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15125

Distr.
LIMITED

UNIDO/IS.393
7 January 1988

UNITED NATIONS
INDUSTRIAL DEVELOPMENT ORGANIZATION

ENGLISH

MARINE BIOTECHNOLOGY AND THE DEVELOPING COUNTRIES*

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V.88-50206

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 prospects for both immediate and long-term rewards 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 genetic engineering and ensures continuous production in the laboratory, eliminating fluctuations caused by weather and climate.

Island and riparian countries should explore the potentialities of marine biotechnology 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 centre.

Workshops, seminar programmes, 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 establishing a marine biotechnology capability.

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

Herein is provided: (1) an overview of advances in marine biotechnology; (2) a summary of their applications to industry; (3) a discussion of the possibilities that marine biotechnology offers to the developing countries; and (4) a draft plan for the development of marine biotechnology appropriate for UNIDO to employ, on request, as a basis for providing advisory services to island or riparian countries wishing to draft national programmes in marine biotechnology or to establish national biotechnology research centres.

INTRODUCTION

Genetic engineering, i.e., applied molecular biology, represents a giant, technological "leap" forward, a major accomplishment 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 the splitting of the atom, and concurrent with the advent of the computer, a revolutionizing of science and society is underway.

Aquaculture

Historically, aquaculture has long been established in China, where for example, approximately 2 million metric tons of fin fish are produced every year, mostly in the form of carp grown in ponds, lakes, reservoirs and ditches. However, 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, crayfish, blue crab, brine shrimp, salmon and other fin fish, oysters, clams, abalone and scallops.

Gene manipulation, i.e., the introduction of new combinations of heritable materials 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 of continued propagation. These methods are being applied to aquaculture, employing larvae of fish and shellfish, including oysters, clams, abalone and other molluscan species.

In vitro manipulations, such as cloning, cell fusion, production of chimeras and other recombinant DNA techniques applied to marine 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 the Chesapeake Bay.

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 and 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 with many species.

The inducer required for induction of Haliotis larval settlement and metamorphosis has been identified and characterized. Intriguingly, 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 rardo. 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 chemosensory 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. Professor D. Morse and his colleagues at the University of California at Santa Barbara have described the stereochemical specificity and binding properties of larval receptors. They have also described regulation of the receptors by endogenous and exogenous factors, including seawater-borne amino acids, and the induced ionic flux resulting in depolarization of the chemosensory membrane, with resulting transduction of the inducing signal. The early sequence of developmental changes, including larval secretions, abscission of the velum, internal organogenesis and shell growth, all of which lead to irreversible commitment to the benthic habitat, have been elucidated, as well as the subsequent 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. 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 approximatly 25 per cent over the mean growth rate within the first few days following metamorphosis, while 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 clones in microbial plasmid vectors, DNA from abalone sperm has been purified, treated with DNA-restriction enzymes and ligated or recombined with 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 the enhancement of nutrient assimilation, increased synthesis of protein and glycogen (meat constituents of greatest value), and an 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 nudibranch 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 1920s, 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, been 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 "artificially" induce the 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 attraction and attachment to a surface, i.e, "set", by Weiner, Bonar, and Colwell. 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 only 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 per cent) of the larvae are still capable of metamorphosis. The unmetamorphosed larvae show increasingly reduced activity and eventually 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 (Lewes, Delaware, Spat Tank isolate, i.e., LST) which has been repeatedly isolated from the substratum surface film to which oyster larvae set.

Inverte' rate 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 released 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.

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 strongly mutualistic and bacterial products can function as mediators.

The bacterium LST adheres very strongly to "cultch", i.e., shell and other solid surface for spat set and other hard surfaces, it forms micro-colonies on cultch, and when present in sufficient numbers during the decline phase of its growth, produces 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 Halochondrea panicea stimulates the bacterium Pseudomonas insolita. Thereby the larvae are able to disseminate the bacterium, and reciprocally, the 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 permits cloning of genes controlling production of these attractants and inducers. Recombinant DNA probes and templates can be used to analyze and control the life-cycle processes of benthic invertebrates.

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 diseases. Vibrio disease, for example, is widespread amongst fish. Viral agents, including infectious pancreatic necrosis virus (IPN), and other viruses, as well as Aeromonas spp. and a variety of other bacteria, cause diseases 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 diseases, 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 occurring in aquaculture of many species of shellfish and fin fish. Furthermore, it offers a means for obtaining presently "recalcitrant" species in culture. Obviously such organisms represent 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 improving success in maintaining animals from the egg through larval stages, presently a high-risk portion of the life cycle of a significant number of cultured species.

Seaweeds

Marine plants offer special opportunities. 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 another 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 many 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 include agar, carrageenan and alginate. 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 U.S. and Canada. Commercial seaweed cultivation is currently being conducted in both enclosed systems and ocean farms in other parts of the world as well. Altogether there are approximately 11 genera (less than 20 species) of seaweeds being cultivated commercially to a significant extent 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 plarts for desirable 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. Laminaria provides a major food product in China.

Some of the recent research employing protoplast fusion-somatic hybridization techniques is aimed at producing new, cultivatable strains of high quality, agar-producing seaweeds. A major advantage is that genetic traits 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 synchronize. Therefore with such methods it should be possible to hybridize species of Glacilaria and Gelidium which does not occur via 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, protopl st isolation has already been accomplished in approximately 28 genera of algae.

Marine pharmaceuticals

Perhaps one of the most dramatic examples of marine biotechnological applications is in marine pharmaceuticals. At a conference held in September 1977 at Norman, Oklahoma, U.S.A., cardiotonic polypeptides isolated from sea anemones were described, as were an adrenergic compound from the sponge, Verongia fistularis and potential anti-cancer agents from Caribbean gorgonians and soft corals. An extensive literature is now available on marine natural products; many appear to have potential pharmacological value. The Porifera (invertebrates), algae and coelenterata were studied and compounds extracted from sponges, coelenterates, algae and seaweed, most of which have biological activity. In general, a high proportion of extracts studied have proven to be cytotoxic in preliminary experiments with many of the anti-bacterial, anti-fungal and anti-viral compounds 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 and 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:toluene (3:1) showed activity against type 1 Herpes simplex virus grown in CV-1 cells (monkey kidney tissue), indicating that it inhibited the growth of the virus. This antiviral activity may also involve anti-tumor 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 anti-tumor 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 a variety of compounds from the sea are described which act on the cardiovascular and central nervous systems. Marine animals and plants have yielded cardiovascular-active substances including 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 provide useful drugs: liver oil from some fish provides excellent sources of vitamins A and D; insulin extracted from whales and tuna fish; and the red alga Digenia simplex, long used as an antihelmintic. Bacteriologists have for many years 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 strains 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 of being 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 anti-carcinogenic, antibiotic, growth-promoting (or inhibiting), hemolytic, analgetic, antispasmodic, hypotensive and hypertensive agents.

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 one, which was held during August 1967 in Rhode Island, U.S.A.

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. 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 the new insecticide nereistoxin was developed from the compound. 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.

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. 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 the structure and function of neurological systems that the toxins are of interest.

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. The latter is an especially important application, since there are no antidotes for many of the toxic venoms of marine animals at the present time. 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} is 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 produced by 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 electrial stimulation of the muscle. The data suggest that epoxylactone and furanoaldehyde groups may be responsible for the potent biological properties of lophotoxin.

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 profit 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 having possibilities for short-term, perhaps 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, ranging from evaporated milk to toothpaste. Agarose is widely employed in electrophoresis and chromatography analyses in the laboratory. Because of this significant economic value, seaweed culture offers an opportunity for gene cloning and transfer in microbial processes that can extend the presently profitable market by providing a stable, bioengineered source of the polypaccharides for production.

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 compounds and metabolites not yet observed from terrestial sources have also been reported, including spatane diterpenoids from the tropical marine alga Stoechospermum marginatum.

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 with. 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 for industry remain to be realized.

Biodegradation in the Marine Environment

In contrast to natural products, man-made compounds are relatively refractory to biodegradation, creating special problems for waste treatment and environmental protection. Usually this happens because organisms naturally present in the environment often cannot produce enzymes necessary for transformation of the original compound. The resulting accumulation of intermediate metabolites can be toxic and/or refractory to further metabolism, i.e., catabolism.

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 comprised of heterotrophic bacteria, photosynthetic bacteria and algae are already in use in Japan for treating selected industrial wastes in reactors. 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 oceans as a depository for mankind'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, contain carcinogenic and mutagenic members and may be more persistent in the environment than DDT and its degradation products. A concerted effort should be made 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.

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, it is possible to employ in vitro recombinant DNA manipulation, in vitro 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 parts per thousand), at 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 that 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 disease, more rapid growth, etc., achievable by genetic engineering.

Interest in marine biotechnology has increased significantly during the past five years, documented by the inclusion of topics dealing with marine biotechnology in national scientific society annual meetings, special symposia and conferences, including a Gordon Research Conference in Marine Biotechnology planned for the summer of 1986. That scientists are viewing this area of research with interest is evidenced by the number of papers on the subject that are currently appearing in journals. Industry is also casting about for a role in marine biotechnology, with special sessions on the subject scheduled and continuing to appear in programmes of industrial organization meetings. Furthermore, companies have already been formed e.g., SeaPharm, located in New Jersey U.S. Thus, investment in marine biotechnology has begun.

A few products have already been marketed, as a result of technological application and marine product development. An insecticide, Cartap chloride, cited above, is marketed in Japan. The insecticide derives from a marine annelid and is active against the rice-stem borer. Also in Japan, the use of tetrodotoxin in treating terminal cancer patients and test trials of lophotoxin and palytoxin as analgesics are in progress, as is the use of halitoxin as an anti-tumor agent.

A large-scale screening of marine bacteria and marine animals and plants for anti-bacterial, anti-tumor, and/or anti-viral activity is underway in several laboratories, with a few such substances already close to clinical trial (W. Fenical, personal communication).

Looking to the future, i.e., in projecting trends for the field of marine biotechnology, one can predict an increasing effort being placed on search and discovery of pharmacologically active substances, notably anti-microbial and anti-neoplastic agents. These products offer immediate economic reward and profits may be used to underwrite research in other, more long-term projects.

Close upon the pharmacologically active substances will be the development of vaccines and/or agents for the treatment of diseases of aquacultured fish and shellfish species. In the U.S. and the United

Kingdom vaccines against Vibrio disease (also called vibriosis, a devastating disease of salmonids in culture) are being "engineered", i.e., genetic engineering is being done to modify agent(s) for use as live vaccines, while new methods of vaccination are also being tested. Control of fish diseases will move conventional aquaculture significantly forward, carrying the industry from the precarious to the predictable, i.e., ensuring profit making. Culture of fin fish in Japan provides evidence of what can be done with currently available methods. Extrapolating benefits expected from the application of genetic engineering permits the prediction of significantly increased production, if the diseases are also fully controllable.

Natural product research is moving forward most impressively and the opportunities for applying strains of genetically engineered bacteria to carry genes for the production of alginates, carrageenins, and/or xanthans, are enormous. This line of research is being pursued by at least one company in the U.S. and very likely other companies in countries such as Japan and Western Europe will soon follow, if they have not already begun to do so. Thus specialty chemicals, notably those useful for food manufacture and processing, offer possibilities for short-term pay-off.

Over the long range, it can be predicted that basic research in aquaculture, seaweed culture, and mariculture will lead to closed-system, controlled production of fish and shellfish. Ultimately, tissue culture of fish and shellfish will provide fish protein for food in many countries, notably developing countries. For island and riparian countries, especially those falling into the category of developing nations, the opportunity for self-sufficiency in protein should be very attractive.

Also long-term, but achieveable, is return from investment in bioengineering of marine bacteria for controlled, closed system biodegradation. This can be both cost-effective and environmentally protective. For developing nations this aspect of marine biotechnology holds the advantage that an economic yield can be attained from by-product industries that can be developed, while the natural resources of the country, e.g. unusual species of animals and plants (a major resource in the years ahead), are protected.

In short, it is clear that future trends can be predicted which show possibilities for both short- and long-term pay-offs from investment in marine biotechnology.

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 R+D 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 are 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 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 being immediate development and market exploitation.

Suggested plan of development for UNIDO

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

The ideal model for a marine biotechnology centre of excellence will require the construction of a modern facility, housing both a molecular biology laboratory facility and a marine biology laboratory with a seawater circulation system, i.e., both open and closed seawater circulation. That is, the facility should include a modern microbiology and biochemistry laboratory and a chemical engineering laboratory with prototype and scale-up capabilities. The techniques that are required for R+D include tissue culture, hybridoma, and molecular genetic laboratory facilities. The seawater system should support open and closed aquaria. A closed system that is isolatable and autoclavable will serve for experimentation with fish disease agents and also for vaccine trials. The capital construction can be expected to require a minimum of about 200,000 square feet (net) fully equipped, with a cost of approximately US $200 per square feet; the total construction costs being estimated at US $40 million. Equipment for the laboratories should include high speed centrifuges, laminar flow hoods, an electron microscope (TEM) with ancillary equipment (such as photographic equipment), ultra-microtomes, etc. Spectrophotometers, scintillation counters, flow cytometers, high pressure liquid chromotography, mass spectrophotometry and associated analytical equipment should also be available to investigators. An estimation of equipment costs would be US $12 million.

The facility would require staffing by knowledgeable marine biologists who are familiar with maintenance, care and nutrition of marine animals and plants. It can also serve as an aquaria with staff who can offer tours and lectures, thereby engendering public support and providing a revenue source. Marine scientists knowledgeable in

reproduction, growth and behaviour of marine animals and plants will be needed. These individuals may also, though not necessarily, be molecular biologists in their interest, training and experience. The cadre of staff must include molecular biologists, genetic engineers, technicians and support staff. The molecular biologists, geneticists and biochemists may also be marine scientists, though not necessarily so. The ideal environment for marine biotechnology is one friendly to interdisciplinary research and training.

The number of people for start-up of a marine biotechnology centre, at the minimum, would include approximately 16 marine scientists, 16 molecularly-geneticist/genetic engineering scientists and 20 support staff. Availability of fellowships for postdoctoral and student level support is strongly recommended - approximately 15 postdoctorals, 20 graduate fellowships and support for 10 and 15 highly capable undergraduate students interested in research would be a good mix.

Linkage of the centre with a university is recommended. The excitement and stimulation of students and the opportunity for the senior staff to teach and direct graduate student research would make the centre active and vital, and hopefully, keep it constantly at the forefront of research. Guest (visiting) scientists should be encouraged and funds for 6 or 7 such appointments ought to be available.

The staffing of a marine biotechnology centre can derive from appointments of newly trained personnel, re-trained molecular biologists (genetic engineers trained in culture and genetic manipulation of marine organisms), marine scientists, and discipline-oriented, molecular biologists/genetic engineers and marine scientists. All categories should be represented.

In the short-term, start-up in staffing can be gradual, with an initial "core" of 50 staff, building to 90 staff within three to five years. Additional personnel can be hired on "soft" money, i.e., research and development grants and contracts, as the centre grows. Thus, at start-up the staffing requirement may cost between US $1.0 - 2.0 million per year.

Cooperation with industry should be emphasized by the centre from the first, including the planning phase. However, research directions of the centre should not be solely product-oriented but must include significant long-term research.

The surrounding environment of a marine biotechnology centre will be critical for its success. Ideally, it should be located on a coast or very close to the sea, but also near a university with access to libraries, computer facilities and other information gathering and processing capabilities.

Because the research will involve radioisotopes and potentially pathogenic and/or toxic organisms, customs service and air transport facilities will have to be considered, as well as regulations of both. If the aquaria can be a museum as well as a research facility, import regulations governing rare species may be less strict.

Clearly, a marine biotechnology centre located in one country may focus on research questions quite different than if it were located in another country, because of climate, available natural resources and diversity of animal and plant species unique to each locale. For example, a tropical country could focus on marine pharmaceuticals because it possesses an abundance of animal and plant species, coupled with the occurrence of toxic or poisonous species of fish and/or marine plants; whereas another country highly dependant on fish as a food staple would choose to emphasize aquaculture and genetic engineering of fish, with the objective of becoming protein self-sufficient. Diverse interests would not be an obstacle and would in fact, argue in favour of the establishment of several centres, as for example, the MIRCENs (Microbiological Resource Centres) which are operated by UNESCO, but on a larger, more focussed scale of operation. Linkage would then be very effective, with all areas of marine biotechnology being addressed, though not necessarily at a single location.

The retraining of scientific personnel is a possibility to consider when staffing the centre(s). It may be relatively easy to retrain a molecular biologist to work with marine systems, but this may not necessarily always be the case. For example, marine physiologists can be encouraged to learn the methods of marine biotechnology. On the other hand, aquaria curators and lower level technicians will be required. Retraining of these people would not be necessary because their duties and respons bilities for marine biotechnology remain essentially the same, i.e., to maintain cultures for laboratory research and development.

In moving from the ideal described above to the more pragmatic possibility, i.e., exploiting existing facilities in building a marine biotechnology centre, it is possible to move in either of two directions, i.e. towards upscaling a marine facility, or re-directing a molecular biology facility to marine biotechnology. As stated above, linkage of existing facilities is a reasonable alternative to construction and staffing of a completely new facility. The options are ranked as follows: a new facility being ideal, linkage of existing facilities a reasonable substitute, adding a marine facility to a molecular biology facility a possibility, adding a molecular biology laboratory to a marine facility also a possibility, linkage with a facility in another region or country an alternative to consider when funds are limiting. None of these options, it should be emphasized, precludes or prevents successful development of a marine biotechnology centre. Only the rate of progress and extent of success over the long term will be influenced, not whether or not a biotechnology centre is feasible.

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. Once the potential of the country is known, viz. marine pharmacology or aquaculture, a plan for development of a centre can be initiated.

Goals of the marine biotechnology effort should be determined. For example, if the goal is to attract new industry, expertise in marine biotechnology research and development will be needed. If local industry is to be strengthened, linkages with universities, especially with molecular genetic and genetic engineering talent should be established. Projects to be undertaken should be a mix of short-term a.d long-term efforts.

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 development expectations of the country.

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

Linkage of developing nation centres with counterparts in developed countries should allow more rapid achievement of goals if the individual country does not internally have the combination of molecular genetic and marine biology facilities and intellectual resources.

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

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