Biotechnological approaches in aquaculture

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The rapid strides made by biotechnology in agriculture, and animal and human health care management are just beginning to be seen in aquaculture. There is much scope for exploiting the vast aquatic resources available in India by the application of modern biotechnological approaches.

THE benefits accrued from the green revolution and the white revolution could soon be wiped out by the rapidly growing population, threatening trends in soil erosion, deforestation, depletion of the ozone layer, air pollution, acid rain, and the consequent loss of plant and animal life[1]. The much-too-frequent vulnerability of India's agriculture to erratic monsoons and fickle climatic trends, and increasing biotic and abiotic stresses constantly pose new challenges to our ability to cope with the growing demands on our food resources[2]. This is where 'sea farming' can play a unique role. The aquatic source is infinitely larger, and bioecologically safer. Thus efforts in this direction can usher in yet another major advance in food production, one that we may call 'blue revolution'.

India is endowed with a 5688-km-long coastline, which can form an ideal habitat for prawn farming and other brackish-water farming. The low-lying coastal belt in Nellore district of Andhra Pradesh, for instance, has a great potential in this regard, but remains completely neglected and far underused. Even private entrepreneurs, who have had considerable success in the Circar districts of Andhra Pradesh, have not come forward to take advantage of the untapped resources of the Nellore coast, where the prized tiger prawn (*Penaeus monodon*) is abundant[3]. Such neglected stretches abound in all parts of the Indian coastline. By adopting modern methods, it is possible to make this coastline highly productive. Besides this, inland aquafarming can profitably replace some agricultural crops that are financially not as rewarding. The high export potential of marine products makes commercial aquaculture even more attractive. Impressive export achievements have stimulated governments to promote this industry with more liberal terms of financing. As a result, the potential of opportunities for increased and predictable returns has led to rapid replacement of conventional sources of and methods of obtaining marine produce by improved techniques of aquaculture.

However, commercial aquafarming has yet to cross several major hurdles. The brood stock of prawns used by farmers, for example even in the most productive state of West Bengal, is of inferior quality. *Metapenaeus dobsonii* represents the bulk of the production (~35% of the total) from brackish-water ponds in Kerala and West Bengal. Although it is favoured because of its hardiness, its very small size is a severe handicap in fetching a competitive price in the international market. Besides, *M. dobsonii* takes as long as 5–7 months to attain marketable size[3]. Faulty design of the culture tanks/farm, inability to control the predators that cause high mortality, lack of proper supplementary diet with a good conversion ratio, and inadequate supply of high-quality seed prawn chiefly contribute to and accentuate the problem, culminating in frequent failures of commercial aquafarming projects.

By adopting an integrated approach that encompasses improvements in hatchery technology and development, mass culture of superior quality brood stock(s), application of recent advances in biotechnology and information technology, and advanced business management principles, it is possible to achieve higher targets, and, at the same time, bring underused resources into optimal use.

Biotechnology, by itself, is not a completely new field, but an assembly of new tools and technologies arising out of better understanding of biological systems and processes, particularly at the molecular level[2]. Information technology has combined with two dramatic advances in biology, viz. recombinant-DNA and hybridoma technology, to form the basis of the revolution in biotechnology. The distinction of this modern technology over the classical genetics or other analogous disciplines is the rapidity and molecular precision of its techniques. Further, the versatility of the modern biotechnological methods is such that they allow study of a microorganism or man with equal facility. In this article I consider some of these techniques and the impact they potentially can have or already have on aquaculture.

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Molecular genetics

Genome mapping

The current international effort to organize projects aimed at generating a physical and genetic map of the entire human genome and determining its complete nucleotide sequence is unprecedented in magnitude in biological research[4]. Marine molecular biologists had initiated, in early 1988, experiments in mapping, cloning and sequencing the genome of *Halobacterium volcanii* DS (synonym, *Haloferax volcanii* DS). Several overlapping cosmid clones of the *H. volcanii* genome have been prepared and analysed by chromosome-walking methods[5]. Because of the advantages of the early initiative and the small size of the bacterial genome the complete genomic sequence of this marine bacterium is likely to be available soon. This will result in a wealth of information on marine prokaryotes about the chromosomal organization, the coordinated regulation of expression and the role of various genes.

Insertion sequences

A novel insertion sequence, IS 492, capable of transposition, is known to cause variation in extracellular polysaccharide production in the marine bacterium *Pseudomonas atlantica*. The 1202-base-pair (bp)-long IS 492 element as well as the site of its insertion in the *eps* locus on the bacterial chromosome have been sequenced. IS 492 contains one large open reading frame (ORF) that encodes a protein of 318 amino acids. It has no direct or inverted terminal repeats and shows no sequence homology with other known transposable elements[6]. Insertion of IS 492 at *eps*, however, has been shown to generate a 5-bp repeat; its excision has also been shown to be precise. Availability of well-characterized transposons such as this one facilitates isolation of several useful variants of marine bacteria using site-directed-mutagenesis techniques.

Marine bacteriophages

Gene expression and regulation in *Halobacterium halobium* bacteriophage IH is under study at the Max Planck Institute in Germany[7]. Suitable engineering of the phage genome, and its use in conjunction with its host *H. halobium* would allow development of a host–vector system for gene expression, and facilitate study of a variety of genes from different organisms, including the marine species.

Molecular cloning of important genes

Several genes have been cloned from a wide variety of aquatic organisms, analysed, transferred and expressed in heterologous host systems ranging from *Escherichia coli* to Atlantic-salmon (*Salmo salar*). Some important considerations in cloning genes from aquatic organisms are: (i) improving our understanding of the structural basis of the function of various genes, (ii) developing suitable methods to modify and/or transfer these genes to new environments capable of giving higher production levels and greater stability of the gene products, (iii) creating products with novel structural and functional properties, and (iv) manufacturing the end-products in a more pure form at reduced cost. Table 1 lists representative examples of genes cloned from various aquatic species.

Actin

Genomic and complementary DNA (cDNA) clones were isolated from the sea star *Pisaster ochraceus*[8]. From Southern-blot hybridization studies, it was concluded that sea stars have four to six nonallelic actin genes, and that at least one of them is prevalent in the tube-foot mRNA populations[8].

Insulin

The gene encoding preproinsulin has been isolated from an EMBL3 library of chum salmon (*Oncorhynchus keta*) DNA[9]. It was found to be quite similar to all the other known insulin genes in organization. It contains two introns, the first one within the 5′-untranslated region and the second in the C-peptide region. The 3′-untranslated region has ~70% homology with DNA sequences reported earlier by others[10], suggesting the presence of more than one nonallelic insulin genes in salmon.

Growth hormone

Molecular cloning and characterization of growth hormone (GH) gene from Japanese eel (*Anguilla japonica*)[11], common carp (*Cyprinus carpio*)[12], flounder (*Paralichtys olivaceus*)[13], chinook salmon (*Oncorhynchus tshawytscha*)[14], rainbow trout (*Salmo gairdneri*)[15], and tilapia (*Oreochromis niloticus*)[16] have been reported. A 1187-bp-long cDNA clone of carp GH contains an ORF that codes for the precursor form, of 210 amino acids. In mature GH serine is the first residue, preceded by a 22-residue-long hydrophobic signal peptide. The deduced amino-acid sequence of carp GH shows 40% homology with the sequences of mammalian and chicken GHs, and about 65% homology with salmon GH[12]. The structural gene corresponding to mature flounder GH consists of only 173 amino acids, making it the smallest of all the known GH species[13]. Rainbow trout has been shown to have two genes for growth

Table 1. Examples of genes cloned from aquatic organisms

Gene	Source organism	Reference	Homology
Actin	Sea star (c, g)*	8	
Insulin	Salmon (g)	9	
	Salmon (c)	10	
Growth hormone	Eel (c)	11	Chum salmon, chicken, rat, human
	Common carp (c)	12	Mammals, salmon
	Flounder (c)	13	
	Chinook salmon (c)	14	
	Rainbow trout (g)	15	
	Tilapia (c)	16	
Metallothionein	Rainbow trout (c)	17	
	Chinook salmon (c)	17	
	Winter flounder (c)	18	
	Antarctic fishes (g)	19	
Anti-freeze protein	Winter flounder (g)	20	
	Yellowtail flounder (g)	20	
Homeobox	*Zebrafish (c)*	23	*Drosophila*, mouse
Opsin	Octopus (c)	24	*Drosophila*, cow, human
Superoxide dismutase	Marine bacterium (c)	25	Eubacteria
Lipoproteins and receptors	Common carp (c)	26	
Prolactin	Salmon (c)	27, 28	
	Carp (c)	29	
	Tilapia (c)	30	
	Trout (c)	31	Teleosts, mammals
Cytochrome P-450	Trout (c)	33	

*A cDNA clone is denoted by (c), and a genomic clone by (g). Taxonomical names of source organisms are given in the text

hormone, tGH1 and tGH2, each containing a 630-nucleotide-long ORF that codes for 210 amino acids, of which 11 are variant[15]. The gene tGH2 has been expressed in *E. coli*[15].

Metallothionein

Specific induction of metallothionein (MT) production in response to treatment with heavy metals such as zinc and cadmium, and synthesis of the 70-kDa heat-shock protein hsp70 in response to a general stress such as heat shock have been demonstrated in fish cell lines derived from rainbow trout and from chinook salmon (*O. tshawytscha*). The expression of MT in both these cell lines is cell-type-specific and is regulated differentially by heavy metals and dexamethasone[17]. cDNA made from hepatic MT mRNA from winter flounder (*Pseudopleuronectus americanus*) has also been cloned[18]. Analysis of restriction patterns, under different stringency conditions, of DNA from three different Antarctic fish, *Parachaenichthys bernacchi*, *Notothenia rossii* and *Chaenocephalus kathleene*, has suggested the occurrence of multiple copies of the *hsp70* gene in them[19]. The RFLP (restriction fragment length polymorphism) profiles have also indicated a closer relationship between the first two species. Transcriptional studies of the corresponding poly(A)$^+$ RNA have shown that a temperature of 8°C is optimal for expression of the *hsp70* gene in these fishes[19]. The *hsp70* promoter from these fishes can be used in the development of efficient gene expression-vector systems that would function at low temperature in these fish hosts, while the RFLP procedures can be extended to study the taxonomic relationships and evolution of various fishes and other aquatic organisms.

Antifreeze proteins, cold adaptation

A number of marine fishes survive in icy waters by producing antifreeze glycoproteins (AFGP) or antifreeze polypeptides (AFP), which lower the freezing temperature of the fish by inhibiting ice-crystal growth. AFP

chemically similar to plasma AFP have also been found in the bladder urine of winter flounder[20]. AFP genes from the winter flounder and yellowtail flounder (*Limanda ferruginea*) have been isolated[20]. Southern-blot analysis of winter flounder and yellowtail flounder genomes using cloned AFP cDNAs as probes has revealed occurrence of 20 tandem repeats of the AFP gene in the former, and 10–12 copies of it, spaced irregularly, in the latter. This difference in AFP gene dosage was found to be consistent with observed differences in the peak serum levels of AFP in midwinter, viz. 9 mg ml^{-1} in winter flounder and 4 mg ml^{-1} in yellowtail flounder[21].

Fishes such as *Trematomas hansoni*, native to the Antarctic region, adapt to the local extremely low temperatures (−2 to +6 C) by developing unique physiological capabilities, e.g. modification of protein-synthesis machinery for optimal function in the range −2 to +2 C rather than at 37°C (optimal for mammals). A 115-bp-long repeat sequence accounting for 10% of total genome length and extreme sensitivity of the genome to restriction endonucleases of bacterial origin, including *Alu*I, also are characteristic of these low-temperature fishes[22].

Homeobox

Comparison of the nucleotide sequence and developmental distribution of the transcripts of the homeobox (HOX) gene in zebrafish (*Brachydanio rerio*) has revealed its close relationship with the *Antennapedia* class of *Drosophila* homeobox genes as well as with the murine HOX gene[23], suggesting evolutionary conservation of these sequences and potential utility of the murine and *Drosophila* gene probes in molecular-genetic studies of zebrafish HOX products and *vice versa*.

Rhodopsin

Ovchinnikov *et al.*[24] determined the nucleotide sequence of opsin cDNA from octopus (*Paroctopus defleini*). Comparison of the amino-acid sequence of the 455-residue-long opsin from octopus with opsin sequences of *Drosophila*, cow and man revealed significant homologies[24].

Superoxide dismutase

The amino-acid sequence deduced from the cDNA sequence of the manganese superoxide dismutase (SOD) gene from the marine bacterium *H. cutirubrum* has a high degree of homology with the sequence of iron and manganese SODs from eubacteria. This relatedness in sequence suggests the possibility of a lateral transfer of the SOD gene between eubacteria and archaebacteria some time after the accumulation of atmospheric oxygen[25].

Lipoproteins and lipoprotein receptors

Binding of low-density lipoproteins (LDL) to LDL receptors leads to heart attacks in humans. Much research is directed at finding ways of reducing this risk, such as by avoiding diets rich in cholesterol, which is believed to contribute to higher levels of LDL. An exciting finding in this regard is that the major lipoproteins in the common carp belong to the high-density group (HDL), in contrast to the predominance of LDL in man. Further, carp very-low-density lipoprotein (VLDL) as well as LDL (but not HDL) complexed with dimyristoyl L-α-phosphatidylcholine (apolipoprotein A-1-DMPC) compete with the specific binding of human LDL to its receptor[26], demonstrating their potential for use in attempts to reduce the incidence of heart attack.

It would also be interesting to isolate the corresponding genes from carp and compare their sequences with those of the human counterparts to detect key sequence variations responsible for this important physiological difference between them. The results will be of great value in our efforts to develop novel procedures or agents for possible preventive or therapeutic intervention in heart attack.

Prolactin

Complementary-DNA clones for prolactin have been isolated from salmon[27,28], carp[29], tilapia[30] and rainbow trout[31]. The deduced amino-acid sequence of trout prolactin consists of 210 residues, including a signal peptide of 23 residues. Two domains in this gene were found to be strongly homologous with the sequences of the genes from teleosts and mammals[31].

Cytochrome P-450

Trout species have well-defined constitutively expressed isozymes of cytochrome P-450 for efficient microsomal metabolism of steroids, fatty acids and xenobiotics. Availability of the cytochromes in highly purified forms[32] has facilitated isolation and sequence analysis of trout P450IA1 cDNA[33].

Gene transfer

In order to develop an efficient gene-transfer system for fish, a fish cell line was transfected with a test plasmid, pSV2CAT. The reporter gene chloramphenicol acetyl transferase (CAT), of bacterial origin, showed appreciable

expression in the transformed fish cell line. Further, *Southern-blot analysis of fish DNA after the plasmid* was microinjected into the cytoplasm of tilapia zygotes and cleavage-stage embryos via the micropyle revealed persistence of the plasmid and restriction-site rearrangements of some of the injected DNA in embryos[34].

When plasmids carrying the bacterial genes for CAT, neomycin phosphotransferase (NPT) and β-galactosidase (β-Gal) were microinjected into the cytoplasm of one-celled embryos of zebrafish and rainbow trout, the reporter enzyme activities were detectable in ~66% of trout gastrulae and eyed embryos, in 20% of juveniles, and in 5% of six-month-old fish. Dot-blot analysis showed that 25% of juvenile trout and 5% of adult zebrafish retained plasmid DNA[35]. These results are very useful in designing transfer vectors with suitable reporter genes that will make screening for transgenic fish easy.

Germline manipulation

Since the dawn of agriculture, man has been constantly manipulating the genetic traits of plants and animals. Consequently, domesticated species known today are in fact the products of decades of genetic alteration effected through selection of superior individuals with desired characteristics. The observation that parental characteristics are transmissible to offspring and the elucidation of the laws of genetics by Mendel have provided the basis of the classical methods of selective breeding[36]. Beautiful multicoloured bougainvillaeas, hybrid rice, juicy broiler chicken and high-milk-yield hybrid cows are examples of the benefits of such selection. However, progress was constrained by the enormous complexity of organisms and the limited range of genetic variation readily available in nature.

The rapid developments in genetics within the last two decades, however, have changed this picture dramatically. The advent of recombinant-DNA technology has made it possible to isolate single genes from any organism. Cloned genes can be manipulated in the laboratory to produce novel recombinants (chimaeras), and introduced into cells of host organisms of the same or a different species to produce transgenic organisms with novel characters[36].

The technique of introducing cloned genes into animals was pioneered in mice[37]. In short, first the fertilized eggs are recovered from superovulated mice. The gene of interest is introduced directly into the male pronucleus by microinjection prior to the fusion of the male and female pronuclei. The eggs are then cultured for 24 h *in vitro*, and the survivors are surgically introduced into the fallopian tube of a pseudopregnant female, which is allowed to carry the embryos to term. Typically, 1 to 3% of the injected eggs develop into transgenic mice[38]. Although a fair degree of success has been recorded by the use of other methods such as retrovirus-mediated transfer of cloned genes and implantation of the inner cell mass of one embryo into that of another which would subsequently grow into a chimaera with a mosaic genome[39], microinjection has been by far the most popular approach for producing transgenic animals[36].

The report of the production of 'super mice' by Palmiter *et al.*[38] after injection of rat growth hormone gene under the control of mouse metallothionein promoter led to great interest in the application of this technique to a wide variety of farm animals such as sheep, pigs, rabbits[40] and cattle[41]. The success rate has been different with different species as candidates for production of transgenic animals, but the protocol is very similar in all these cases[36].

The role of the growth-hormone cascade on growth in vertebrates is well documented. It stimulates the hepatic production of insulin-like growth factor-I (IGF-I), which, in turn, stimulates and leads to the proliferation of peripheral tissues. Palmiter *et al.*[38] showed that it is possible to manipulate this pathway by introducing rat growth hormone (rGH) gene, or even the more distantly related human growth hormone (hGH) gene, in a fusion construct with mouse metallothionein-I (MT-I) gene promoter, into fertilized mouse eggs, and bring about extrapituitary production of GH in the transgenic mice at levels that are several hundred times higher than those normally produced in the pituitary[38]. These studies can be extended to prawn or fish using GH gene of either human or fish origin.

Transgenic prawns

A hypothetical strategy for devloping transgenic prawns, carrying, for instance, a heterologous GH gene, would involve making a chimaeric gene construct by fusing the promoter region of the MT-I gene of flounder to the structural portion of the gene encoding GH in flounder, trout or carp. About a thousand copies of this fusion gene construct can be microinjected into the male pronucleus of fertilized prawn eggs. Approximately 1 to 3% of the prawns derived from the microinjected eggs would have stably incorporated the fusion genes into their genomes, show high concentrations of GH in body fluid, and grow significantly larger than normal prawns. Further, augmented synthesis of GH can be induced by an MT-I inducer, say cadmium or zinc. The chimaeric prawns expressing MT GH would not only be significantly larger in size and mass, but would also prove to be valuable as high-quality brood stock. With standardized procedures and brood stocks with desirable characteristics, selected traits can be tagged with genetic markers

such as RFLP pattern, and ribosomal RNA (rRNA) or mitochondrial DNA (mtDNA) profile.

In 1987 Dunham *et al.*[42] reported stable integration of MT–hGH fusion gene constructs into the chromosomal DNA of one-cell embryos of channel catfish (*Ictalurus punctatus*). In an effort to improve the rate and efficiency of growth of carp, a transgenic species has been developed at the Alabama Experiment Station of Auburn University in the US by hatching carp eggs injected with rainbow trout GH gene (rtGH)[43]. Similarly, Fletcher *et al.*[44] produced transgenic Atlantic salmon by microinjecting linearized AFP gene (1×10^6 copies) into the micropyle of fertilized but nonactivated salmon eggs. More than 2000 eggs were injected, and a remarkably high survival rate of 80% was recorded. The transferred AFP gene was found to be stably integrated in the salmon genome and to be expressed well. Efforts are under way to improve the AFP levels expressed in transgenic salmon.

Engineering and transfer of cloned AFP gene from winter flounder can potentially have valuable application in protecting local varieties of fish from seasonal damage due to the temperature dip experienced during winter.

Fish as living bioreactors

Another potential use of transgenic fish is as biological factories for production of important biomedical substances and other useful proteins. As *E. coli* lacks appropriate cellular machinery for post-translational modifications such as glycosylation, several alternative host systems have been explored for proper expression of cloned eukaryotic genes. In this regard, insect cells and larvae have shown great promise as appropriate hosts[45]. It is interesting and useful to see if aquatic organisms can also serve the purpose equally well. A positive finding will be exciting because one can then express the protein(s) of interest in an edible organism such as fish, prawn or snail, which can be consumed directly, thus perhaps avoiding the tedium and expense of purifying the product from the host.

A similar strategy has been pioneered by John Clark of the Edinburgh Institute of Animal Physiology in the UK to produce novel therapeutic agents in transgenic sheep and other farm animals. Clark's group cloned the human gene encoding factor IX, tagged it to a control DNA sequence, introduced it into sheep and obtained recombinant factor IX in the sheep's mammary gland. Factor IX is a blood protein essential for clotting in the initial stages of wound healing. The absence of a functional factor IX or the presence of defective copies of it is responsible for the continuous bleeding in haemophiliacs[46]. Because of the high copy number of the AFP gene, and the high serum levels of AFP in flounder, one can consider large-scale production of important proteins by expressing the cognate genes under the control of the AFP promoter of winter flounder using flounders or other fish as low-cost bioreactors. In a recent example of the use of the AFP promoter, Du *et al.*[14] achieved significant growth enhancement in transgenic Atlantic salmon by expression of an 'all-fish' chimaeric GH gene construct consisting of chinook salmon GH cDNA placed under the control of ocean pout AFP promoter.

Diagnosis and prevention of diseases

Bacterial and viral infections

A major problem in aquaculture is disease, which may occur as infections and epidemics[47]. The most common agents include *Vibrio*, *Aeromonas*, *Myxobacter* and other bacteria, and infectious pancreatic necrosis (IPN), Egtved, and herpes viruses[48]. Monodon baculovirus (MBV) disease of giant tiger prawn is also of major concern to prawn farmers[49].

Fungal diseases

Aspergillus terreus is a fungal pathogen of fish that is highly prevalent in places where effluents from sugar factories are disposed of[50]. *Branchiomyces* and *Saprolegnia* are common fungal pathogens of Indian major carps. The protozoans *Myxobolus*, *Trichodina* and *Epistylis*, trematode parasites, and crustaceans also cause major losses in fish culture. More importantly, infection of the Indian major carp *Catla catla* by *Myxobolus* sp. results in necrosis and degeneration of renal tubules and glomeruli, and culminates at times in 100% mortality within five days after infection.

Leukaemias

Soft shell clams, *Mya arenaria*, develop fatal leukaemias in the haemolymph. Tissue sections and haemolymph samples from normal and tumour-bearing clams were tested with an antileukaemic cell-specific monoclonal antibody (Mab) IEII. Evaluation of leukaemic cells and normal haemocytes by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and Western blot analyses showed that Mab IEII binds to a large protein of approximately 200 kDa from tumour cells, but not to any protein from the normal cells[51].

Disease prevention

To control bacteria-induced mortality, the current practice of adding antibiotics to water can be replaced

by the addition of relatively less expensive but more reliable anti-bacterial substances derived from insects. The antibacterial peptides cecropins[52] and attacins[53] from species of silk moth offer good possibilities in this regard. Since the genes encoding these antibacterial proteins have already been cloned[52,53], one can introduce suitably engineered versions of these genes into prawns, fish and molluscs. Intraperitoneal injection of the extract of *Ecteinascidia turbinata* (Ete)[54], previously shown to enhance haemocyte function by causing increase in haemocyte count[55], may also be useful in rendering prawns and fish more resistant to bacterial infections.

The epoch-making invention by Kohler and Milstein[56] of a method of producing endless quantities of highly pure monoclonal antibodies from immortalized cell cultures has revolutionized the entire field of medical diagnosis and therapeutics, especially with regard to cancer. However, because of their rodent origin, the value and life of monoclonal antibodies as direct therapeutic agents were limited, until Winter and coworkers[57] devised ingenious ways to disguise monoclonal antibodies made in mice to appear as though they were made in human cells by introducing the gene encoding the reactive part of the mouse antibodies into human producer cell cultures[57]. It is extremely useful to develop various systems and protocols that facilitate production of heterologous antibodies or functional regions of antibodies to aquatic toxins and pathogens. Availability of monoclonal antibodies to various antigens of pathogens that afflict aquatic organisms is certain to improve management practices in aquaculture projects.

Approaches for preventing viral infections include isolation and characterization of attenuated strains (e.g. temperature-sensitive (ts) mutants of the virus) and their use as live vaccines. Studies of the antigenic relationships of various isolates of a virus should facilitate development of polyvalent antisera and effective vaccines against the virus. cDNA cloning, sequencing and expression of the glycoprotein gene of infectious haematopoietic necrosis virus, a fish rhabdovirus, have been reported[58]. The cDNA is 1609 bp long and encodes a polypeptide of 508 residues and 66 kDa in insect cell cultures when expressed under the control of an insect virus promoter (AcNPV polyhedrin).

Algal farming

The many uses of algae include production of valuable commercial compounds such as glycerol and β-carotene from *Dunaliella*; arachidonic acid, polysaccharides and pigments from *Porphyridium*; agar-agar, vitamins, phytol, steroids, amino acids, flocculants, polyols and other carbohydrates, pharmaceuticals and other bioactive substances, liquid fuels, waxes, phospholipids and lecithins, essential fatty acids, and prostaglandins from various other members of algae; biological control of undesirable plants in fish tanks; use as biofertilizers, soil conditioners, plant growth regulators, and food supplement (for example, *Spirulina* has a cholesterol-lowering effect)[59]. Genetic engineering of both prokaryotic (cyanobacteria) and eukaryotic algae should help achieve improved product yields, expansion of product range, and improved growth and cell characteristics.

One can also embark profitably on a project to develop and provide the technology base for collection, characterization and improvement of microalgal strains that can provide in outdoor culture sustained and reliable productivity of large amounts of lipids that can be converted to gasoline or to an ester fuel. Once superior strains are developed or identified, the relevant genes involved can be cloned, characterized, manipulated and transferred to suitable hosts for making the products in large quantities.

Summary

The powerful and sophisticated experimental approaches of modern biotechnology have made a profound impact on clinical medicine and agriculture in the past two decades. I have examined the prospects and implications of this innovative technology for aquaculture and provided examples in specific aspects of current research in aquaculture biotechnology. Judicious application of this technology will not only aid isolation, characterization and manipulation of various genes of aquatic organisms but will also help preserve and potentially improve the rich aquatic resources in more harmonious and beneficial ways.

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1. World Watch Institute, *State of the World 1990 Report*, Washington, DC, 1990.
2. Swaminathan, M. S., *Proceedings of Regional Seminar on Public Policy Implications of Biotechnology for Asian Agriculture*, Asian and Pacific Development Center, Kuala Lumpur, 1989.
3. Surendra Babu, K., PhD thesis, Sri Venkateswara University, Tirupati, 1989.
4. Green, E. D. and Waterston, R. H., *J. Am. Med. Assoc.*, 1991, **266**, 1966.

5. Charlesbois, R. L., Hofman, J. D., Schalkwyk, L. C., Lam, W. L. and Doolittle, W. F., *Can. J. Microbiol.*, 1989, **35**, 21.
6. Bartlett, D. H. and Silverman, M., *J. Bacteriol.*, 1989, **171**, 1763.
7. Gropp, F., Palm, P. and Zilling, W., in *Molecular Biology of Archaebacteria* (eds. Matheson, A. T. and Dennis, P. P.), 1989, p. 182.
8. Kovesdi, I., Preugschat, F., Stuerzl, M. and Smith, M. J., *Biochim. Biophys. Acta*, 1984, **782**, 76.
9. Koval, A. P., Petrenko, A. I. and Kavsan, V. M., *Nucleic Acids Res.*, 1989, **17**, 1758.
10. Sorokin, A. V. *et al.*, *Gene*, 1982, **20**, 367.
11. Saito *et al.*, *Gene*, 1988, **73**, 545.
12. Koren, Y., Sarid, S., Ber, R. and Daniel, V., *Gene*, 1989, **77**, 309.
13. Momota, H., Kosugi, R., Ohgai, H., Hara, A. and Ishioka, H., *Nucleic Acids Res.*, 1988, **16**, 10362.
14. Du, S. J. *et al.*, *Bio/Technology*, 1992, **10**, 176.
15. Rentier-Delrue, F. *et al.*, *DNA*, 1989, **8**, 109.
16. Rentier-Delrue, F. *et al.*, *DNA*, 1989, **8**, 271.
17. Misra, S., Zafarullah, M., Price-Haughey, J. and Gedamu, L., *Biochim. Biophys. Acta*, 1989, **1007**, 325.
18. Ming, C. K., Davidson, W. S., Hew, C. L. and Fletcher, G. L., *Can. J. Zool.*, 1989, **67**, 2520.
19. Maresca, B., Patriarca, E., Goldenberg, C. and Sacco, M., *Comp Biochem. Physiol.*, 1988, **B90**, 623.
20. Fletcher, G. L., King, M. J., Kao, M. H. and Shears, M. A., *Fish Physiol. Biochem.*, 1989, **6**, 121.
21. Scott, G. K., Davies, P. L., Kao, M. H. and Fletcher, G. L., *J. Mol. Etol.*, 1988, **27**, 29.
22. Beccari, E. *et al.*, *Nucleic Acids Res.*, 1986, **14**, 7633.
23. Njoelstad, P. R., Molven, A. and F. Jose, A., *FEBS Lett.*, 1988, **230**, 25.
24. Ovchinnikov, Y. A. *et al.*, *FEBS Lett.*, 1988, **232**, 69.
25. May, B. P. *et al.*, *Can. J. Microbiol.*, 1989, **35**, 171.
26. Fainaru, M., Schafer, Z., Gavish, D., Harel, A. and Schwartz, M., *Comp. Biochem. Physiol.*, 1988, **B91**, 331
27. Song, S. *et al.*, *Eur. J. Biochem.*, 1988, **172**, 279.
28. Kuwana, Y. *et al.*, *J. Agric. Biol. Chem.*, 1988, **82**, 1033.
29. Shiow-Chyi, Fu-Ming, P. and Wen-Chang, C., *Nucleic Acids Res.*, 1988, **16**, 9350.
30. Rentier-Delrue, F., Swennen, D., Prunet, P., Lion, M. and Martial, J. A., *DNA*, 1989, **8**, 261.
31. Mercier, L. *et al.*, *DNA*, 1989, **8**, 119.
32. Miranda, C. L., Wang, J.-L., Henderson, M. C. and Buhler, D. R., *Arch. Biochem. Biophys.*, 1989, **268**, 227.
33. Heilman, L. J., Cheen, Y. Y., Bigelow, S. W. and Nebert, D. W., *DNA*, *1988*, **7**, *379*.
34. Indig, F. E. and Moav, B., in Proceedings of French–Israeli Symposium on Reproduction in Fish (eds Zohar, Y. and Breton, B.), Inst Natl. Rech Agron., Versailles, 1988, p. 221.
35. Gibbs, P. D. L., Gray, A. and Thorgaard, G. H., Proceedings of Aquacultural International Congress, Vancouver, 1988, p. 56.
36. Bialy, H., *Bio/Technology*, 1991, **9**, 786.
37. Jaenisch, R. and Mintz, B., *Proc. Natl. Acad. Sci. USA*, 1974, **71**, 1250.
38. Palmiter, R. D., Norstedt, G., Gelinas, R. E., Hammer, R. E. and Brinster, R. L., *Science*, 1983, **222**, 809.
39. Jahner, D., Haase, K., Mulligan, R. and Jaenisch, R., *Proc. Natl. Acad. Sci. USA*, 1985, **82**, 6927.
40. Hammer, R. E. *et al.*, *Nature*, 1985, **315**, 680.
41. Lathe, R. *et al.*, in *Exploiting New Technologies in Animal Breeding* (eds. Smith, C., King, J. and Mckay, J.), Oxford University Press, New York, 1986, p. 99.
42. Dunham, R. A., Eash, J., Askins, J. and Townes, T. M., *Trans. Am. Fish. Soc.*, 1987, **116**, 87.
43. Zhang, P. *et al.*, *Mol. Reprod. Dev.*, 1990, **25**, 3.
44. Fletcher, G. L., Shears, M. A., King, M. J., Davies, P. L. and Hew, C. L., *Can. J. Fish. Aquat. Sci.*, 1988, **45**, 352.
45. Choudary, P. V., Kamita, S. G. and Maeda, S., in *Methods in Molecular Biology—Baculovirus Expression Protocols* (ed. Richardson, C. D.), Humana Press, Clifton, 1992, in press.
46. Clark, A. J. *et al.*, *Genome*, 1989, **31**, 950.
47. Colwell, R. R., *Science*, 1983, **222**, 19.
48. Ahne, W., in *Fish Diseases*, Springer, New York, 1980
49. Fukuda, H., Momoyama, K. and Sano, T., *Nippon Suisan Gakkaishi/Bull. Jap. Soc. Sci. Fish.*, 1988, **54**, 45.
50. Bhattacharya, U., Prasad, J. and Dubey, N. K. *Curr Sci.*, 1988, **57**, 622.
51. Miosky, D. L., Smolowitz, R. M. and Reinisch, C. L., *J. Invertebr. Pathol.*, 1989, **53**, 32.
52. Qu, X.-M., Steiner, H., Engstrom, A., Bennich, H. and Boman, H. G., *Eur. J. Biochem*, 1982, **127**, 219.
53. Hultmark, D. *et al.*, *EMBO J.*, 1983, **2**, 571.
54. Sigel, M. M. *et al.*, Food-Drugs from the Sea: Proceedings, 1969, (ed. Youngken, H. W., Jr.), Marine Technology Society, Washington, DC, 1970, p 281.
55. Sigel, M. M. *et al.*, *Am. Zool.*, 1983, **23** 221.
56. Kohler, G. and Milstein, C., *Nature*, 1975, **256**, 495.
57. Winter, G. and Milstein, C., *Nature*, 1991, **349**, 293.
58 Feyereisen-koener, J. M., *Diss. Abstr. Int.*, 1988, **B48**, DA 8724474
59 Singleton, F. L. and Kramer, J. G., *Genet. Engg. Biotechnol. Monitor*, 1988, **25**, 83.
60. Axelrod, H. R., Burgess, W. E., Pronek, N and Walls, J. G., in *Dr Axelrod's Atlas of Freshwater Aquarium Fishes*, 3rd edn, TFH Publications, Neptune, 1989, p 78