied to these animals provide an impetus for major advances in fish and shellfish genetics. Successful aquaculture of many species of invertebrate animals has been achieved and the stage set for the realization of the potential of genetic engineering, since very large populations of shellfish--in the form of larvae and intermediate stages--can be manipulated and their genes cloned. Work at the University of Maryland has recently yielded a "gene bank" for *Crassostrea virginica*, a commercially important shellfish species in Chesapeake Bay.

Gene manipulation, i.e., introduction of new combinations of heritable material by insertion into any virus, bacterial plasmid or other vector system of nucleic acid molecules (the basic genetic material of all organisms except some viruses), allows for the incorporation of heritable material into a host organism in which it does not naturally occur, but in which it becomes capable to continued propagation. These methods are being applied to aquaculture, employing larvae of fish and shellfish, including oysters, clams, abalone and molluscan species.

Historically, aquaculture has long been established in China, where, for example, ca. 2 million metric tons of finfish are now produced every year, mostly in the form of carp grown in ponds, lakes, reservoirs and ditches. The rate of knowledge-gathering in marine biology and advances made in technical expertise for applying discoveries in marine biology to aquaculture has increased significantly over the past decade. Research is underway on marine shrimp, freshwater prawn, crawfish, blue crab, brine shrimp, salmon and other finfish, oysters, clams, abalone and scallops.

For developing countries, this aspect of marine biotechnology is of great importance, since the traditional approaches to aquaculture have benefited by modernization and promise to be even more favorably influenced by genetic engineering. As one of several examples, the reproduction and growth of gastropod molluscs of the genus Haliotis can now be controlled. It has been

shown that spawning is normally regulated by prostaglandins,hormones regulating reproduction in humans and other animals, with the rate-limiting process in the mollusc being enzymatic synthesis of the prostaglandins. Synthesis of the latter in reproductive tissue is controlled by very small amounts of hydrogen peroxide. Incidentally, enzymatic synthesis of prostaglandins occurs not only in the reproductive tissues of Haliotis, but in that tissue of many other molluscs as well. Spawning in a large number of these species can be induced conveniently, reliably and inexpensively simply by adding a low concentration of peroxide to the surrounding seawater. In fact, thirteen species of abalone, including Haliotis gigantea from Japan, four species of Crassostrea, three species of Mytilis, two species each of Tridacna, Cellana and Trochus amd several other genera, have been found to spawn in response to stimulation with hydrogen peroxide.

After spawning and development of the fertilized eggs to the larval stage, settlement and metamorphosis has been shown to be under similarly stringent biochemical control, dependent upon larval recognition of specific molecular signals, deduced early on from the patterns of substrate-specific recruitment of larvae, an observation made for many species.

The inducer required for induction of Haliotis larval settlement and metamorphosis has been identified and characterized. Interestingly, the inducer is a chemical uniquely available to

the larvae only at the surface of crustose red algae, including species of Lithothamnium, Lithophyllam philippi and Hildenbrandia nardo. Larval contact with the inducers at the algae surface triggers rapid settlement and metamorphosis, accounting for the substrate-specific recruitment of Haliotis larvae to crustose red algae in the benthic environment.

Inducer molecules extracted from the natural recruiting algae and active for the abalone are derivatives of aminobutyric acid (GABA), a simple amino acid neurotransmitter. The GABA-related molecule appears to be recognized by stereochemically specific chemosensary receptors on the larval epithelium. GABA alone will induce rapid and complete settlement and metamorphosis when an available substrate is provided.

Interestingly, binding of the neurotransmitter-like GABA-related inducing molecule to larval receptors activates the behavioral and development sequence, resulting in settlement and metamorphosis. Morse and his colleagues at the University of California at Santa Barbara have described the stereochemical specificity and binding properties of the larval receptors, regulation of the receptors by endogenous and exogenous factors, including seawater-borne amino acids; the induced ionic flux resulting in depolarization of the chemosensory membrane, with resulting transduction of the inducing signal; and the early sequence of developmental changes, including larval secretions, abscission of the velum, internal organogenesis and shell growth,

leading to irreversible commitment to the benthic habitat, and initiation of growth of the attached juvenile.

Along with the ability to control spawning, larval settlement, and induction of metamorphosis, enhancement of growth will affect aquaculture of the abalone significantly, since the animal grows relatively slowly. In fact, abalone require several years to mature, with significant heterogeneity in growth parameters. Professor Morse has shown that postlarval abalone growth can be accelerated significantly by addition of specific, exogenous peptide hormones. The growth-regulating hormones, insulin, and growth hormone, isolated from mammals, have proven effective, and both act in a concentration-dependent manner to accelerate early growth, yielding accelerations of ca. 25% over the mean growth rate within the first few days following metamorphosis, also reducing heterogeneity in growth rates and sizes. Apparently, the active hormones increase efficiency of nutrient assimilation and utilization, rather than increase feeding activity and ingestion.

To scale-up the production and provide for the use of homologous, molluscan growth-regulating peptide hormones, normally encoded by the Haliotis DNA cloned in microbial plasmid vectors, DNA from abalone sperm has been purified, treated the DNA with DNA-restriction enzymes and ligated or recombined the resulting DNA fragments of the Haliotis genome (both en masse and as electrophoretically purified, separate genes) with the DNA of genetically modified, autonomously replicating plasmids. The

plasmids used as cloning vectors were selected for proven ability to amplify the production of peptide hormones from cloned DNA templates, when introduced into cells of rapidly dividing producer strains of bacteria or yeast.

The abalone gene-bank and its counterpart in the Eastern oyster, Crassostrea virginica, and individually cloned genes of these animals will prove useful for economical production of safe, homologous (molluscan) peptide hormones for enhancement of nutrient assimilation, increased synthesis of protein and glycogen (meat constituents of greatest value), and acceleration of growth.

A role of prostaglandin has also been shown for barnacles in egg hatching, with the major marine invertebrate source of prostaglandins being the gorgonian, Plexaura homomalla. The larvae of the coral-eating hudibranch Phestilla sibogae settle and metamorphose specifically in response to a soluble, coral-produced substance. They undergo slow, but complete metamorphosis, in response to choline, GABA, and related compounds. For the chiton Tonicella lineata, a relatively high molecular weight factor (60,000 - 100,000 daltons) associated with food of the Chiton, the coralline alga Lithothamnion, induces settlement of chiton larvae.

Clearly, major advances have been made since the late 1920's, at which time, and before, it was generally assumed that metamorphosis of marine larvae was simply a function of the

developmental state of the animal, i.e., once larvae developed the ability to metamorphose, they would do so, and for the few which by chance "fell upon good ground" and survived, the vast majority would "fall by the wayside and be lost." With the most recently gained, new information, it is evident that planktonic larvae of many benthic invertebrates settle and metamorphose in response to specific substances, or conditions, in their environment, and may delay metamorphosis indefinitely in the absence of those substances. Metamorphosis-stimulating factors have, in almost every case, shown to originate from, or be related to, some feature of the preferred adult environment. These features include presence of other individuals of the same species, algal or bacterial films, specific types of substrata, or certain plant or animal species, often those upon which the metamorphasing species will feed as an adult. For any particular species, two or more of these factors may act together as the appropriate metamorphic stimulus. Thus, once the factor(s) can be identified, the genes cloned, and the production of the factors exogenously amplified, controlled aquaculture is possible.

At the University of Maryland, studies on bacterial films implicate bacterial by-products as factors responsible for induction of metamorphosis of the Eastern oyster, Crassostrea virginica. Serotonin (5-hydroxytryptamine), succinylcholine chloride, or acetyl-beta-methylcholine chloride have routinely been used to induce "artificially" metamorphosis of larvae of the gastropod, Ilyanassa obsoleta. Veitch and Hidu demonstrated that thyroxine

and related iodinated compounds stimulated setting of Crassostrea virginica.

L-DOPA has been found to be active in promoting oyster set by Weiner, Bonar, and Colwell, at the University of Maryland. Crassostrea virginica larvae become competent to metamorphose one to two days following the appearance of pigmented eyespots and this developmental stage is typically reached at a specific shell size (about 260 micrometers). Interestingly, it was discovered that competent larvae have only a few days during which they can metamorphose and then if presented with an attractive substratum. Once competence is acquired, the ability of a group of larvae to metamorphose declines, so that after eight days, only a small percentage (approximately 11%) of the larvae are still capable of metamorphosis. The unmetamorphosed larvae show increasingly reduced activity and subsequently die.

Metamorphosis will occur on a suitable substratum, and evidence shows that oyster larvae have a predilection for surfaces coated with periphytic microbiota. Consistently present in this primary film is a bacterium, LST, which has been repeatedly isolated from the substratum surface film to which oyster larvae set.

Invertebrate species about which the effect of periphytic organisms on induced metamorphosis has been studied include the sea urchin Lytechinus pictus, cnidarians Hydractinina echinata and Cassiopea andromeda, and the annelid Janua brasiliensis. For

Lytechinus, the responsible factor is a low molecular weight, less than 5,000 daltons, bacterial byproduct, very likely a protein. Muller and his associates observed that planulae larvae of Hydractinia metamorphose in response to a product eneitled by certain marine, gram-negative bacteria at the end of their exponential growth phase. If the bacterial cultures are subjected to osmotic shock, the activity shows up in the supernatant, suggesting it to be a soluble factor rather than a bound one.

Cole and Knight-Jones found that the larvae of Ostrea edulis, the European oyster, prefer setting on surfaces covered with a film of bacteria and diatoms.

In addition to the well-documented case involving LST and Crassostrea virginica larvae, cited above, preliminary evidence points to other potential examples of bacterial-invertebrate symbioses. Melanin has been reported to protect organisms in the marine environment and, in addition, it has been shown that some procaryotes, viz. vibrios, survive longer when they are associated with invertebrate chitin. Therefore, it is concluded that associations between bacteria and invertebrates are stongly mutualistic and bacterial products can function as mediators.

The bacterium LST adheres very strongly to cultch and other hard surfaces, forms micro-colonies on cultch, and, in sufficient numbers, during the decline phase of its growth, produce a high concentration of pigment, sufficient to attract Crassostrea virginica larvae. The larvae can feed on LST and, as noted earlier,

induce reproduction of the bacterium, much as a lectin produced by Halachondrea panicea stimulates the bacterium Pseudomonas insolita. Thereby, the larvae are able to disseminate the bacterium, and, reciprocally a bacterial metabolite can, by production of a hormone-like compound, stimulate larval development and metamorphosis.

It is significant that the natural molecular inducer required for Haliotis recruitment, settlement and metamorphosis is an amino acid derived, neurotransmitter-related, small molecule linked to a large (protein) polymer. As stated above, this class of molecular structure has been documented to be involved significantly in the induction of larvae settlement and metamorphosis. Crassostrea virginica larvae are induced to settle and undergo metamorphosis by contact with melanin-like polymers of dihydroxyphenylalanine (DOPA) produced by marine bacteria, and by various analogues of the amino acid derived, neurotransmitter-related compound, DOPA.

Application of genetic engineering and modern biotechnology should permit cloning of genes controlling production of these attractants and inducers. Recombinant-DNA probes and templates will make it possible to analyze and control the life-cycle processes of benthic invertebrates. Clearly, the implication for developing countries where aquaculture of marine invertebrates is important, is that production can now be controlled.

Vaccine production

Another important area in which genetic engineering and advances in applied molecular biology can be applied to aquaculture is in the control of microbially-mediated disease. Vibrio disease, for example, is wide-spread amongst fish. Viral agents, including IPN, egtved, and other viruses, as well as Aeromonas spp. and a variety of other bacteria also cause disease and loss of hatchery stocks. Production of vaccine strains, employing genetic engineering for excision of virulence factors, as has been done for Vibrio cholerae and other agents of human disease, should be equally effective for controlling vibrio diseases of fish and shellfish.

An extract from Ecteinascidia turbinada (Ete) has been shown to enhance hemocyte function of invertebrates, e.g., the blue crab (Callinectes sapidus), crayfish (Procambarus clarkii, and prawn (Macrobrachium rosenbergii), possibly rendering the animals more resistant to infection. Interestingly, intraperitoneal injection of Ete renders eel strongly resistant to Aeromonas hydrophila and appears to potentiate phagocytic activity. Ete also causes changes in the concentration of peripheral blood leucocytes.

Thus, biotechnology offers opportunities for control of diseases in aquaculture of the many species of shellfish and finfish already available in culture. Furthermore, it offers a means for

obtaining presently "recalcitant" species in culture. Obviously there are excellent opportunities for gene selection, manipulation and amplification.

Production of vaccines, employing both hybridoma technology and genetic engineering, can advance aquaculture significantly, especially in increasing productivity and improved success in maintaining animals from the egg through larval stages, presently a high-risk portion of the life cycle of cultured species.

Seaweeds

Marine plants offer special opportunities, and genetic engineering of osmoregulation, for example, is being studied. Plants which are halophytes can be introduced to agricultural areas where the soil has become too salty for conventional agriculture. Halophytes, as well as selected stocks of marine and estuarine grasses, can be beneficial in managing erosion and shoreline losses. This represents a unique opportunity for island and riparian countries with arid regions.

Seaweeds are far more economically important than generally realized. They are used as human and animal food, in medicine and agriculture, and as a source of raw materials for numerous industries. The Porphyra, or nori, industry in Japan alone is estimated to involve over 60,000 hectares in cultivation area and to be worth more than $730 million annually. In fact, Porphyra

is the most important mariculture crop in Japan at the present time.

In the western hemisphere, seaweeds are principally utilized as a source of phycocolloids, which induce agar, carrageenan and aliginate. These three phycocolloids have a combined current world market value in excess of $250 million annually.

Many workers have been successful in cultivating agar and carrageenan-producing seaweeds on a small, experimental scale in both the United States and Canada. Commercial seaweed cultivation is currently being conducted in both enclosed systems and ocean farms in other parts of the world. Altogether, there are approximately 11 genera (less than 20 species) of seaweeds being cultivated to a significant extent commercially on a worldwide basis. For some developing countries, seaweeds represent an important food source.

In general, the application of genetic modification/improvement techniques to seaweeds is recent and is, as yet, somewhat limited. The most widely used approach has been that of simple strain selection, i.e., the screening of wild plants for deisrable traits such as rapid growth. Strain selection experiments have been conducted on several economically important seaweeds, including Chondrus.

Perhaps the most notable success to date in genetic improvement of seaweeds has been that of Chinese researchers working

with the kelp Laminaria japonica, a plant not native to Chinese waters. Through the use of a variety of techniques, including intensive inbreeding and selection, X-ray induced mutations and colchicine treatment, new and improved stains have been produced that have resulted in higher yields and extensive geographical expansion of the Laminaria culture industry in China.

Some of the recent research is aimed at producing new, cultivable strains of high quality agar-producing seaweeds employing protoplast fusion-somatic hybridization techniques. A major advantage can be transferred from one species to another without involving (or requiring) sexual reproduction. Thus, it is theoretically possible to hybridize individuals from different (sexually incompatible) species (or genera), as well as from the same species (or genus). Furthermore, somatic hybridization offers the potential of hybridizing sterile individuals, as well as species in which male and female reproductive structures are rare and/or difficult to snychronize. Therefore, with such methods, it should be possible to hybridize species of Gracilaria and Gelidium, which does not ocur in a sexual hybridization.

Interestingly, a large body of literature is available concerning protoplast isolation and fusion in "higher" (i.e., seed) plants supporting the notion that, with increased emphasis placed on marine plants, breakthroughs can be expected within the near future. In fact, protoplast isolation has already been ccomplished in approximately 28 genera of algae.

Marine pharmaceuticals

Perhaps one of the most dramatic examples of marine biotechnological applications is in marine pharmaceuticals. A conference was held at Norman, Oklahoma, in the United States, in September, 1977 and at that time there were described cardiotonic polypeptides isolated from sea anemones, an adrenergic compound from the sponge, Verongia fistularis, and potential anticancer agents from Caribbean gorgonians and soft corals. An extensive literature is now available on marine natural products, many of which appear to have potential pharmacological value. The Porifera, algae and coelenterata were studied and compounds extractd from sponges, coelenterates, algae, and seaweed, most of which have biological activity have been described. In general, a high proportion of extracts that have been studies have proven to be cytotoxic, in preliminary experiments, with many of the antibacterial, antifungal and antiviral compounds that have been isolated. In many cases, biological activities have been confirmed in more extensive assays employing tumor cells, pathogenic microorganisms, and viruses. Of special interest are the didemnins, depsipeptides isolated from a didemnid tunicate, inhibiting several RNA and DNA viruses and exhibiting potent cytotoxicity against tumor cell lines.

Extracts prepared from the Caribbean tunicate, an ascidian or sea squirt of the family Didemnidae, inhibit growth of DNA ad

RNA viruses, as well as L1210 leukemic cells. These depsipeptides--termed didemnins after the name of the tunicate family, Didemnidae, from which they are isolated--are closely related, but vary in activity. The discovery indicates that the subphylum Tunicata or Urochordata (phylum Chordate) may be an abundant source of bioactive compounds of pharmaceutical interest. The tunicate of the Tridemnum genus, when extracted with methanol-tolulene (3:1) showed activity against herpes simplex virus, type 1, grown in CV-1 cells (monkey kidney tissue), indicating that it inhibited the growth of the virus. This antiviral activity may also involve antitumor activity. When tested against other viruses, essentially all extracts of the tunicate collected at a number of sites showed activity in inhibiting both RNA and DNA viruses. The suggestion that the extracts might also have antitumor properties was evidenced from their high potency against L1210 murine leukemic cells. The novelty of the didemnins results from a new structural unit for depsipeptides, hydroxy-isovalerylpropionate and a new stereoisomer of the higher unusual amino acid, statine.

In the literature, there is described a variety of compounds from the sea, which act on the cardiovascular and central nervous systems. Marine animals and plants have yielded cardiovascular-active substances, and these include histamine and N-methylated histamines of sponges, viz. Verongia fistularis, asystolic nucleosides from the sponge, Dasychalina cyathina, and the nucleoside, spongosine, isolated from Cryptotethya crypta.

Several marine organisms have provided useful drugs: liver oil from some fish provides excellent sources of vitamins A and D; insulin has been extracted from whales and tuna fish; and the red alga, Digenia simplex, has long been used as an anthelmintic. Bacteriologists, for many years, have incorporated agar and alginic acids into laboratory media. In general, it has been uneconomical to extract and purify a drug from an organism which has to be captured in large quantities from remote corners of the world. Thus, only a few marine organisms are currently sources of useful drugs. Genetic engineering can change this situation dramatically, opening up a vast and diverse range of marine life to probing for valuable pharmacological compounds, if the genes coding for production of the compounds can be cloned into laboratory stains of microorganisms. In the long run, these opportunities will open as the tools for gene cloning are sharpened and the applications broadened.

Marine Toxins

Of particular interest in marine systems are toxins produced by marine organisms. A toxin is a substance possessing a specific functional group arranged in the molecule(s) and showing strong physiological activity. A toxin has the potential to be applied as a drug or pharmacological reagent. Furthermore, even if direct use as a drug is not feasible, because of potent or harmful side effects, the toxin can serve as a model for synthesis or improvement of other drugs. Many attempts have been

made to develop useful drugs from the sea by screening for anticarcinogenic, antibiotic, growth-promoting (or inhibitory), hemolytic, analgetic, antispasmodic, hypotensive and hypertensive agents.

Two successes demonstrate the potential. Tetrodotoxin, the main action of which is paralysis of peripheral nerves, is a valuable pharmacological reagent because it specifically inhibits the sodium permeability of nerve membranes. It has been valuable for elucidating the excitation mechanism. However, it must be emphasized that, for the moment, applications of marine toxins are limited, to say the least. It is mainly in the area of understanding structure and function of neurological systems that the toxins have an interest.

A second success, i.e., a commercial success, because it represents a marketed product, is an insecticide developed from nereistoxin. Fishermen are familiar with the fact that flies die when they come into contact with the dead marine annelid, Lumbrineris (Lumbriconeris) brevicirra, commonly used as bait. A toxin of the annelid was first isolated in 1934, and once its structure was determined, a new insecticide was developed from the compound nereistoxin. Cartap hydrochloride, the name of the commercial product, is one of the synthesized derivatives. Active against the rice stem borer and other insect pests, it has been marketed since 1966. Unlike DDT and BHC, it does not appear

to be toxic for warm blooded animals, and resistant strains of insects do not readily develop.

With increased interest in marine toxins or bioactive substances in marine organisms, research on these substances has increased in recent years, with a number of monographs and reviews appearing in the literature. The burgeoning research work in this area has provided the focal topics of symposia in conferences since "Drugs from the Sea," the first, which was held in Rhode Island, in the United States, August, 1967.

Marine toxins show great promise as pharmacological reagents, viz., tetrodotoxin, and as models for development of new synthetic chemicals. Recently, ciguatoxin, palytoxin and halitoxin have also been investigated and provide interesting, new information.

Ciguatera is a human disease caused by the ingestion of a wide variety of coral reef fishes that contain toxins accumulated via the marine food web. The principal toxin of ciguatera poisoning is a heat-stable, lipid-soluble compound named ciguatoxin, and the source of ciguatera toxin(s) is a photosynthetic, benthic dinoflagellate, Gambierdiscus toxicus Adachi and Fukuyo. Origin of the toxin is unknown but may be derived by the fish from ingestion of toxic tropical red tide dinoflagellates such as Pyrodinium bahamense.

Because the source of ciguatoxin has not been clearly identified, application of gene cloning techniques is not yet feasible, should this compound prove of potential pharmaceutical use. However, hybridoma technology will be invaluable in identifying, isolating and characterizing this and other toxins, as well as for producing diagnostic and therapeutic reagents for treatment.

Applications of hybridoma technology in marine pharmacology are practically unlimited, including study of the structure and function of the toxins, as well as for production of anti-toxins for treatment, an especially important application since there are no antidotes for many of the toxic venoms of marine animals at the present time and the incidence of ichthyosarcotoxism and toxic effects of poisonous fish and invertebrates are not uncommon, especially in native populations of island communities.

Halitoxin, a toxic complex of several marine sponges of the genus Haliclona, has been isolated, partially purified, spectrally characterized and chemically degraded, yielding a proposed chemical structure for the toxin. The toxin has proved to be a complex mixture of high molecular weight, toxic pyridinium salts and can be isolated from the sponges, Haliclona rubens, H. viridis, and H. erina. The sponge extracts are toxic for fish and mice (LD^{50} 275 mg/kg) and also inhibit the growth of Ehrlich

ascites tumors. Thus, the halitoxin(s) may prove to be an antitumor agent or agents. Hybridoma technology applied to characterize these compounds should permit screening for the toxin amongst a variety of sponges, as well as for subsequent purification and testing.

Lophotoxin, a neuromuscular toxin isolated from several Pacific gorgonians of the genus Lophogorgia, has been isolated and purified. Originally discovered during a search for chemical defense adaptations of marine organisms, a variety of horny corals or gorgonians (sea fans and whips; phylum Cnidaria, order Gorgonaceae) in tropical or subtropical waters were studied and cytotoxic, ichthyotoxic and antibacterial activity was noted. Lophotoxin inhibits nerve-stimulated contraction without affecting contraction evoked by direct electrical stimulation of the muscle. The data suggest that epoxylactone and furanoaldehyde groups may be responsible for the potent biological properties of lophotoxin.

Palytoxin, an extremely poisonous, water-soluble substance from marine coelenterates belonging to the genus Palythoa has recently been described and its structure elucidated. The molecular weight of the toxin is 2700, with three nitrogens in the molecule. A complete stereochemical structure has been proposed.

The palytoxin represents an interesting challenge for cloning of the gene(s) synthesizing the toxin, but the general principles of gene manipulation should apply.

The need, at the moment, in marine biotechnology is for strategies for collecting, culturing, and screening marine organisms, from which bioactive agents can be isolated and characterized. Most likely, the immediate successes will occur in discoveries of novel anti-bacterials or antibiotics produced by marine bacteria. However, the potential for engineering the production of the more complex pharmaceuticals and polysaccharides of commercial value exists. Ingenuity will certainly provide the means and the initiative.

Industrial Chemicals

Marine toxins are fascinating, from a scientific point of view, but it is more likely, in the short term, that the marketable products will come from marine polysaccharides, carotenoids and specialty chemicals, such as unusual sugars, enzymes, and algal lipids. These represent products of short-term, and rather immediate pay-off.

In fact, carrageenan is a major product from the red seaweeds and is widely used as an extender in foods and related products, from evaporated milk to toothpaste. Agarose is widely employed in electrophoresis and chromatography analyses in the laboratory. Thus, seaweed culture offers an opportunity for gene cloning and transfer in microbial processes that can extend the presently profitable market.

Specialty chemicals from salt-tolerant microbial systems, notably polysaccharides and lipids, offer the greatest potential in the immediate future.

Besides toxins and biologically active substances and those substances already exploited commercially, such as carrageenin, chitin, and agarose, a variety of interesting compound metabolites have also been reported, including spatane diterpenoids, from the tropical marine alga Stoechospermum marginatum, not yet observed from terrestrial sources.

Sponges and gorgonians have been useful sources of biologically active metabolites because they are frequently abundant, permitting pursuit of trace metabolites. These unusual compounds may be pathway intermediates and offer potential sources of new chemicals. Sea hares provide the advantage of being rich sources of interesting metabolites, but the ultimate source of the latter is not always the animal itself, often proving to be algae on which it feeds or is associated. Extracts of the sea hare, Aplysia dactylomela show both cytotoxicity and in vivo antitumor activity.

Recently the cloning and expression of sea urchin histone genes, using SV-40 DNA as a vector has been reported. Thus, the cloning of genes of marine animals has begun, but the opportunities remain to be realized.

Biodegradation in the Marine Environment

In contrast to natural products, man-made compounds are relatively refractory to biodegradation, usually because organisms naturally present often cannot produce enzymes necessary for transformation of the original compound, such that the resulting intermediates can enter into common metabolic pathways to be metabolized completely, creating special problems for waste treatment and environmental protection.

Required steps to initiate biodegradation are reasonably well understood. Halogenated compounds, are known to be persistent because of the location of the halogen atom, the halide involved, and the extent of halogenation. Selective use of microorganisms, including actinomycetes, fungi, bacteria, phototrophic microorganisms, anaerobic bacteria and oligotrophic bacteria, is not new but represents a common practice in certain applications, such as wastewater treatment for biological removal of nitrogen via sequential nitrification and denitrification. Controlled mixed cultures are already in use in Japan for treating selected industrial wastes in reactors, comprised of heterotrophic bacteria, photosynthetic bacteria and algae. Population selection based on the use of various methods of genetic engineering to develop optimized proliferation and maintenance of selected populations will certainly become widely used.

What has not yet been widely applied, however, is the engineering of microorganisms to be added to wastes that are to be discharged into the marine environment. It is obvious that, with increased use of the world oceans for man's waste, attention must be paid to the problems of marine pollution. Pollutants entering the ocean that can interfere with the integrity of ecosystems include synthetic organics, chlorination products, dredged spoils, litter, artificial radionuclides, trace metals and fossil fuel compounds. Toxaphene, a group of slightly under 200 compounds, i.e., chlorinated hydrocarbons produced by chlorination

under ultraviolet light of wood waste products, camphors, contains carcinogenic and mutagenic members and may be more persistent in the environment than DDT and its degradation products. A concerted effort to develop marine microorganisms that can be added to waste effluent prior to discharge, in order to ensure degradation of the recalcitrant species of compound should be made.

The problems of *in situ* degradation are much greater than for contained application. The modifications of genetic information resident in microorganisms that are useful in pollution control are: 1) amplification of enzyme concentrations in an organism, either by selection of constitutive mutants, increase in the number of copies of the gene for the enzyme, or both, 2) rearrangement of regulatory mechanisms controlling the expression of specific genes in response to specific stimuli, 3) introduction of new enzymatic functions into organisms not possessing them, and 4) alteration of the characteristics of specific enzymes, viz. substrate specificity, kinetic constants (K_m and V_{max}) or factors such as pH optimum. To achieve these modifications, *in vitro* recombinant DNA manipulation, *in vivo* modification via transposon mutagenesis or other transposon-mediated gene manipulation, genetic exchange via transduction, transformation, or conjugation, protoplast fusion, specific site mutagenesis, and specialized selection procedures to enrich for mutants can be

employed. What has not been considered to date is the engineering of microorganisms capable of flourishing in the marine environment. Ability to grow at low temperatures in a high saline environment (35%), of a relatively high pH (8.2) and, in the deep sea, under elevated hydrostatic pressure, are characteristics of organisms which should be engineered for use in treatment of recalcitrant wastes that are dumped into the ocean.

Of the achievements in marine biotechnology cited above, those of particular relevance to developing countries are in marine pharmacology, aquaculture, and marine plants. The products which can be expected include new drugs, food products, and specialty chemicals. Of particular interest, however, is the possibility of establishing aquacultured species which have enhanced genetic traits, i.e., resistance to desire, more rapid growth, etc., achieved by genetic engineering.

Plan of Action for Island and Riparian Developing Countries

In order for developing countries to achieve progress from applied marine biotechnology, a cadre of trained marine biotechnologists must be produced and the necessary facilities provided. It would be most efficient to link existing marine biotechnology laboratories with molecular genetic laboratories if both exist in the developing countries. If this is not the case, it would be effective to link with a developed country and to establish an exchange of students and faculty.

Technology transfer can be achieved effectively by workshops, training courses, and visiting lecture series. However, practical experience must be included, so that the procedures and methods of genetic engineering can be instituted within the developing country.

Because of the diversity and abundance of unusual marine animals and plants in island and riparian countries, the opportunities for discovery of new drugs, food sources, and specialty chemicals is great. Thus, the attraction of marine biotechnology for such countries is significant.

To ensure results from research in marine biotechnology are made available to the relevant industries, a close linkage between the research laboratory and the industry should be encouraged. In fact, investment by industry in marine biotechnology ventures will catalyze development, allowing industry access to new products with the result of immediate development and market exploitation.

Suggested Plan of Development for UNIDO

A brief outline of plan of action which UNIDO may follow and employ, on request, as a basis for providing advisory services to island or riparian countries that may wish to draft national programs in marine biotechnology or to establish national marine biotechnology research centers is provided in the following section.

Having established the will to move into marine biotechnology, an assessment of the strengths and/or weaknesses of the country with respect to molecular biology and marine sciences should be done. This can be accomplished by engaging consultants, i.e., either internal or external experts. Knowing the potential for the country, viz. marine pharmacology or aquaculture, a plan for development of a center can be initiated.

Goals of the marine biotechnology effort should be determined. For example, if the goal is to attract new industry, a strength in marine biotechnology research and development should be initiated. If local industry is to be strengthened, linkages with universities, especially with molecular genetic and genetic engineering talent should be established.

A financial plan, i.e., costing of staff, facilities, and core support will be needed, but the scale of effort should be appropriate to the long range developme..t expectations of the country.

Finally, recruitment and/or training of a cadre of biotechnologists appropriate to the long-term goals will be required.

Projects to be undertaken should be a mix of short-term and long-term efforts.

Linkage of developing nation centers with counterparts in dveloped countries should allow a by-pass, i.e., more rapid achievement of goals.

The potential of marine biotechnology from developing countries is especially great. The opportunities now awaiting should not be lost.

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