From Basic Research To New Industries Within Marine Biotechnology: Successes And Failures In Norway

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Introduction

In this paper we present a summary of the development within "marine biotechnology" in Tromsoe/Norway, covering

a) Infrastructure in Tromsoe
b) R & D programmes (main areas)
c) R & D results
d) New commercial companies, and
e) Experience from development of these companies.

In the city of Tromsoe, Norway, a network of academic research, higher education and commercial activities within fisheries and aquaculture has been developed, creating a good environment for the development of new biotechnological industries.

Development Of Infrastructure In Tromsoe/Norway

As a result of overall planning at governmental levels in Norway during late 60's and early 70's, combining:

a) Norway's strong position and interest in harvesting fish from the sea and improving our production of food products, and
b) politics for regional development,

the Norwegian government decided to establish the following new institutions:

- The University of Tromsoe, with the Norwegian College of Fishery Science (NFH) (1972),
- The Norwegian Fishery Research Council (NFFR) (head office in Trondheim), (1973),
- Institute of Fishery Technology Research (FTFI) (1974). Since 1991, it has become the Norwegian Institute of Fisheries and Aquaculture (Tromsoe).

Tromsoe is located far north in Norway, where fishing and, frequently aquaculture are the backbone activities of commercial life. Approximately two-thirds of the total catch in Norway is caught in the sea off the coast of northern Norway. The city is a center of higher education and research; in relation to areas such as marine biotechnology and aquaculture it has the status of a national center. The Norwegian College of Fisheries Science under the University of Tromsoe, and the Norwegian Institute of Fisheries and Aquaculture are the two institutions in Tromsoe which are responsible for education, research and industrial innovations. The Institute has the primary purpose of transfering the results from basic and applied research into practical applica-

Note: This paper was presented at the Seminar by Dr Strøm.

tion and to formulate problems, tasks and challenges which may influence the research priorities within the University. These two institutions have throughout the last 15 years worked closely together, both with regards to personnel and research projects. This has been one very important factor for a successful transfer of research results into commercial products and processes.

The development in Tromsoe has been financed by R & D grants from the Research Council, by funds directly from the Norwegian Ministry of Fisheries for investments in infrastructure (offices, laboratories, etc) and by funds for regional development from other governmental ministries.

R & D Programmes

The social environment and affiliated commercial activities of Tromsoe and northern Norway were (and are) strongly dominated by fisheries. Recently aquaculture is also playing an important role. The exposure of the R & D institutions and their researchers to the daily realities of these activities, has influenced (and influences) strongly the choice of R & D. With the advantage of starting new activities in the mid 70's from scratch, a concentration of R & D in a few selected areas was easily accomplished, thereby making good use of limited funds.

Two initial areas of research with major implications for academic research and establishment of commercial companies in the marine biotechnology sector of Tromsoe were:

- utilization of fish processing waste, primarily fish viscera, and
- fish diseases and the microbiology of marine bacteria which are pathogenic to fish (*Vibrio* spp.).

The first project was a joint R & D project between NFH and FTFI, wherein the researchers at NFH concentrated on fundamental questions related to fish silage and its application, and in which FTFI developed processes for industrial production and applications.

Scientists at NFH were also involved in R & D related to aquaculture, including disease prevention by vaccination and other aspects of fish health. Thus, with the development of aquaculture in Norway, an overall approach to R & D within marine biotechnology, combining activities/possibilities in fisheries and aquaculture, was gradually developed (See Fig. 1).

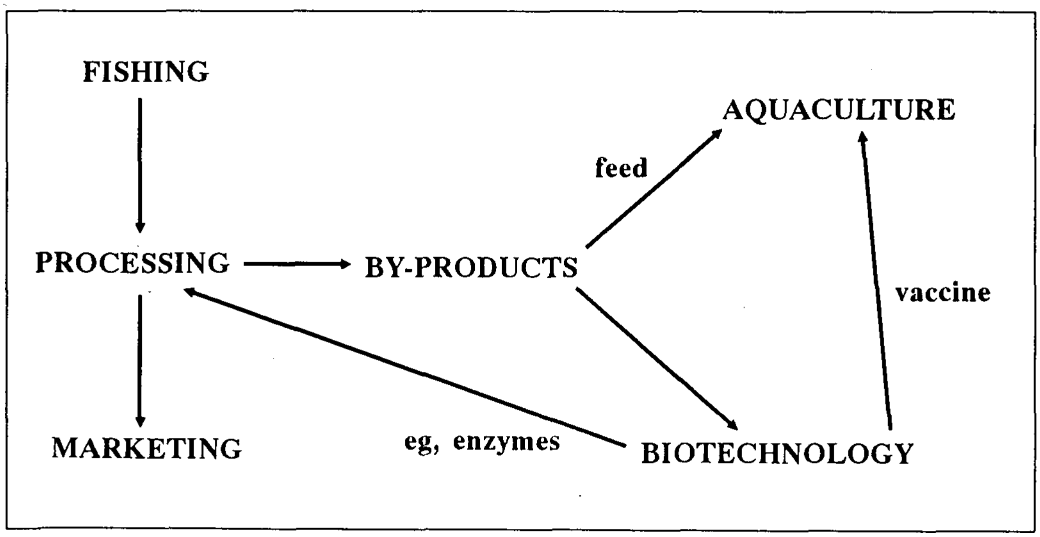

Fig. 1. Schematic illustration of fish processing and utilization of by-products in fish processing and aquaculture.

R & D Results And Commercial Applications

Some of the R & D results from the activities in Tromsoe will be described in the following with reference to application and commercial production. More details of the commercial companies involved are given in a later chapter.

Biochemicals From Fish Waste

Utilization of fish processing waste; the silage technology

Fish processing waste, and in particular fish guts, was a pollution problem in Norway when NFH/FTFI started to look into the potential applications of this very interesting raw material, containing enzymes and other interesting biomolecules. The first product to be developed was a deoiled fish silage which was used as feed for domestic animals, including farmed fish. For review see Raa and Gildberg (1982).

A silage of fish guts preserved by adding acid will be digested by enzymes present in the gut and thereby become liquid with a layer of oil on the surface. Studies of the biochemistry of this autolytic process (Raa and Gildberg, 1976) and of the enzymes involved (Gildberg, 1982) indicated new possible uses of fish viscera. A process for the industrial production of silage concentrate was developed (Raa, Gildberg and Strøm, 1983). Basic studies on the mode of attack by fish enzymes on various biological tissues of fish and other organisms gave rise to ideas for new applications of fish enzymes in the processing of food (Gildberg and Almås, 1983).

Liquid Fish Protein Concentrate

Based on the silage technology, A/S Rieber & Co produced a concentrate (45% dry weigth) of the aqueous solution which results from autolysis of fish waste. This concentrate is used in extruded fish feeds and is added as a liquid prior to the extrusion process. The feed pellets which contain this concentrate have good functional properties and there is less dust both in the production plant and in the final product. The concentrate has a protein value corresponding to the best quality LT-fish meal (low temperature dried) and apparently also contributes to the attractant properties of the feed. The production plant in Tromsoe has a capacity to produce 20,000 mt per year.

Proteases and peptone

Enzymes and peptone from fish viscera are produced by Marine Biochemicals A/S in a process which involves

- acidification
- autolysis by enzymes present in the fish guts, and
- ultrafiltration and purification of the aqueous phase, yielding a mixture of enzymes (mainly pepsins) and an ultrafiltrate containing a mixture of amino acids and peptides.

Pepsins in the stomach of fish are stable in acid and can be filtered off after complete liquefaction of an acid silage of fish viscera. The properties of pepsins from fish differ significantly from those of pepsins from warm-blooded animals; fish pepsins will therefore not necessarily compete with other pepsins on the market.

The ultrafiltrate, after removal of enzymes from acidified and autolysed fish viscera, is rich in amino acids, peptides and other extractives of fish. When the ultrafiltrate is neutralized, the clear liquid is spray-dried to produce a peptone powder. This powder dissolves completely in water and forms a clear solution that is a better growth medium for certain bacteria than other commercial growth media (Clausen, Gildberg and Raa, 1985). This marine peptone particularly favours growth of marine bacteria and of lactic acid bacteria (Vecht-Lifskitz, Almås and Zomer, 1990). Therefore potential commercial applications are in the production of fish vaccines and of lactic acid bacteria used as starter cultures in preservation of grass silages, and eventually also of a fermented fish silage.

Nucleotides/nucleosides

In a joint venture with the fish processing industry in Norway, Marine Biochemicals A/S produces DNA from fish milt, and nucleosides from the same DNA by enzymatic hydrolysis (Gildberg and Almås, 1986). The market for the former product is the cosmetics industry; the latter is used as raw materials in the biochemical industry.

Enzymes from shrimp; alkaline phosphatase

When frozen blocks of shrimp are thawed in water before processing, enzymes are eluted. One of these enzymes is alkaline phosphatase. After removal of particles, the water is ultrafiltered and the alkaline phosphatase purified by an exchange chromatography (Olsen, Johansen and Myrnes, 1990). This enzyme has properties that are important in its use as a tool in diagnostic analysis. Sales have begun to producers of diagnostic kits.

The Use Of Enzymes In Fish Processing

The pepsins and other proteolytic enzymes (trypsins, chymotrypsins, peptidases) present in fish and other marine organisms differ significantly from their counterparts in warm-blooded animals and offer distinct advantages for certain applications(Gildberg, 1982). For example, the fish pepsins have a higher pH optimum than other pepsins and they are active at low temperature and they are very resistant to autolysis at low pH. It has also been demonstrated that fish enzymes may have distinctly different pH optima when acting on different tissues. Lysozymes of scallops and blue mussels are active at acid pH and they retain 40% of maximal activity at 4°C. Moreover, these lysozymes are able to attack the cell wall of Gram-negative bacteria without the presence of chelators (Myrnes, 1991).

Fish tissues and the tissues of marine invertebrates differ very much from corresponding tissues of warm-blooded animals with regard to sensitivity to various enzymes. This understanding of the mode of action of marine enzymes on various tissues, combined with knowledge of the sensitivity of various tissues of marine organisms to a long series of enzymes, also of plant and animal origin, formed the basis for a very specialized expertise in Tromsø. Specifically, this relates to the use of enzymes as processing aids, and the abilities to biochemically dissect and separate biological tissues.

A number of such enzymatic processing methods have been further developed by the company KS Biotec-Mackzymal to a state of high sophistication. This company can now provide complete processing lines, designed according to the specifications given by the enzyme technologists. Some of these processes are summarised below.

Enzymatic caviar production

The eggs of fatty fishes are firmly attached to the connective tissues of the roe sacs. Even though mechanical processing of roe is feasible with several fish species this does not apply to many fatty fishes whose eggs are too fragile to survive the treatment. Mechanical caviar production of roe from such fishes normally involves rubbing the roe against a sieve or screen. This results in high damage and poor yield. Thus, there are a number of fish species from which the roe cannot be converted into caviar, but in which the extent of roe maturation may also make processing impossible. The raw materials available for the production of high value caviar for the delicatessen market are therefore limited.

However, the eggs can be released from the connective tissues using enzymes that selectively degrade the connective tissues without damaging the eggs. The strength of individual eggs can also be adjusted at will to meet the requirements of various markets. KS Biotec-Mackzymal supplies the enzymes necessary for performing that process and the processing lines where such caviar is produced.

The main advantages of enzymatically produced caviar compared with that produced mechanically can be summarized as follows:

- Better recovery (up to 92%, depending on species).
- Less damage to the eggs (makes production of other roe types and a wider variety of maturity possible).
- Cleanliness of product with no residues of connective tissues.
- Less drip loss during thawing if the resulting caviar is frozen.
- Less labour is required for processing.
- Good hygiene in production.

KS Biotec-Mackzymal has demonstrated the viability of the technology with the following salmonid fishes:

- Chum salmon (*Oncorhynchus keta*)
- Rainbow trout (*Oncorhynchus mykiss*, previously *Salmo gairdneri*)
- Pink salmon (*Oncorhynchus gorbuscha*)
- Atlantic salmon (*Salmo salar*)
- Coho salmon (*Oncorhynchus kisutch*)
- Sockeye salmon (*Oncorhynchus nerka*)

The method can most likely also be used to produce caviar from flying fish (*Hilsa* spp.), ocean catfish (wolffish) (*Anarhicaslupus*), paddlefish, sturgeon, catfish, mullet, carps (eg, *Cyprinus carpio*), whitefish and bowfin.

Complete processing units are delivered for the production of caviar by the enzyme method, and outside Norway the technology is being licenced to customers in the Soviet Union, Finland, Denmark, Faroe Islands, United Kingdom, Australia, USA and Canada.

Enzymatic removal of squid skins

Specific squid skin degrading enzymes have been found in the intestine of the squid itself, and in various plants. However, preparations of such enzymes also contain proteases which degrade the muscle. For practical use it was therefore necessary to make an enzyme preparation in which the protease activity was low. With such a preparation an industrial squid deskinning line which produces skinless tubes, wings and tentacles from various squid species could be established.

KS Biotec-Mackzymal in collaboration with the Danish company Carnitech, has developed a processing line for deskinning of squid. The method can be used on the following types and genera of squid:

- *Illex* spp.
- Flying squid
- *Todarodes* spp.
- *Nototodarus* spp.
- Cuttlefish

The muscle and tentacles of many squid species are too tough to be accepted well on certain markets. By including more of the proteases in the same enzyme preparation used for deskinning, the product can be tenderised simultaneously. The enzymatic tenderising process also results in improved taste of the product, in particular of the tentacles.

Some other enzyme processes

Some enzyme processing methods other than those described above have been shown to work in practice and to have clear economic advantages. But development work necessary for market introduction has not yet been carried out. Examples of such processes are the use of enzymes to remove scales from fish, to remove or tenderise skin of herring and tuna. One process which has been developed, namely loosening of shrimp shells from the muscle, is now ready to be adopted in industrial peeling of shrimp (patent pending).

The Use Of Enzymes In Fish Feeds

Studies of the digestive processes of fish suggest certain applications of enzymes as digestive aids in feed for fish.

Feed pellets disintegrate quickly in the stomach of fish. The slurry which is formed passes on through the pylorus node after a relatively short time of exposure to the stomach pepsins. It is therefore assumed that a large proportion of protein particles rather than soluble peptides are transported to the gut when fish consume pelleted feeds. Natural preys are, in contrast, digested from the surface and inwards in a process which releases a soup of peptides. It is further assumed that protein particles in the gut may create an overloading of its digestive capacity. To compensate for the short exposure time in the stomach, fish pepsins may be added to the fish feed as a digestive aid. The pepsins produced by Marine Biochemicals A/S have been shown to enhance growth of fish, and the company manufactures and exports pepsins for this application.

Fish Vaccines, Adjuvants And Prophylactic Agents

Vaccines

Studies of the microbiology of marine vibrios that cause disease in farmed salmonids were made at the University of Tromsoe in 1973. Cold-adapted strains of *Vibrio anguillarum* were isolated from farmed fish in north Norway and it was shown that environmental contaminants reduce the resistance of fish to vibrioses (Egidius *et al*, 1982; Olafsen, Christie and Raa, 1981). A novel disease in Norwegian fish farms was discovered in 1979. Researchers in Tromsoe and at the Marine Institute in Bergen were, in 1981, able to demonstrate that the disease was caused by a hitherto undescribed *Vibrio* species. The disease, which has been called "Hitra-disease", soon became the most serious threat to the Norwegian salmon farming industry, causing losses, which, in 1987 amounted to more than 200 million Norwegian kroner (30 million US$).

The research community in Tromsoe had the necessary qualifications and experience to quickly develop a vaccine against this disease (Espelid, Hjelmeland and Jørgensen, 1987). After a thorough description of the biochemistry and growth characteristics of the disease causing organism, which researchers in Bergen designated *Vibrio salmonicida*, the time was ripe for pilot-scale production and efficacy-testing of a vaccine. This was produced on a commercial basis by Apothekernes Laboratorium A/S, a pharmaceutical company which diversified into this sector when it established the fish vaccine production plant in Tromsoe.

The vaccine against "Hitra-disease" is very efficient, causing a more than 90% protection against the disease. As a result, the use of antibiotics in Norwegian fish farms dropped drastically and fish farmers have avoided potential losses in the range of 300-400 million Norwegian kroner a year. In other words the economic gain from this project is of an order of magnitude higher than the total costs of the research and development work which formed the basis for the vaccine.

Furunculosis in salmon, caused by *Aeromonas salmonicida*, first became a serious problem for Norwegian fish farmers a few years ago. The bacterium quickly develops resistance to antibiotics and, moreover, has the ability to escape the action of the immune system of fish. Conventional vaccines against this disease have therefore not been very effective.

Adjuvants/immunostimulants

Recently a new adjuvant and general immunostimulant has been developed in Tromsø and is now being produced commercially by the company KS Biotec-Mackzymal. The immunostimulant is a ß-1.3/ß-1.6-glucan derived from yeast. Its trade name is MacroGard.

A series of experiments have demonstrated that MacroGard alone causes a substantial increase of resistance in fish to a number of different diseases (Robertson *et al*, 1990). The compound is effective, both after injection and when administered in the feed. When used as an adjuvant in a vaccine prepared from killed *Aeromonas salmonicida*, the degree of protection has been about 90%. A furunculosis vaccine with MacroGard as adjuvant has recently come in commercial production in Norway.

MacroGard induces increased resistance against many diseases simultaneously, by activating the macrophages. When animals are treated with glucans, their macrophages produce more interleukin, a polypeptide which activates B- and T-lymphocytes. The lymphocytes then produce interferone-like molecules which activate the macrophages so they become more active in engulfing and killing of bacteria. This in turn leads to a cascade of events which results in higher general resistance to infection by both bacteria and viruses.

Scientific Expertise In Tromsoe

Based on the activities in Tromsoe over the last 15 years a strong scientific network has been established. Applied fundamental research is carried out as well as applied R & D within:

- *Enzyme biotechnology*; purification, industrial production and application of enzymes.
- *Fish health*; production of vaccines against bacterial fish diseases and application of feed additives (eg, glucans) in preventive health treatment.
- *Fish immunology*; research on defence mechanisms against fish diseases.
- *Fish nutrition*; development of feed, eg, starter feed for marine larvae and fry, fermented feed and fish silage.
- *Microbial ecology*; studies of the influence of the environment on micro-organisms and fish health.

New Commercial Companies In Tromsoe

In the previous chapter we have given a brief description of some R & D results from projects carried out in Tromsoe. These projects have given results that have been and are being commercialized. Initially, the researchers studied the possibility of improving the utilization of fish raw material, eg, fish waste. Some of these options looked commercially promising and the companies involved are scaling up for production based on market opportunities and requirements.

Commercial Production And Companies In Tromsoe

In Tromsoe (and northern Norway generally) few companies have the financial resources, know-how and production of products to take commercial advantage of these R & D results. In fact, very few companies in all of Norway are strategically and finacially able to do so. In the mid-80's the environment favoured the starting up new businesses, in high-technology production fields, including marine biotechnology and aquaculture. Contacts at personal and institutional levels resulted in the establishment of the companies in Tromsoe as shown in Table 1.

The products that these companies are producing and developing on commercial scale which have been presented in the earlier chapter, are summarized below:

1. KS Biotec-Mackzymal	Enzymatic processing, adjuvants, others
2. Marine Biochemicals	Biochemicals: pepsin, peptone, DNA, ALP
3. Apothekernes Lab.	Vaccines

Additionally, the production of fish silage concentrate is carried out by Rieber & Co, Tromsoe. This company has long been involved in small scale production of marine lipids. The combined production of lipids and silage concentrate is carried out in a large scale operation (20,000 mt per year).

These Tromsoe companies represent a powerful base for expansion into viable commercial operations. This opens the door to possible production of a wide range of products made from marine raw materials, for use in fish processing, in pharmaceuticals and in other items.

Table 1. Marine biotechnology companies established in Tromsoe.

Company	Year	Owners
AS Biotec	1984	- Professor J. Raa and graduate students
	1985	- 90% Selmer Sande Biocomp. (SABICO)
	1990	- Initial owners 100%
Mackzymal	1986	- AS Biotec and L. Macks Brewery, Tromsoe
KS Biotec-Mackzymal	1990	- AS Biotec, L. Macks Brewery and Provesta (Phillips Petroleum Company)
Marine Biochemicals AS	1985	- Norsk Hydro (largest Norwegian Company)
	1991	- Employees: T. Strøm and J. Raa
Apothekernes Lab. Production unit in Tromsoe (vaccine)	1986	- Apothekernes Lab. AS

Factors For Success And Failure

Documentation/Markets

The overall aim in commercial applications is to meet market requirements. For new products, extensive documentation is required to convince potential buyers to make their first purchases. The new companies in Tromsoe have found that documentations compiled in collaboration with scientists from universities or R & D institutes have high credibility and build confidence in the new enterprises.

Production

Production of the vaccine developed in Tromsoe against salmon disease began after a heavy investment in a modern production plant. With close cooperation from government agencies in approving the vaccine and by strong marketing efforts, full scale production and sales quickly developed.

The other companies experienced a more traumatic development, resulting in financial difficulties after 2-5 years and final sale of the companies to new owners. These included the founders and key personel of these companies. One reason for this outcome was lack of investment in production facilities; the financers required reliable cash flow analyses prior to investment, which the project leaders were not able to give. Without this investment, the companies could only "sell" potential production on the basis of laboratory-scale production. This slowed down the demonstration of commercial applications to actual customers.

Both KS Biotec-Mackzymal and Marine Biochemicals AS have now industrial production facilities for their products.

Time From R & D To Positive Results

New companies in Norway have generally found that the time required for developing R & D results into commercially viable production is 5-10 years. This is a longer period than expected by researchers and economists in the mid-80's. In addition, the general situation in aquaculture was not so promising in 1990, as it had been a few years previously. As a result, the economists who had supported foundation of two of the new companies lost faith in their investment possibilities. This resulted in sale of the companies to new owners.

Equity Capital

The new Tromsoe companies have made heavy investments in equipment, in the order of 100 million NOK (15-20 million US $). Only companies with strong financial base are willing to invest in such high risk/long term projects, as the projects above admittedly are.

However, no industry can be developed without risk capital to finance the final preparations for pilot-scale and industrial productions. And more money is needed when the time comes to invest in production facilities, product control and market introduction.

Summary Of Our Experience

On the basis of 15 years' experience in R & D and industrialization, we offer the following summary of requirements important to success:

1. Development based on natural advantages related to resources and industrial traditions
2. Network with academic R & D groups
3. Entrepreneurs with innovative strength, intuition and profound knowledge of the products and its applications
4. Investment in pilot plants and industrial production facilities prior to reliable cash-flow analysis
5. Government funding support
6. Long term investors (5-10 years)
7. Personal involvement in the companies of key employees.

In addition, our work in Tromsoe since 1975 indicates that there are very few short cuts to success. Success requires hard work at every stage, from research to commercial-scale production and marketing.

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Discussion

Dr Strøm informed the meeting that the enzymes used in the production of high-quality caviar are commercially available and are distributed by a Norwegian company.

Because very few peptones are produced worldwide, documentation on peptones is required.