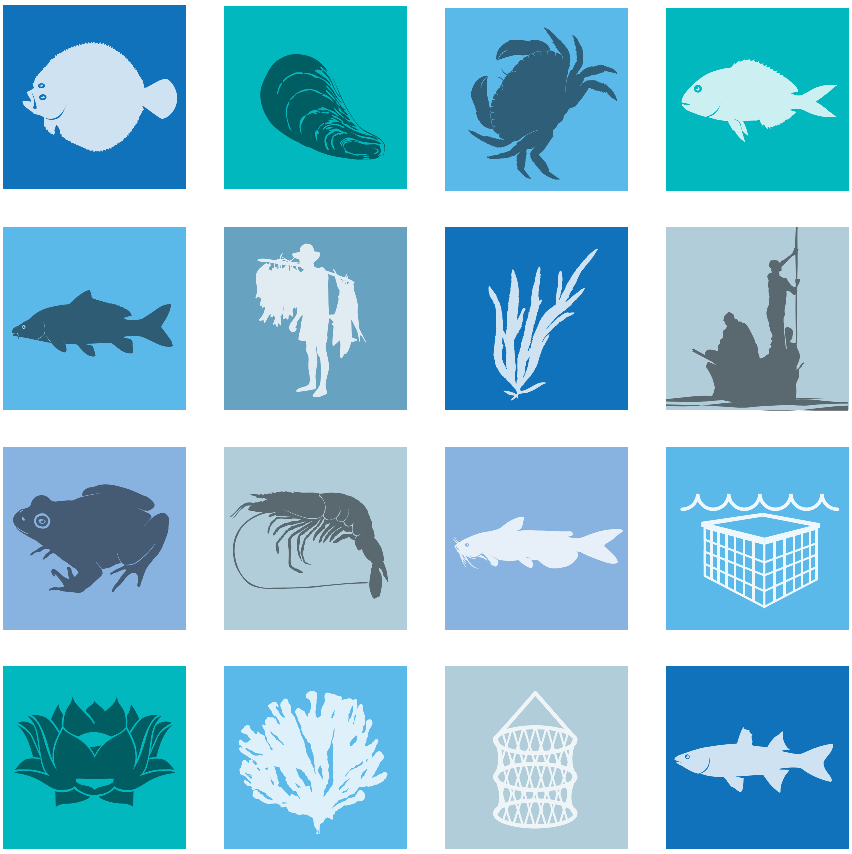
Food and Agriculture
Organization of the
United Nations

THEMATIC BACKGROUND STUDY

Genetic resources for microorganisms of current and potential use in aquaculture



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Genetic resources for microorganisms of current and potential use in aquaculture

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Contents

Tables

Abbreviations and acronyms

AEM	applied and environmental microbiology
AFLP	Amplified Fragment Length Polymorphism
DNA	deoxyribonucleic acid
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
ICN2	Second International Conference on Nutrition LED light-emitting diode
RAPD	Random Amplified Polymorphic DNA
RNA	ribonucleic acid
RNAi	RNA interference

1. INTRODUCTION

Aquaculture is the farming of aquatic organisms ranging from microbes to shellfish and finfish. Fisheries production from the capture of wild fish has remained fairly constant since the late 1980s and it is the increase in production from aquaculture that has led to substantial growth in fish production for human consumption, with aquaculture contributing more than wild-caught fisheries for the first time in 2014 (FAO, 2016) and this trend is likely to continue. Global aquaculture production accounted for 44.1 percent of total global fish production, including production for non-food uses, in 2014. The share of fish produced by aquaculture for human consumption increased from 26 percent in 1994 to about 50 percent in 2014, with 73.8 million tonnes of fish valued at USD 160 billion being harvested from aquaculture in 2014 (FAO, 2016). In facing the challenge of providing food to a growing human population predicted to reach 9.7 billion by 2050, fish consumption, especially produced from aquaculture, has an important role to play. The Second International Conference on Nutrition (ICN2) held in 2014 adopted the Rome Declaration on Nutrition that highlighted the key role of fish in meeting the nutritional needs of this growing population (FAO, 2016). Global per capita fish consumption has increased from under 10 kg in the 1960s to approach 20 kg in 2014 and 2015 and now provides over 3.1 billion people with approaching 20 percent of their animal protein intake, enhancing people's diets around the world (FAO, 2016).

Microbes play a critically important role in the cycling of nutrients in terrestrial and aquatic ecosystems globally. Marine microbes are responsible for approximately half of global primary production and play a huge role in the cycling of carbon, nitrogen, phosphorus and other nutrients (Arrigo, 2005). Microbes have a central role in sustaining life on earth and lie at the centre of issues such as sustainability and climate change (Kowalchuk *et al*., 2008). Microbes also have a direct, central and critically important role in fisheries and aquaculture. Microbes in natural marine and freshwater ecosystems are key components of food webs, primary and secondary production and nutrient cycling. A wide range of microbes are used directly in aquaculture as live feeds, probiotics, and in filtration systems. Aquatic microorganisms are therefore indispensable resources for growth of shellfish and finfish in natural aquatic ecosystems and in aquaculture.

The provision of suitable feeds in aquaculture has been identified as an important constraint in the growth of aquaculture (FAO, 2016). Fishmeal and fish oil are important ingredients in many farmed fish feeds but there has been a reduction in the amount of fishmeal and fish oil included in feeds as the cost of these ingredients has risen. Microbes such as rotifers, *Artemia* spp. and microalgae can be important substitutes for fishmeal and fish oil. Suitable aquaculture feeds are particularly important in developing countries where there is a need to ensure that fish farmers have economical and balanced feeds to meet the nutritional needs of various life stages of their production species. Improvements in feed quality and availability could increase production and reduce costs (Hasan and New, 2013). In many cases, in particular for early life stages of fish and shellfish, these feeds include microbes grown specifically to fulfil the nutritional needs of aquaculture species. Microbes are also important for non-fed aquaculture species, including for microalgae grown as crops, and for filter feeding fish species that consume microbes naturally present in production systems. It has been estimated that 30.8 percent of world fish production by aquaculture comprises non-fed species, notably carp, and bivalve molluscs such as clams, oysters and mussels (FAO, 2016). This thematic background study on the genetic resources of key microorganisms on which aquaculture depends focuses on microbes grown to feed aquaculture species. However, the microbiology of non-fed aquaculture species is also worth considering. Rapid advances in technologies used to study microbial ecology mean that it is now possible to rapidly determine the community composition of complex natural microbial communities, such as those grazed on by species grown in non-fed aquaculture, by genomic approaches and to monitor changes in the diversity of those communities.

Application of these genomic monitoring approaches may lead to the ability to alter environmental parameters in growth systems to enhance growth of microbes that are best suited to support the nutritional needs of the production species.

The importance of aquatic microbial genetic resources in current aquaculture production and in the future of aquaculture is often underappreciated. This thematic background study provides information on the genetic resources of key microorganisms on which aquaculture depends.

2. SCOPE

This thematic background study provides information on the genetic resources of key microorganisms on which aquaculture depends. These microorganisms fall into the microbial groups of: microalgae, fungi and fungal-like organisms; bacteria, including cyanobacteria; and zooplankton. Many microalgal species are important in aquaculture, with different species being suitable as feed for shellfish and finfish larviculture, as components of green water widely used to enhance survival and growth of larval and adult fish, and as feeds to enhance the nutritional quality of *Artemia* and rotifers. Microalgae are also grown in aquaculture to produce pigments and fatty acids important to fish aquaculture and as human nutraceuticals. Bacteria that are used in aquaculture include cyanobacteria such as *Arthrospira* spp. used for human diet supplements and a rapidly growing suite of probiotic bacteria. These probiotic bacteria include species that improve survival and growth of fish and shellfish larval and adult stages. Probiotic bacteria are expected to become increasingly important for disease prevention in aquaculture as antibiotic use is further curtailed and species are grown in more intensive aquaculture systems. Bacteria also play an important role in filtration systems needed in recirculating aquaculture systems. Zooplankton, specifically *Artemia* spp. and rotifers, have a long history and wide application as feed for the aquaculture industry. Several species of *Artemia* are used, with *A. franciscana* being the most important. Of more than 2 000 species of rotifers, *Brachionus plicatilis* and *B. rotundiformis* are most commonly used. Other zooplankton used in aquaculture include copepods that are growing in importance and Cladocera such as *Daphnia* spp. that are widely used in freshwater larviculture.

The scope of this report is generally limited to production and use of specific microorganisms in aquaculture. The scope excludes the role of microbes in diseases in aquaculture as well as the critically important roles of microbes in natural aquatic ecosystems, including as natural food sources for non-fed aquaculture species such as fish and shellfish species that are filter feeders consuming the microbes that occur naturally in some production systems. However, this latter topic of microbial communities that sustain non-fed aquaculture species is considered in the context of new technologies (Section 7: Genetic and genomic characterization) where it is pointed out that it is now possible to rapidly determine community composition of complex natural microbial communities by genomic approaches and this may have application in future improvements in production of non-fed aquaculture species.

The geographic scope of this report is global and examples of the use and production of microbes in aquaculture are given from many countries. Particular emphasis has been placed on the genetic resources of key microorganisms on which aquaculture depends in the major aquaculture nations. China is by far the world's biggest aquaculture producer. Other major aquaculture producer countries include Indonesia, India, Viet Nam and the Philippines and together with China these countries make up over 80 percent of world aquaculture production (FAO, 2016).

3. MICROORGANISMS USED IN AQUACULTURE

3.1 Microalgae, fungi and fungal-like organisms

3.1.1 Uses of microalgae, fungi and fungal-like organisms

Uses of microalgae

The uses of microalgae in aquaculture can be divided into four categories: green water approaches; direct feeding; indirect feeding; and production of pigments for aquaculture. Each is considered separately below.

Green water approaches

Green water is water in which microalgae grow naturally, or to which microalgae are added. The latter approach in which cultured microalgae are added to fish larval rearing tanks is sometimes termed pseudo-green water (Shields and Lupatsch, 2012). Extensive freshwater fish and shrimp culture is done in man-made enclosures that are fertilized by agricultural or domestic waste or commercial fertilizer to produce blooms of microalgae. The resultant green water has been described as an important sector in world aquaculture because of the huge size of freshwater fish aquaculture that it supports (Neori, 2011; 2013). Several of the most important freshwater fish species consume green water plankton which is comprised of mainly microalgae, although zooplankton, protozoa and bacteria are also present (Neori, 2011). These fish and shrimp are raised without additional aquaculture feed. The quantity of green water microalgae consumed by fish and shrimp has been conservatively estimated at about 250 million tonnes per annum, based on the tonnage of fish and shrimp produced in green water polyculture ponds, the feed conversion ratio, and the proportion of microalgae first consumed by zooplankton before being eaten by fish or shrimp. The productivity of these complex communities of microalgae in open ponds can be high. There are few reports in which the microalgal species composition of green water has been determined. Species composition is likely to be dynamic, changing according to environmental parameters, grazing by protozoa and ingestion by fish and shrimps and other factors. Application of molecular approaches for rapid identification and monitoring of microalgal communities may enable insights into optimal microalgal community composition.

Production of green water is also used on a smaller scale by the addition of microalgae to tanks in which larval fish or prawns are being raised. This green water technique is used by many fish and shrimp hatcheries. The benefits derived from this approach include improved larval growth and survival although the reasons for these improvements have not always been scientifically studied. Zmora et al. (2013) lists documented benefits that include enhancement of the nutritional quality of live prey such as *Artemia* and rotifers, antibacterial activity and improvement of water quality by acting as an *in situ* biological filter. The microalgal species that are widely used for green water production are *Nannochloropsis* spp., *Chlorella vulgaris*, *Isochrysis* spp. and *Tetraselmis* spp.

Direct feeding

Microalgae are produced by many aquaculture facilities for direct feeding to finfish, shrimp, crab, bivalve, abalone and sea cucumber larval and juvenile stages. Of the many hundreds of microalgal species, a large number have been tried as aquaculture feeds and around 20-30 species are now widely used. Hemaiswarya *et al.*, 2011 lists *Nannochloropsis* spp., *Pavlova* spp., *Isochrysis* spp., *Tetraselmis* spp., *Thalassiosira weissflogii*, *Dunaliella* spp. and *Chaetoceros* spp. as microalgal species that are commercially used. Zmora *et al*. (2013) lists some additional microalgal genetic resources that are important in feeding crab and shrimp larvae, abalone juveniles and bivalves, including *Thalassiosira pseudonana*, *Skeletonema* spp., *Rhodomonas* spp., *Pyramimonas* spp., *Navicula* spp., *Nitzschia* spp., *Cocconeis* spp., and *Amphora* spp. Table 1 reports a compiled listing from several recent reviews of microalgal species and genera used in aquaculture and the applications for which they are used.

TABLE 1.
Microalgal species used in aquaculture

Phylum or Class	Genus and species	Application	Citation
Chlorophyta	*Chlamydomonas khaki*	Bivalve molluscs; rotifer and freshwater zooplankton live prey	Becker, 2013
	Chlorella spp.	Bivalve molluscs and crustacean larvae; formulated feed ingredient; rotifer and *Artemia* live prey	Shields, 2011; Becker, 2013.
	Chlorella vulgaris	Formulated feed ingredient; rotifer live prey	Shields, 2011
	Chlorella vulgaris B12	Rotifer live prey; green water	Zmora *et al*. 2013
	Chlorella spp. (marine)	Crustacean (crab) larvae	Zmora *et al*. 2013
	Chlorella minutissima	Formulated feed ingredient; rotifer live prey	Shields, 2011
	Chlorella virginica	Formulated feed ingredient; rotifer live prey	Shields, 2011
	Chlorella grossii	Formulated feed ingredient; rotifer live prey	Shields, 2011
	Dunaliella tertiolecta	Bivalve molluscs; formulated feed ingredient; rotifer and *Artemia* live prey	Shields, 2011; Becker, 2013
	Dunaliella salina	Formulated feed ingredient	Shields, 2011
	Dunaliella spp.	Mollusc hatcheries	Hemaiswarya *et al*., 2011; Muller-Feuga, 2013
	Haematococcus pluvialis	Bivalve molluscs; formulated feed ingredient	Shields, 2011; Becker, 2013
	Micromonas pussila	Bivalve molluscs	Becker, 2013
	Pyramimonas virginica	Bivalve molluscs	Becker, 2013; Muller-Feuga, 2013; Becker, 2013
	Scenedesmus obliquus	Rotifer and *Artemia* live prey	Becker, 2013
	Scenedesmus quadricauda	Rotifer and *Artemia* live prey	Becker, 2013
	Tetraselmis spp.	Bivalve molluscs and crustacean larvae; rotifer live prey	Hemaiswarya *et al*., 2011
	Tetraselmis chui	Bivalve molluscs and crustacean larvae	Shields, 2011
	Tetraselmis suecica	Bivalve molluscs and crustacean larvae; Rotifer and *Artemia* live prey	Muller-Feuga, 2013; Shields, 2011; Becker, 2013
Ochrophyta	*Nannochloropsis oculata*	Rotifer live prey; green water	Shields, 2011
	Nannochloropsis spp.	Crustacean (crab) larvae; zooplankton feed fin fish hatcheries; shrimp hatcheries	Hemaiswarya *et al*., 2011; Muller-Feuga, 2013; Zmora *et al*., 2013
Labyrinthulomycetes	*Schizochytrium* spp.	Rotifer and *Artemia* live prey	Shields, 2011
	Ulkenia spp.	Rotifer and *Artemia* live prey	Shields, 2011

(cont.)

Phylum or Class	Genus and species	Application	Citation
Bacillariophyta (diatoms)	*Actinocyclus normanii*	Bivalve molluscs	Becker, 2013
	Amphora spp.	Gastropod molluscs and sea urchins	Shields, 2011
	Amphora ovalis	Crustacean larvae	Becker, 2013
	Bellerochea polymorpha	Bivalve molluscs	Becker, 2013
	Chaetoceros affinis	Bivalve molluscs and crustacean larvae; *Artemia* live prey	Becker, 2013
	Chaetoceros calcitrans	Bivalve molluscs and crustacean larvae	Shields, 2011, Becker, 2013; Muller-Feuga, 2013; Zmora *et al.*, 2013.
	Chaetoceros gracilis	Bivalve molluscs and crustacean larvae	Shields, 2011; Muller-Feuga, 2013; Zmora *et al.* 2013
	Chaetoceros muelleri	Bivalve molluscs and crustacean larvae; *Artemia* live prey	Becker, 2013
	Chaetoceros neogracile	Bivalve molluscs	Zmora *et al.*, 2013
	Chaetoceros spp.	Shrimp hatcheries	Hemaiswarya *et al.*, 2011
	Cocconeis duplex	Abalone larvae	Becker, 2013
	Cyclotella cryptica	Aquaculture, unspecified	Muller-Feuga, 2013
	Cyclotella nana	*Artemia* live prey	Becker, 2013
	Cylindrotheca closterium	Crustacean larvae	Becker, 2013
	Navicula spp.	Bivalve mollusc larvae, gastropod molluscs and sea urchins	Shields, 2011; Becker, 2013; Zmora *et al.*, 2013
	Nitzschia spp.	Gastropod molluscs and sea urchins	Shields, 2011; Zmora *et al.* 2013
	Nitzschia closterium	*Artemia* live prey	Becker, 2013
	Nitzschia paleacea	*Artemia* live prey	Becker, 2013
	Phaeodactylum sp.	Crustacean (crab) larvae	Zmora *et al.* 2013
	Phaeodactylum tricornutum	Mollusc hatcheries; Bivalve molluscs and crustacean larvae	Becker, 2013; Muller-Feuga, 2013
	Skeletonema costatum	Bivalve molluscs and crustacean larvae	Shields, 2011; Becker, 2013; Muller-Feuga, 2013
	Skeletonema spp.	Crustacean larvae	Zmora *et al.*, 2013
	Thalassiosira pseudonana	Bivalve molluscs and crustacean larvae	Shields, 2011; Becker, 2013
	Thalassiosira pseudonana, clone 3H	Mollusc hatcheries	Muller-Feuga, 2013
	Thalassiosira weissflogii	Bivalve molluscs and crustacean larvae; copepod and *Artemia* live prey	Hemaiswarya *et al.*, 2011
Haptophyta	*Coccolithus huxleyi*	Bivalve molluscs	Becker, 2013
	Cricosphaera elongata	Bivalve molluscs	Becker, 2013
	Dicrateria spp.	Bivalve molluscs	Becker, 2013
	Isochrysis spp.	Copepod and *Artemia* live prey; bivalve molluscs	Hemaiswarya *et al.*, 2011
	Isochrysis galbana	Mollusc hatcheries	Muller-Feuga, 2013
	Isochrysis galbana affinis "Tahiti" *(T.* iso*)*	Bivalve molluscs and crustacean larvae Bivalves; rotifer and *Artemia* live prey	Shields, 2011; Becker, 2013; Muller-Feuga, 2013; Zmora *et al.* 2013

(cont.)

Phylum or Class	Genus and species	Application	Citation
	Olisthodiscus luteus	Freshwater zooplankton live feed	Becker, 2013
	Pavlova lutheri	Bivalve molluscs Rotifer and *Artemia* live prey Mollusc hatcheries	Shields, 2011; Becker, 2013; Muller-Feuga, 2013; Zmora *et al.* 2013
	Pavlova pinguis	Bivalve molluscs Rotifer and *Artemia* live prey Mollusc hatcheries	Becker, 2013
	Pavlova spp.	Bivalve molluscs Rotifer live prey	Hemaiswarya *et al*., 2011
	Pseudoisochrysis paradoxa	Bivalve molluscs and crustacean larvae	Becker, 2013
Cryptophyta	*Cryptomonas* spp.	Bivalve molluscs	Becker, 2013
	Rhodomonas salina	Bivalve molluscs	Becker, 2013
	Chroomonas salina	Bivalve molluscs	Becker, 2013
Dinophyta	*Crypthecodinium cohnii*	Rotifer and *Artemia* live prey	Shields, 2011

Note: Compiled from reviews by Hemaiswarya *et al*., 2011; Becker, 2013; Muller-Feuga, 2013; Zmora *et al*., 2013.1. For Zmora *et al.,* 2013, microalgae with applications designated as "most popular" are included.

A group that warrants separate mention is the thraustochytrids. Thraustochytrids are marine microorganisms that were previously classified as fungi but are here considered as microalgae, although there is ongoing debate about the classification of thraustochytrids. Many thraustochytrids are rich sources of long-chain polyunsaturated fatty acids and therefore have potential as feed additives in aquaculture. Unlike other microalgae used in aquaculture that are photosynthetic, thraustochytrids are heterotrophs that can grow on rich media in the absence of light and can therefore be readily cultured using industrial fermenters. The genus *Schizochytrium* within the thraustochytrids is the genus most commonly used in aquaculture. Several products based on thraustochytrids from the genus *Schizochytrium* have been marketed through Aquafauna Biomarine and Sanders Brine Shrimp. These products have high concentrations of docosahexaenoic acid (DHA), which is important for human health (Guedes and Malcata, 2012). Dried algae *Schizochytrium* from Advanced BioNutrition Corp. was shown to be effective in enhancing weight gain, feed efficiency ratio and long-chain polyunsaturated fatty acid content when included in the diets of channel catfish *Ictalurus punctatus* (Li *et al*., 2009). *Schizochytrium* spp., have been used as a replacement for fish oil in the diets of jade perch (*Scortum barcoo*) juveniles which stored more n-3 long chain polyunsaturated fatty acids, in particular DHA (Van Hoestenberghe *et al*., 2014). In a recent study, fish oil was completely replaced with dried whole cells of *Schizochytrium* in the diet of Nile tilapia, resulting in significantly higher weight gain and an improved feed conversion ratio, as well as improved deposition of long chain polyunsaturated fatty acids in the tilapia filets (Sarker *et al*., 2016). Although the genus *Schizochytrium* is now most commonly used, new isolates from the diverse thraustochytrid group have high future potential. For example, the newly isolated microalga *Aurantiochytrium* sp. KRS101 was found to have a high DHA content and ability to grow on cheap substrates such as molasses (Hong *et al*., 2011). An interesting approach to reduce the cost of thraustochytrid biomass is to grow these organisms on wastewater from marine aquaculture, which has the added advantage of removing nutrients from the wastewater prior to discharge (Jung and Lovitt, 2010). The dinoflagellate *Crypthecodinium cohnii* can also be grown autotrophically and has the advantage of producing DHA in high concentrations, and without a mixture of other polyunsaturated fatty acids (Mendes *et al*., 2009).

Indirect feeding

Indirect feeding includes feeding microalgae to zooplankton to improve nutritional value. Microalgae are used as a food source for zooplankton such as rotifers and *Artemia* spp., both to grow the zooplankton and to enrich their nutritional value once they have reached the appropriate size to be fed to the species being grown in aquaculture.

Rotifers are grown in batch, semi-continuous or continuous culture, and commonly used microalgal diets for these rotifers include *Chlorella vulgaris*, *Isochrysis* spp., *Pavlova* spp., and *Nannochloropsis* spp. (Hemaiswarya *et al*., 2011; Guedes and Malcata, 2012; Zmora *et al*., 2013).

Thraustochytrids have also been used to enrich *Artemia* spp. and rotifers with long-chain polyunsaturated fatty acids (Yamasaki *et al*., 2007). *Schizochytrium limacinum*, a thraustochytrid with high DHA content, was found to be effective in increasing the DHA content of rotifer *Brachionus plicatilis* and *Artemia franciscana*. Turbot (*Scophthalmus maximus*) juveniles fed with these enriched rotifers and *Artemia* nauplii were found to have a reduced rate of pseudoalbinism compared with a control group that received yeast-fed rotifers and *Artemia* spp. (Song *et al*., 2007).

Crypthecodinium cohnii phospholipid extract and meal have also been used to enrich rotifers and *Artemia*, resulting in high levels of DHA. A 60 percent replacement of menhaden oil with *Crypthecodinium cohnii*-derived algal oil resulted in no change in growth rates in striped bass (Harel *et al*., 2002).

Production of pigments of importance to aquaculture

The microalga *Haematococcus pluvialis* is increasingly used as a natural source of the carotenoid pigment astaxanthin that has application as a nutraceutical for human health and as a pigment to provide a desirable pink or red colour in species grown in aquaculture. The biology and commercial aspects of astaxanthin pigment production by *H. pluvialis* has been well reviewed by Han *et al*. (2013). Natural astaxanthin produced by *H. pluvialis* competes in the marketplace with synthetic astaxanthin. Synthetic astaxanthin costs about USD 2 000 per kg (Li *et al*., 2011a) and dominates the market that was estimated to total USD 447 million in 2014 (Wade *et al*., 2015). Natural astaxanthin fetches a much higher price and has advantages as a feed additive in aquaculture because it provides better pigmentation in some fish species and is preferred by consumers because of perceived safety benefits (Han *et al*., 2013). Natural and synthetic astaxanthins are often used in diet formulations for crustaceans such as shrimp that are grown in intensive aquaculture systems and are also widely used for farmed salmon and trout (Wade *et al*., 2015).

Uses of fungi and fungal-like organisms

Some species of yeast have been used as probiotics, dietary supplements and sources of pigments in aquaculture. Many species of yeast have been found as part of the normal microbiota of fish. Most of the reports of probiotic effects of yeast are focused primarily on two species, *Saccharomyces cerevisiae* and *Debaryomyces hansenii* (Navarrete and D. Tovar-Ramírez, 2014). A number of commercial products comprising yeast and yeast components are available commercially, including the preparations MacroGard®, Betagard A®, EcoActiva®, NuPro® (a yeast- derived protein source), Nutriferm®, Fibosel®, Levucell® (derived from *S. boulardii*). These products are sold as additives for agriculture, including in pig and poultry diets, as well as human nutraceuticals. The products MacroGard® Betagard A® and Levucell® SB20 all provided some increased resistance to challenges with the bacterial pathogen *Edwardsiella ictaluri* in juvenile channel catfish (*Ictalurus punctatus*) (Welker *et al*., 2012). The effects of β-glucans derived from yeast on fish immunity was recently reviewed by Vetvika *et al*. (2013) and found to be satisfactory in eliciting immunity and already to be widely used in commercial aquaculture, although there is a need for more efficient administration methods before glucans are prophylactically used routinely in aquaculture. Yeast extracts added to diets have been shown to have beneficial effects on health and growth rate in both marine fish (e.g. cobia; Lunger *et al*., 2006) and freshwater fish (e.g. Nile tilapia; Berto *et al.*, 2015).

The yeast *S. cerevisiae* has also been tested as a dietary component. Addition at 15 percent dry weight substitution of fish meal was palatable to juvenile tilapia without affecting body composition (Ozório *et al*., 2012). Yeast can be an economic substitution because it is available cheaply as brewer's yeast and as a by-product from ethanol fermentation, although dried yeast from these sources may vary in quality from batch to batch.

The red yeast *Rhodosporidium paludigenum* was found to enhance the growth performance and antioxidant performance of the widely cultured tropical shrimp *Litopenaeus vannamei* and has the potential to be a promising probiotic (Yang *et al*., 2010).

The pink yeast *Phaffia rhodozyma*, contains astaxanthin at about 0.4 percent and was used in the past as a source of this pigment for colouring of salmonids (Choubert *et al*., 1995). More recently, the microalgae *Haematococcus* that contains 1.5–4.0 percent of astaxanthin has been used instead (see above). In a study comparing commercially synthesized astaxanthin with that derived from *P. rhodozyma* marketed as Ecotone™. Ecotone™ was found to be a more effective astaxanthin source for pigmentation of Atlantic salmon muscle than the synthetic astaxanthin (Bjerkeng *et al*., 2007). The major market for this pigment is aquaculture, more than 95 percent of the market share belongs to synthetic astaxanthin but with interest in natural rather than synthesized astaxanthin increasing because of consumer preference for natural products.

3.1.2 Production strategies and systems Microalgae

Microalgae are divided into photoautotrophic microalgae that require light for growth and heterotrophic microalgae that grow on organic nutrients and are not light requiring. Many different systems are used for in-house production of microalgae by aquaculture hatcheries, including carboys, hanging polyethylene bags, and bubble or airlift columns. Generally, production at a small scale is by batch culture, although continuous culture has been proposed as an attractive alternative because it can achieve reduced costs through automation and provide better environmental controls leading to more consistent quality of microalgae (Marchetti *et al*., 2012). For larger scale production, the two major cultivation systems are open ponds, tanks or raceways and closed photobioreactors. Open outdoor systems are susceptible to contamination and are therefore generally used for microalgae that can be grown under selective conditions, such as *Chlorella* spp. that can tolerate high concentrations of nutrients or *Dunaliella* spp. that thrive under high-salt conditions. For closed systems, many configurations of photobioreactors have been used, generally comprising flat panel photobioreactors or tubular photobioreactors. Important variables in all these systems are the type of aeration that is provided and the light source, ranging from natural light in open outdoor systems to incandescent, fluorescent and light-emitting diode (LED) lighting of varied intensity and wavelengths in closed systems. Microalgal culture facilities for aquaculture are reviewed in more detail in Zmora *et al*., 2013 and in Guedes and Malcata, 2012.

Neori (2011; 2013) has described green water production as the most important sector within aquaculture because of the vast freshwater fish aquaculture that this approach supports. Green water production strategies include reliance on natural algal blooms and addition of fertilizer and domestic waste to ponds to stimulate natural bloom production. Production of pseudo-green water by addition of microalgae to fish larval rearing tanks relies on microalgal production by one of the approaches described above.

Heterotrophic microalgae including thraustochytrids such as *Schizochytrium* spp. and the dinoflagellate *Crypthecodinium cohnii* can be grown in fermenters. Growth in fermenters can achieve very high cell densities and have economic advantages. *Crypthecodinium cohnii* is grown on an industrial scale to produce DHA by Royal DSM (previously Martek Corp.) in Maryland, United States of America. The freshwater photoautotrophic microalga *Chlorella* spp. can also be grown heterotrophically. *Chlorella* spp. is grown commercially in heterotrophic systems in Taiwan Province of China, and Japan with an annual production of about 1 100 tonnes (Liu and Hu, 2013) and the total annual production of *Chlorella* biomass in both phototrophic and autotrophic conditions, exceeds 2 000 tonnes per annum (Spolaore *et al*., 2006).

Fungi and fungal-like organisms

Due to the importance of fungi in a wide range of commercial processes, there is an extensive base of knowledge for the intensive cultivation of fungi. Most of these species of potential application in aquaculture can readily be cultivated economically on a large scale by tapping into the existing technologies for fungal cultivation.

3.1.3 Genetic technologies

Microalgae

Genetic technologies can be applied to microalgae of importance in aquaculture in several different ways. Genome sequencing and transcriptomics can be used to understand the systems biology of important strains, including patterns of gene expression under different conditions. Sequencing of genes that serve as phylogenetic markers, such as the cytochrome *c* oxidase subunit 1 (CO1), 18S and 16S rRNA genes can be used to rapidly and unequivocally identify individual strains. A study of 16S and 18S rRNA gene molecular markers showed that 18 strains of microalgae could be well differentiated at the genus level but that better databases of reference sequences may be needed before species-level identification can be reliably achieved using these marker genes (Alonso *et al*., 2012). It is important to correctly identify microalgae in order to obtain reproducibility and reliability in their application in aquaculture. It is also important that gene-based identifications are accurate because they can be particularly useful to differentiate strains that are morphologically similar. Metagenomic approaches based on sequencing of large numbers of genes that provide phylogenetic information can be used to fully characterize the species composition of complex microalgal communities (Uyaguari *et al*., 2016), such as in green water. Even more extensive sequencing of total DNA and RNA extracted from complex communities such as those found in green water could be used to reveal phylogeny and patterns of gene expression in all of the microalgae and bacteria making up these complex communities.

Rapidly advancing genetic and genomic technologies offer the potential to genetically modify microalgal strains to enhance desired characteristics, including optimizing microalgal quality for aquaculture feeds and other applications.The greatly reduced cost of DNA sequencing has facilitated the sequencing of the genomes of many microalgae. Recently, for example, the genomes of five species of *Nannochloropsis* were sequenced, giving new information on the diversity of lipid synthesis genes in this microalgal genus that is widely used in aquaculture (Wang *et al*., 2014). Bioinformatic analysis of genome sequences can give new insights into the pathways encoding compounds of interest and facilitate genetic manipulation.

Much of the impetus for research on genetic manipulation of microalgae over the past decade has come from the interest in exploiting microalgae for biofuel production. The ability of microalgae to synthesize many useful products and for genetic manipulation to establish microalgae as a widely used platform for the production of many high-value products was reviewed by Rosenberg *et al*. (2008).

A critical step in genetic manipulation of microalgae is genetic transformation to get DNA into the cells. Widely used methods for introduction of DNA into microalgal cells include biolistics in which cells are bombarded with DNA-coated particles and electroporation in which electric current is used to permeabilize the cell membrane. Other methods that have been used include agitation of microalgae lacking cell walls with glass beads coated with DNA and agitation with silicon carbide "whiskers" that can pierce through cell walls and inject DNA into algal cells. Many species of microalgae have now been successfully transformed with DNA introduced into either the nucleus, chloroplast or mitochondrion of the microalga. Successful genetic transformation has now been demonstrated in more than 30 species including most of the microalgae that are important in aquaculture (see Enzing *et al*., 2012 for a list).

Once transformation is achieved, selection methods are needed to select the cells that have been successfully transformed. Several selection systems are available for *Chlamydomonas reinhardtii* and *Volvox carteri* but few systems exist for other microalgae. A widely used selection system is *ble*, a protein conferring resistance to bleomycin, phleomycin and zeomycin (Stevens, Rochaix and Purton, 1996). Marker and reporter genes for use in microalgae are listed by Gangl *et al*. (2015).

Several promoters are available to drive the expression of genes that are inserted into microalgae. Promoters from highly expressed endogenous microalgal genes such as ribulose bisphosphate carboxylase are used for nuclear expression (Walker, Collet and Purton, 2005). Genes encoding core photosynthetic subunits are used for chloroplast expression (Purton, 2007).

Even once genes are successfully inserted into green algae, stable long-term expression of transgenic proteins has seldom been obtained except in *C. reinhardtii* and *V. carteri*, possibly due to microRNA gene regulatory systems causing transgene silencing (Rosenberg *et al*., 2008). One of the first examples of successful genetic manipulation was the transformation of *V. carteri* with a hexose transporter gene that resulted in the microalga being able to grow heterotrophically on hexose rather than by photosynthesis (Hallmann and Sumper, 1996). Trophic conversion was also achieved in the diatom *Phaeodactylum tricornutum* by introducing a gene encoding a glucose transporter (Zaslavskaia *et al*., 2001). These cases of trophic conversion demonstrate the potential of genetic manipulation of microalgae to change production from light-dependent photosynthesis to heterotrophic fermentation for large-scale commercial growth, which may have economic benefits for some production systems.

The microalgal genetic manipulation of *C. reinhardtii* has provided many new products, including erythropoietin, interferon, proinsulin and human fibronectin (Rasala *et al*., 2010). There are few examples of development of transgenic algae for food and feed production because of challenges including public acceptance and regulatory issues (Enzing *et al*., 2014).

Gressel (2013) comprehensively reviews the characteristics that are beneficial to genetically engineer into algal platform strains for wide applicability in aquaculture (and for other applications, including biofuels). If strains are to be grown on a large scale in open ponds, resistance to contamination is important; the approach that Gressel (2013) discusses to prevent contamination by competing algae is to engineer the desired strain with genes encoding herbicide resistance and then apply herbicide treatment to maintain the desired strain. Engineering of microalgal strains to produce short-chain antimicrobial peptides could be useful in reducing contamination by bacteria and fungi (Gressel, 2013). The antimicrobial peptide lactoferrin was successfully produced in a transgenic strain of *Nannochloropsis oculata* and shown to be effective when fed to medaka fish in reducing infection of those fish by the bacterial pathogen *Vibrio parahaemolyticus* (Li and Tsai, 2008). Gressel (2013) proposes that the insecticide avermectin may be effective against many species of zooplankton and algae engineered to produce avermectin may be resistant to zooplankton contamination that can very rapidly decimate dense cultures of microalgae. He and others previously patented a method to attain resistance to viral infections, by isolating and amplifying the viral nucleic acid, splicing it into an expression cassette and transforming into the microalgae. Survivors with the right orientation of the cassette would become resistant to the viral infection (Gressel, Chen and Danon, 2010).

Other desirable traits listed by Gressel (2013) that could be engineered into microalgae are the ability to overcome the quorum sensing processes that may cause algal crashes once high densities are reached, heat tolerance to resist the high temperatures in closed bioreactors or ponds in sunny

climates, and changes to photosynthetic efficiency that overcome photoinhibition at high light intensities. Truncated light-harvesting chlorophyll antenna size *(tla)* strains in *Chlamydomonas reinhardtii* operated with improved solar energy conversion efficiency (Melis, 2009). Reducing or truncating the size of the chlorophyll antennae has been shown to increase the light intensity at which photosynthesis saturates in two strains of the diatom *Cytothella* sp. (Huesemann *et al*., 2009). A strain of *Chlorella vulgaris* in which reduced chlorophyll antennae size was obtained by chemical mutagenesis achieved 44.5 percent improvement in biomass productivity under high light conditions (Shin *et al*., 2016). Random mutagenesis was also effective in producing a strain of *Chlorella sorokiniana* with reduced chlorophyll content and truncated antennae that showed higher productivity that the wild type and yielded 30 percent higher biomass in photobioreactors (Cazzaniga *et al.,* 2014). RNA interference (RNAi) technology was used to silence all 20 light-harvesting complex genes in *C. reinhardtii* resulting in a chlorophyll/cell reduction of 68 percent and cells that were less susceptible to light inhibition and grew at a faster rate (Mussgnug *et al*., 2007). Gene silencing technology such as RNAi is emerging as a useful tool in genetic engineering and functional analysis of microalgae, but progress is limited by incomplete understanding of the highly diverse silencing systems present in most microalgal species (Kim *et al.,* 2015).

Fungi and fungal-like organisms

The genome of the yeast *Saccharomyces cerevisiae* that has probiotic applications in aquaculture was the first eukaryote to have its genome sequenced, in the early 1990s (Goffeau *et al.,* 1996) and there are extensive genetic and genomic resources available for this yeast. The yeast *Debaryomyces hansenii* that also has probiotic effects has a draft genome sequence for two strains that will provide insights into the halotolerance of this yeast (Kumar *et al*., 2012). Although no genome sequence is available for the *Phaffia rhodozyma* that has potential as a source of the important pigment astaxanthin, progress was made with the understanding and potential use of this species, including methods for isolation of mutants that are affected in carotenoid biosynthesis as well as techniques for isolation and analysis of carotenoids (Lin *et al*., 2012). These approaches could be applied to enhance pigment production.

3.1.4 Producer countries and production trends Microalgae

China is the major global producer of microalgal biomass and production trends in China are covered in detail in the recent extensive review by Chen *et al*. (2016). The four major microalgae produced in order of tonnage are *Arthrospira* spp., (see section 3.2.4), *Chlorella* spp., *Dunaliella* spp., and *Haematococcus* spp. Commercial production of all of the species of microalgae of significance to aquaculture has recently been reviewed in individual chapters in Richmond and Hu (2013).

3.2 Bacteria

3.2.1 Uses of bacteria

Arthrospira (Spirulina)

Arthrospira (*Spirulina*) is a genus of photosynthetic cyanobacteria and is therefore correctly discussed under bacteria rather than microalgae, although in many texts, *Arthrospira* (*Spirulina*) is categorized under microalgae. Members of the genus *Arthrospira* are filamentous, multicellular cyanobacteria with a characteristic helical filament shape. The two most important species in terms of production for food and feed are *Arthrospira platensis* and *Arthrospira maxima*. In an analysis based on 16S rRNA gene sequence analysis of five commercial strains of "Spirulina", four of the strains were found to be closely related to the genus *Arthrospira* and the fifth strain was affiliated with the genus *Halospirulina* (Kwei *et al*., 2011). This and other recent phylogenetic studies based on molecular, morphological and biochemical characterization are all consistent with the most important producer organisms being two closely related species in the genus *Arthrospira, A. platensis* and *A. maxima*.

Arthospira platensis and *A. maxima* are found naturally occurring in alkaline waters with high carbonate and bicarbonate concentrations, in tropical and subtropical waters. Under the extreme conditions in alkaline and saline lakes, *Arthrospira* spp. can grow to high concentrations and be the dominant microbe present. The growth characteristics of *Arthrospira* spp. are useful in maintaining almost pure cultures of this genus in production facilities (see 3.2.2. below).

Arthrospira spp. have been used as a vitamin and protein supplement in aquaculture (Habib *et al*., 2008). The widespread use and potential of *Arthrospira* spp. in aquaculture is reviewed by Becker (2013). Raw *A. platensis* was effective as a feed for larval tilapia (Lu *et al*., 2002). *Arthrospira platensis* was used as a probiotic for effective growth and immunity promotion in Nile tilapia (*Oreochromis niloticus*) that were challenged with the bacterial pathogen *Aeromonas hydrophila* (Abdel-Tawwab and Ahmad, 2009). In tests of *Arthrospira* spp. as feed for several species of carp fry, *Cyprinus carpio* (common carp), *Hypophthalmichthys molitrix* (silver carp), and *Ctenopharyngodon idella* (grass carp), addition of 10 percent *Arthrospira* spp. to other diet ingredients generally resulted in better performance of the fry (Ayyappan, 1992). Additional examples are given in Becker (2013) who points out the broad range of applications of *Arthrospira* spp. in commercial aquaculture.

Probiotic bacteria

Bacteria are increasingly used in aquaculture as probiotics to reduce the effects of the many pathogens that can infect aquaculture species. Probiotic bacteria have been most extensively used in China and South America. There are now many hundreds of examples in the literature of the use of bacteria to improve disease resistance or growth of aquaculture species. The use of probiotics for disease control in aquaculture has recently been comprehensively reviewed by Newaj-Fyzul *et al*. (2014) who list 18 species of Gram-negative bacteria and 19 species of Gram- positive bacteria that have been considered for use in aquaculture. Modes of action may include competitive exclusion or immunostimulation as well as improvement in appetite or feed conversion that leads to better growth (Newaj-Fyzul *et al.,* 2014).

Here, some very recent examples are provided as well as a detailed description of some use of probiotic bacteria in China because of the importance of the aquaculture industry in that country. In recent work, supplementation of the diets of the important tropical freshwater fish Indian carp (*Labeo rohita*) with the Gram-positive bacteria *Bacillus subtilis* and *Terribacillus saccharophillus* was found to significantly increase the immune and humoral response, suggesting an improvement in fish innate immunity (Sumathi *et al*., 2016).

With the demand of environmentally friendly aquaculture practices, the use of probiotic products is an increasingly common practice in many fish or shellfish hatcheries and farms in China (Zhang *et al*., 2014; Han and Sun, 2016). The probiotics used in Chinese aquaculture are mainly photosynthetic bacteria (PSB), antagonistic bacteria, microorganisms for nutritional and enzymatic contribution to the digestion (lactic acid bacteria, yeast, etc.), bacteria for improving water quality (nitrifying bacteria, denitrifiers, etc.), *Bdellovibrio* spp., and other probiotics. The species of photosynthetic bacteria currently used in Chinese aquaculture include *Rhodopseudomonas palustris*, *Rubrivivax gelatinosus*, *Rhodobacter capsulatus*, *Rhodobacter sphaeroides*, and *Phaeospirillum fulvum* (Qi *et al.*, 2009). Purple non-sulfur bacteria were traditionally used in aquaculture in China since 1980s (Zhang *et al.*, 1988). These bacteria were reported to be able to stimulate shrimp and fish growth, increase the survival rate of fish larvae and elevate the production of scallop seeds. Instead of using homemade photosynthetic bacterial products, many farmers today are using concentrated and encapsulated commercial photosynthetic bacterial products (Qi *et al*., 2009).

In addition to the above applications, photosynthetic bacteria are being applied to improve the water quality of aquaculture ponds (Li *et al.,* 2011b). Bacterial antagonism plays an increasing major role in the equilibrium between competing beneficial and potentially pathogenic microorganisms (Qi *et al.*, 2009). *Flavobacterium odoratum* (Mo *et al*., 2007), *Alteromonas* spp. (Li *et al*., 2001)*, Phaeobacter inhibens* (Dong *et al*., 2007), *Vibrio natriegens, V. alginolyticus* (Li, 2008) were isolated and identified as effective aquaculture antagonistic bacteria that are capable of inhibiting pathogens in the culture ponds for aquaculture animals (Mo *et al*. 2007; Dong *et al*., 2007; Li *et al*., 2001). Several strains of the genera *Bacillus* and *Rhodobacter* have recently been identified from healthy *Litopenaeus vannamei* and shown to have safe digestive enzyme ability in juvenile shrimp (Dou *et al*., 2016). At present, probiotics research and development are focusing more onto the added amount, additive type, safety and drug compatibility issues of discovered probiotic bacteria; the exploration and development of new probiotic resources to protect aquatic animals from specific pathogens; and the *in vivo* proliferation characteristics of probiotics in aquatic animals and the function and interaction of these probiotics with aquatic animals' immune system (Han and Sun, 2016).

Bacteria used in filtration systems

Bacteria play an important role in filtration in aquaculture systems. Recirculating aquaculture systems have great promise as a sustainable way for farming marine fish. A fully contained recirculating aquaculture system achieved efficient biological waste treatment and water recycling by combining aerobic nitrification with simultaneous anaerobic denitrification and anaerobic ammonium oxidation mediated by efficient microbial filters. In addition, excess organic carbon remaining after denitrification was converted to methane gas by methanogenic microbes (Tal *et al*., 2009). The microbial diversity of biological filters in recirculating aquaculture systems is extensive and includes the genera *Nitrosomonas* (ammonium oxidation), *Nitrospira* (nitrite oxidation), *Thiomicrospira*, *Thiothrix*, *Rhodobacter*, and *Hydrogenophaga* (autotrophic sulfide-dependent denitrification), *Pseudomonas* and *Paracoccus* (heterotrophic denitrification), various genera of Proteobacteria and Firmicutes (dissimilatory nitrate reduction to ammonia), *Planctomycetes* and *Brocadia* (anaerobic ammonium oxidation), *Desulfovibrio*, *Dethiosulfovibrio*, *Fusibacter* and *Bacteroides* (sulfate reduction), *Thiomicrospira* (sulfide oxidation) and methanogenic archaea (methanogenesis) (Schreier *et al*., 2010). Metagenomic approaches that provide insights into metabolic functions and studies to quantify expression of individual genes for the entire community will assist in design optimization and guide bioaugmentation strategies (Schreier *et al*., 2010).

3.2.2 Production strategies and systems Arthrospira (Spirulina)

There is a history of *Arthrospira* spp. harvested from blooms in natural ecosystems such as the soda lakes in Kenya and other parts of East Africa and in Lake Texcoco, near Mexico City, Mexico. Industrial production strategies have been reviewed in an FAO report (Habib *et al*., 2008) and by Belay (2013). Small-scale production has advantages over traditional agriculture in yielding a protein rich-product, requiring no arable land and little water and being efficient in terms of energy use (Habib *et al*., 2008). Commercial and mass cultivation has four key steps: growing the algae; harvesting; drying; and packaging of the biomass (Belay, 2013). Production is generally carried out in shallow raceway ponds with mixing achieved by paddlewheels. Critical parameters include use of an appropriate strain and control of pH, nutrient concentration, the light environment, and contamination (Belay, 2013). Harvesting is generally by filtration and this step is important in

the overall economics of the process. Careful and quick drying is essential to maintain a high-quality product. Some of the technology being developed in attempts to grow microalgae economically and on large scales may have application in improving *Arthrospira* spp. production systems (Belay, 2013).

Arthrospira spp. are phototrophic organisms and have traditionally been considered to be obligate autotrophs that are dependent on light for growth and cannot grow in the dark (Habib *et al*., 2008). However, 34 of 35 axenic *Arthrospira* spp. strains were found to be capable of heterotrophic growth on glucose as a carbon source and 10 strains tested for photoheterotrophy grew with glucose and maltose but not with fructose or sucrose (Muhling, Belay and Whitton, 2005). This raises the interesting possibility that *Arthrospira* spp. could be commercially grown to high biomass concentrations under mixotrophic conditions (Belay, 2013).

Probiotic bacteria

The very wide range of bacteria that have been proposed as probiotics (Newaj-Fyzul, Al-Harbi and Austin, 2014) means that many different media and growth conditions are used to grow these bacteria. One example of bacteria that are used on a commercial scale is the photobacteria

Chromatium perty and *C. okenii* in flat panel photobioreactors by the Chinese company Yantai Rich-Bio Science and Technology Ltd. These bacteria are used to reduce mortality in juvenile sea cucumbers (Zmora *et al*., 2013).

3.2.3 Genetic technologies Arthrospira (Spirulina)

Many cyanobacteria, in particular members of the genus *Arthrospira*, contain large numbers of repeated sequences dispersed throughout their genomes and this characteristic makes sequencing of *Arthrospira* spp. genomes more challenging. Draft genome sequences are now available for several strains of *Arthrospira* spp., including *Arthrospira* sp. PCC 8005 (Janssen *et al*., 2010), *A. platensis* NIES-39 (Fujisawa *et al*., 2010), and *A. platensis* C1 (PCC9438) (Cheevadhanarak *et al*., 2012). *A. platensis* C1 is a widely used laboratory strain that has the advantage of forming single colonies on agar plates because it is non-motile, without the ability to glide on surfaces (Cheevadhanarak *et al*., 2012). Whole genome sequencing was recently done on *A. platensis* YZ, followed by detailed comparative genomic analysis with the other available draft sequences of *Arthrospira* spp. (Xu *et al*., 2016). This study revealed extensive lateral transfer between different *Arthrospira* spp., as well as abundant restriction modification systems.

Genetic manipulation of *Arthrospira* spp. has been challenging and little progress has been made because of the difficulties in transformation of cyanobacteria in this genus. The extensive restriction-modification systems found in this genus are likely one of the factors that have resulted in limited success in introducing DNA into *Arthrospira* spp. There are a few reports of transformation of *A. platensis* strains (Kawata *et al*., 2004; Gaoge *et al*., 2004).

3.2.4 Producer countries and production trends Arthrospira (Spirulina)

Since large-scale production of *Arthrospira* spp. started in Japan in the 1960s, production has expanded to at least 22 countries according to the FAO; production figures vary widely and better monitoring of global *Arthrospira* spp. production is needed (Habib *et al*., 2008). It is generally accepted that the largest *Arthrospira* spp. producing nation is China, with estimates of annual production ranging from 1 000 tonnes to 3 500 tonnes (Lu *et al*., 2011) to the most optimistic figure, based on the websites of a number of companies, of about 10 000 tonnes (Belay, 2013). A large producer is Hainan Simai Enterprising Ltd. in Hainan Province, with an annual production of 200 tonnes (Mostafa, 2012). It was estimated in 2012 that there were more than 60 *Arthrospira* spp. production facilities in China producing around 10 000 tonnes per year (Zhang and Xue, 2012) with an annual growth rate of about 10 percent (Chen *et al*., 2016).

The two largest producers in the United States of America are Earthrise Nutritionals in California and Cyanotech Corp. in Hawaii. Other significant producers are in India, and Thailand.

3.3 Zooplankton

3.3.1 Uses of zooplankton

Artemia

Artemia is a genus of planktonic crustaceans in the class Branchiopoda found around the world occurring naturally in hypersaline environments such as salt lakes. *Artemia*, also known as brine shrimp, produce cysts that are highly resistant to desiccation, thermal fluctuations and UV radiation and retain viability for many years. On rehydration, *Artemia* cysts hatch to produce larval nauplii of about 0.4 mm in size that are very widely used as live feed in aquaculture. The use of *Artemia* spp. as feed for fish larvae in place of their natural diets began in the 1930s and was an important step in the establishment of commercially important aquaculture (Sorgeloos, 1980).

Artemia spp. comprises both zygogenetic and parthenogenetic groups. Seven zygogenetic species are generally recognized (Dhont and Van Steppen, 2003). Several species of *Artemia* are used in aquaculture, with *A. franciscana* found in the Americas being the most important. There are also many geographic strains with their own strain-specific characteristics that have developed in the many hundreds of salt lakes and artificial salterns around the world and these strains provide a resource for selection of characteristics desirable in aquaculture, in particular nutritional value (Dhont and Van Steppen, 2003). *Artemia* spp. can be grown on many different food sources, including microalgae, dried algae, bacteria and yeasts and particulate products from food processing (Dhont and Van Steppen, 2003).

Several different life stages of *Artemia* spp., including decapsulated cysts, non-feeding nauplii (instar I), enriched nauplii (instar II and subsequent stages) and the adult stages, can be used as feed for fish larvae (Dhont and Van Steppen, 2003). *Artemia* spp. are used as live feed for many species in fish larviculture, including seabream, seabass, halibut, flounder and commercially important crustaceans including shrimp, crabs and lobsters (Dhont and Van Steppen, 2003). In 1997, 80–85 percent of *Artemia* spp. went to shrimp hatcheries with the rest going to marine finfish larviculture (FAO, 2011). Because of the cost and as a result of periods of limited *Artemia* spp. supply, there has been some reduction in use. Consumption of cysts in shrimp hatcheries fell from about 10 kg per million postlarvae to less than 5 kg by 2011. In seabream and seabass hatcheries, the reduction has been even more dramatic, from 600–700 kg of cysts per million larvae in 1990 to less than 100 kg in 2011 (FAO, 2011).

Rotifers

The phylum Rotifera comprises three classes, Eurotatoria (including the sub-classes Monogononta and Bdelloidea), Pararotatoria and Seisonidea, with the Monogononta being the largest sub-class with about 1 500 genera, including the genus *Brachionus* which is the most important genus used in aquaculture, although other genera are used by farmers in Asia (X. Zhou, pers. comm.). Two members of this genus, *B. plicatilis* and *B. rotundiformis* are most commonly used as feed for larval stages of marine fish and are euryhaline, able to grow in a wide range of salinities including seawater. *Brachionus plicatilis* ranges in size from 200–360 μm and is known as the L-strain (large) and *B. rotundiformis* is 150–220 μm in size and known as the S-strain (small). *Brachionus calyciflorus* and *B. rubens* are two freshwater rotifers that have been produced in freshwater mass cultures (FAO, 1996). Rotifers have the advantages of small size, the characteristic of growing to very high densities in mass culture systems and can serve vessels for desired nutrients because their nutrient composition can be improved by feeding them with specialized enrichment diets (Delbos and Scharwz ,2009).

Brachionus plicatilis was first identified by Japanese researchers as a pest in eel aquaculture in the 1950s. Soon after it was used as a live food organism for the larval stages of fish species and is suitable for this application because of its small size and slow swimming velocity that make them suitable prey for fish larvae that have just resorbed their yolk sacs but are not yet large enough to be able to feed on *Artemia* spp. (FAO, 1996). Rotifers have been important as feed for the larval stages of many major fish species in aquaculture, including yellowtail (*Seriola quinqueradiata*), red seabream (*Pagrus major*), barramundi (*Lates calcarifer*), gilthead seabream (*Sparus aurata*) and the European seabass (*Dicentrarchus labrax*) as well as penaeid shrimp and crab (Lubzens and Zmora, 2003). Challenges in the use of rotifers include the fact that very large numbers, up to several billions per day, can be required for raising marine fish larvae in commercial aquaculture and the nutritional quality of the rotifers must be carefully controlled by appropriate enrichment methods (Lubzens and Zmora, 2003). One of the most important parameters in the nutritional quality of rotifers is their lipid composition. The lipid content of rotifers typically varies between 9 and 28 percent of their dry weight and phospholipids and triacylglycerols are affected by the lipids provided in their diet. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are well known as essential fatty acids for the survival of marine fish larvae. The ratio of these and other fatty acids can be optimized in rotifers depending on the fish larval requirements. Rotifers cultured on yeast can be nutritionally inadequate because they lack sufficient essential fatty acids; the appropriate lipid content can be achieved by feeding the rotifers on lipid emulsions or on specific algae that provide the desired lipids, such as EPA-rich *Nannochloropsis* spp. or DHA-rich *Isochrysis* spp. (Lubzens and Zmora, 2003).

Copepods and other zooplankton

Copepods, the dominant zooplankton in marine waters, are highly diverse comprising about 2 400 genera and are the natural food source for most marine fish larvae. Free-living copepods most commonly used in aquaculture belong to three of the ten copepod orders: Calanoida, Harpacticoida and Cyclopoida (Støttrup, 2003). The interest in using copepods in aquaculture has been stimulated by efforts to expand the diversity of fish species grown in aquaculture, including ornamental species, some of which have larval stages that accept only copepod-sized prey. The calanoid copepod *Acartia tonsa* is widely cultured for research and has been used as feed for fish larvae in mariculture. Interest in harpacticoid copepods has been stimulated because some of these copepods, including *Tisbe biminiensis*, have fast population growth and high concentrations of highly unsaturated fatty acids such as EPA and DHA, compared to rotifers and *Artemia* spp. (de Lima *et al*., 2013). In general, mass production of copepods as live feed is still at the experimental stage and success has been achieved with a limited number of species. The tropical harpacticoid *Pararobertsonia* in terms of its rapid reproduction (Zaleha and Busra, 2013). Other zooplankton used in aquaculture include cladocerans such as *Daphnia* that are widely used in freshwater larviculture.

3.3.2 Production strategies and systems

Artemia

The two primary methods of *Artemia* spp. production are the harvesting of naturally occurring *Artemia* blooms in salt lakes and the intentional production of *Artemia* cysts in man-made solar saltworks or salterns. Initially, there were only two commercial sources of *Artemia* spp., the Great Salt Lake, Utah, United States of America, and the San Francisco Bay coastal saltworks, California, United States of America. Demand for *Artemia* spp. had increased by the 1970s and at the same time harvests from the Great Salt Lake had decreased, causing a shortage and concomitant price increase that stimulated harvesting of natural *Artemia* resources from other sources, including Argentina, Australia, Canada, Columbia, and France (Dhont and Van Steppen, 2003) as well as southern Siberia, Russian Federation; Kazakhstan and China (FAO, 2011).

Intensive *Artemia* cyst production in salterns was started in the 1970s in Brazil, followed by the Philippines, China and Thailand and is now widespread in East Asia and Latin America with particular success in Viet Nam. The process involved deliberate transplantation of *Artemia* cysts and is beneficial for salt production because the *Artemia* control populations of hypersaline microalgae that can interfere with the salt production process (FAO, 2011).

Rotifers

Production strategies for rotifers are largely determined by the need for a continuous supply of rotifers from live cultures. Rotifers can deteriorate rapidly in their nutritional quality as cultures age. Generally, small stock cultures are maintained separately from the main growth facility to serve as a reserve if the main cultures "crash" or fail as a result of technical errors of infection with pathogens. The systems for mass culture of rotifers have been described in detail by Lubzens and Zmora (2003). The three methods commonly used are batch culture, semi-continuous culture and continuous culture. Some batch culture is done at very high density by feeding with condensed Chlorella enriched with vitamin B12. These high-density compact systems have the advantage of enabling maintenance of different rotifer strains or growth to different sizes, for feeding to different fish species. Semi-continuous systems are generally in tank volumes ranging from 3 000–300 000 litres with low rotifer density of about 100–300 rotifers ml-1, with harvesting at the rate of about 6–7 percent of rotifer biomass per day. Continuous cultures are highly controlled and based on the chemostats used in microbial fermentations. Compact continuous culture systems of 1 000 litres can provide, for example, 1.7–3.5 billion *Brachionus rotundiformis* per day but these systems have a high initial capital cost (Lubzens and Zmora, 2003). The commercial diets for rotifers have been shown to vary considerably in quality and this variation can result in rotifers that are below the minimum requirements for feeding fish larvae (Hamre, 2016). Recently, high-density stable culture of about 20 000–30 000 rotifers ml-1 has been achieved on a commercial scale in Japan, with ultra-high density culture of 160 000 rotifers ml-1 also being successfully attained (Yoshimatsu and Hossain, 2014). Important growth parameters, depending on the species and strain of rotifer being grown and its intended use, typically include salinity, temperature, dissolved oxygen, pH, and ammonia concentration (FAO, 1996). One challenge in all rotifer production systems is to maintain the health of the rotifer cultures. Early warning on deterioration of rotifer culture health can be obtained by careful monitoring of parameters such as egg ratio, swimming velocity, ingestion rate, viscosity of the culture medium increasing with the age of the culture, enzyme activity, and direct detection of diseases that could lead to the eventual collapse of the culture (Lubzens and Zmora, 2003).

Copepods and other zooplankton

Production methods for copepods are reviewed in detail by Støttrup (2003). In many cases, the copepods that have been used to culture marine fish species have been collected from the wild, in fjords or confined waterbodies where the copepods occur naturally at high densities. Filtration devices with appropriate mesh sizes have been developed to facilitate collection. Some copepod production has occurred in enclosed areas in Norway where cod larvae have been successfully raised. Production in outdoor ponds or large tanks has been carried out in Europe and Asia for the culture of cod, grouper and turbot, using filtered seawater that contains phytoplankton from which the zooplankton grazers have been excluded. There have also been some attempts at the intensive culture of copepods. Small calanoid copepods with fast generation times in the genera *Acartia*, *Centropages*, *Eurytemora*, and *Temora* have been successfully cultured. These copepods originate in coastal waters and are tolerant of variations in salinity and temperature (Støttrup, 2003). Some harpacticoid copepods, in particular *Tigriopus japonicus*, have been successfully cultured and Støttrup, (2003) lists several advantages of using harpacticoids, including their high tolerance to a wide range of environmental conditions, ability to live on a range of inert or live diets, high reproductive capacity and short life cycles, and ability to be cultured at high densities.

3.3.3 Genetic technologies Artemia

Genetic technologies are not yet well developed for *Artemia*. Most of the genetic studies of *Artemia* have focused on the phylogeny of this genus. The mitochondrial DNA sequence is available for *A. franciscana* (Valverde *et al*., 1994). A study using Random Amplified Polymorphic DNA (RAPD) analysis confirmed the separation and homologous clustering within four known bisexual species, the American *A. franciscana* and *A. persimilis*, the Mediterranean *A. salina*, and the *Artemia* species from China (Badaracco *et al*., 1995). RAPD analysis was also used to show that 14 *Artemia* strains from the Caribbean belonged to *A. franciscana* and were divergent from *A. persimilis* (Argentina) (Camargo *et al*., 2002). Phylogenetic analysis has also been done by Amplified Fragment Length Polymorphism (AFLP) marker analysis of 15 strains of *Artemia* demonstrating that *A. tibetiana* could be differentiated from *A. sinica* (Sun *et al*., 1999). Use of AFLP analysis also showed that all bisexual European and North African *Artemia* populations are conspecific (Triantaphyllidis *et al*., 1997). Microsatellite markers were developed for characterization of two populations of *A. franciscana* and two populations of diploid parthenogenetic *Artemia* spp., demonstrating the utility of these microsatellites in studies on population genetics and tracking invasive processes in *Artemia* spp. (Muñoz *et al*., 2008). Phylogenetic analysis using DNA barcoding based on the cytochrome c oxidase subunit 1 (COI) gene revealed clear differences between *Artemia* spp. from five salt lakes on the Tibetan Plateau and other *Artemia* populations in China (Wang *et al*., 2008). One recent study moved beyond phylogenetic analysis to establish an AFLP-based genetic linkage map for *A. franciscana* and used this map to explore the sex-determining region as well as provide a genome size estimation for *A. franciscana* of 0.93 Gb (De Vos *et al*., 2013). This comparatively small genome size makes the genome sequencing of *A. franciscana* an obvious next step.

Rotifers

In a major study, the phylogeny of the *Brachionus plicatilis* species complex was investigated by analysis of CO1 and internal transcribed spacer 1 (ITS1) gene sequences from 1 273 isolates of the *B. plicatilis* complex, revealing the existence of 15 species within the complex. This study showed that some traits such as body length were related to phylogeny whereas others such as genome size were not (Mills *et al*., 2016). The genome sizes within the *B. plicatilis* species complex were found to be highly variable with a seven fold range from 55 to 407 megabases. This range of variation was unexpected and is even higher than that among distantly related rotifer species belonging to different genera. There were indications that whole genome duplications have played a role in the evolution of the *Brachionus* "Austria" lineage (Stelzer *et al*., 2011). The heterogeneity in genome size would make it difficult to select a "typical" *B. plicatilis* as the best candidate for full genome sequencing.

Copepods and other zooplankton

Genomics research on copepods has been reviewed by Bron *et al*. (2011). In 2011, there were eight mitochondrial genome sequences but no assembled genomes for copepods. Genomic resources comprised mainly expressed sequence tags for the parasitic species *Lepeophtheirus salmonis* and *Caligus rogercresseyi* (Bron *et al*., 2011).

3.3.4 Producer countries and production trends Artemia

The total global demand for *Artemia* cysts is currently 2 500–3 000 tonnes per annum and is likely to increase. Reliable production statistics are available for the Great Salt Lake, Utah, United States of America, and the Mekong Delta, Viet Nam. For the Great Salt Lake ecosystem, total weight in tonnes of raw biomass harvested varied from less than 1 000 tonnes to almost 12 000 tonnes in annual production figures from 1985 to 2009 (FAO, 2011). The unpredictable and widely varying harvest from the Great Salt Lake is largely a result of changing natural hydrological and climactic conditions. This large variation causes major fluctuations in price and availability. Production in tonnes of wet weight from the Vinh Chau and Bac Lieu districts of the Mekong Delta, Viet Nam, ranged from less than 1 tonne to more than 50 tonnes and stabilized around 15 tonnes from 2004–2009 (FAO, 2011).

The largest demand is from China with an annual consumption of 1 500 tonnes of which approximately half is domestically produced, and the other half imported from the Russian Federation and Kazakhstan (Dhont *et al*., 2013). In China, the *Artemia* can be divided into two broad groups, one in the coastal salt pans to the north of the Yangtze River, including the coastal areas at Liaodong Bay, Bohai Bay, and Laizhou Bay; the other in inland salt lakes, such as Ebi Lake in Xinjiang Uyghur Autonomous Region, Yuncheng Salt Lake in Shanxi Province, and the salt pans on the west coast of Hainan Island (Yan, 2008). China possesses extensive *Artemia* spp. sources as it has coastal salt pans with an entire area of over 7 billion square metres and over 500 inland salt lakes whose area is over 1 000 square metres individually. These unique geographic characteristics have supported the discovery and establishment of over 70 *Artemia* strains and an annual productivity of 800–1 200 tonnes (dry weight) consisting one third of the annual global productivity. Some provinces in China are constituting various regional laws or regulations to protect their local *Artemia* bioresources. Qinghai Province, which has the most abundant salt lakes in China, constituted the Interim Measure for *Artemia* Source Protection in 2003 and later on amending this to become an official measure in 2009.

A study on the diversity of *Artemia* strains in salt pans along the coast of Hebei Province revealed *A. franciscana*, and *A. sinica* both of which reproduce sexually as well as local strains that have parthenogenetic reproduction (Kexin, 2006). Another study focusing on the *Artemia* spp. Bioresources in inland salt lakes of Alxa League in Inner Mongolia discovered that the local *Artemia* strains possess oocytes of medium size diameter, red colour, and resistant to high temperature. Since they live in salt lakes in the natural massive deserts, their productivity and quality are heavily impacted by local severe weather and climate conditions (Fuyi, 2005).

Artemia production in the Russian Federation is focused on about 100 *Artemia* lakes in western Siberia with harvesting of cysts typically taking place in 20–40 of these lakes each year. The total annual harvest is 550 tonnes, with 350 tonnes in the Altai region and 200 tonnes from other Russian regions (Litvinenko *et al*., 2015).

Farmed production of *Artemia* spp. in salterns has been particularly successful in Viet Nam, starting in the Mekong Delta in the 1980s and expanding to more than 1 000 hectares of salterns in the Vinh Chau and Bac Lieu areas and resulting in around 50 tonnes per annum of high quality *Artemia* cysts for domestic use and export (FAO, 2011).

There is potential for growth of *Artemia* production in sub-Saharan Africa. *Artemia* populations are present along the coast of Kenya and *A. franciscana* occurs in eight salt works in Kenya. Artemia production in Kenya has been proposed as an important asset to the local aquaculture industry that could create thousands of employment opportunities (Ogello *et al*., 2014).

Rotifers

Because of the need for a continuous supply of rotifers from live cultures, rotifer production is a highly distributed process with large hatcheries typically producing their own rotifers. The distributed nature of rotifer production makes it difficult to obtain good figures for production levels in various countries. Rotifer mass production in three locations in Israel was reported at circa 1.2–3.0 × 10^{10} rotifers per day (Lubzens *et al*., 1997).

Copepods and other zooplankton

Information on production of copepods is limited. A few culture methods have been applied to mass culture in commercial hatcheries Støttrup (2003) gives the example of the Danish hatchery Maximus A/S that was producing half a million turbot juveniles per year based on copepods supplemented with *Artemia* spp. for later larval stages.

4. DRIVERS AFFECTING PRODUCTION OF MICROORGANISMS

The role of microorganisms in aquaculture is likely to expand, with key drivers being the growth of the aquaculture industry, economic factors in cases where use of microbes can reduce costs and the need to reduce the environmental impact of aquaculture. The environmental impact and scarcity of fish meal and fish oil as feed for aquaculture is a major driver in development of alternative, microbe-based feeds. There is great potential in exploring the about 2 400 genera of copepods to identify additional copepod species that can be readily grown in mass production systems to economically produce copepods for live feed.

Increased use of recirculating aquaculture systems for freshwater and marine species will benefit from rigorous studies on the diversity of bacteria used in their filtration systems, including establishment of defined inocula to be able to rapidly commission new recirculating systems, recommission systems after maintenance and rapidly obtain optimal filtration efficiencies.

The potential of microalgae to be grown at large scale to produce biofuel has received intensive renewed interest over the past decade. Microalgae can be grown on non-arable land using brackish water and have the potential to produce high concentrations of lipids that can be converted to biodiesel or the microalgal biomass can be converted to biocrude by hydrothermal liquefaction. There are still major challenges to produce algal biofuels economically at a scale that would have a meaningful impact on the supply of liquid fuels for transportation (Hannon *et al*., 2010).

Nevertheless, the investment in this field is providing new technologies for economically growing microalgae at large scales that may benefit aquaculture by reducing costs of microalgal production. It is also possible that if microalgal biofuels are produced at scale, there may be synergies between algal biofuels and availability of microalgae for nutrition in aquaculture.

5. CULTURE COLLECTIONS AND CONSERVATION STRATEGIES

There is a great need for maintenance of microbes that have critically important roles in the aquaculture industry to be maintained and generally available in culture collections. Because of the diversity of microbes that are used in aquaculture and the fact that some of them are difficult to preserve, this is not a straightforward issue. Good conservation strategies make it less labour- intensive and more economical to preserve strains. For each class of microbes, preservation strategies are discussed and examples of culture collections and other sources for these microbes are provided.

5.1 Microalgae and fungal-like organisms

Gressel (2013) points out that "domesticated" microalgal strains have a tendency to revert back to their "wild-type" characteristics, making it important to maintain stock cultures and to check cultures before using them as starters for new cultures. It is not always straightforward to determine appropriate conditions for long-term storage of microalgae. Cryopreservation is by far the preferable method for long-term storage because it preserves the genetic integrity of strains and is more economical. However, conditions for cryopreservation have to be established for each algal species and strain.

The Bigelow National Center for Marine Algae and Microbiota (https://ncma.bigelow.org/) maintains the largest collection of publicly available marine algal strains in the world, with very good taxonomic and geographic representation. The collection has more than 1 700 cryopreserved algal strains maintained in liquid nitrogen. Another major algal strain collection in the United States of America is UTEX, the Culture Collection of Algae at the University of Texas at Austin (https://utex.org).

Microalgal culture collections in China include the Freshwater Algae Culture Collection at the Institute of Hydrobiology (http://algae.ihb.ac.cn/english/). In Taiwan Province of China, 31 species (49 strains) of microalgae are maintained at the Tungkang Marine Laboratory (Su *et al*., 1997).

Microalgal collections in Japan include the Culture Collection of the National Institute for Environmental Studies https://www.nies.go.jp/kenkyu/yusyo/index.html (Japanese website) and the Chlorella Industry Co. Ltd. which has a large culture collection of *Chlorella* species and provides condensed *Chlorella vulgaris* products for culturing rotifers.

The Australian National Algae Culture Collection, at the Commonwealth Scientific and Industrial Research Organisation (http://www.csiro.au/en/Research/Collections/ANACC), holds living cultures of more than 1 000 strains of more than 300 microalgal species.

A useful list of microalgal culture collections in several countries can be found at http://mcc.nies.go.jp/AOACC/Facilities.html

5.2 Bacteria

A very large number of bacterial species have been proposed as probiotic agents. Also, many different microbial species including bacteria and archaea, the majority of which are still poorly characterized, are important in biological filters in recirculating aquaculture systems. Fortunately, bacteria and archaea are generally readily preserved for long periods of time using cryopreservation or lyophilization. Cryopreservation is often accomplished by addition of glycerol at 15–30 percent in the growth medium that is suitable for the bacterium, followed by freezing at -80 °C in an ultra-low temperature freezer or in liquid nitrogen. Freeze-drying or lyophilization effectively preserves many species of bacteria although not all strains can be recovered after lyophilization, so individual testing is required. A major challenge here is that many different research groups have isolates that have been described as having probiotic properties and in many cases these strains are maintained by the individual research group, which does not provide for long-term security of the strains. Most countries have national culture collections and it is very important that significant strains are deposited in the culture collections to ensure their long-term availability to the research community and the aquaculture industry. Once the necessary intellectual property protection has been obtained and strains are described in the literature, they should be deposited in culture collections. This is a requirement for patent filing. For those strains on which patents are not obtained and are described in the scientific literature, it is a requirement of some journals that strains be deposited in culture collections. For example, the instructions to authors for the American Society for Microbiology journal Applied and Environmental Microbiology (AEM) state that "AEM expects authors to deposit important strains in publicly accessible culture collections and to refer to the collections and strain numbers in the text." If this requirement was also put in place by all journals publishing work on bacterial strains of significance in aquaculture, key bacterial strains would be better secured for future research and industrial application.

The World Federation for Culture Collections (http://www.wfcc.info/index.php/collections/display/) lists 589 culture collections in 68 countries.

5.3 Zooplankton

Artemia

The highly resistant cysts of *Artemia* spp. make this organism eminently suited to easy long-term storage. INVE Aquaculture (http://www.inveaquaculture.com/) offers a wide range of *Artemia* cysts.

Rotifers

Rotifer eggs can be cryopreserved after treatment with suitable cryoprotectant agents such as dimethylsulfoxide or propane-diol (Lubzens and Zmora, 2003). Short-term storage can be achieved at 4 °C but this is for periods of weeks to months rather than for long-term storage.

In Japan, Chlorella Industry Co. Ltd. sells rotifers as culture starters (http://www.chlorella.co.jp/; Japanese website). The laboratory of Dr. Atsushi Hagiwara at the Graduate School of Fisheries and Environmental Sciences, Nagasaki University, maintains rotifer cultures of 110 strains together with some copepod and crustacean species, which are used mainly for academic research and provided only for academic purposes (http://www2.fish.nagasaki-u.ac.jp/FISH/KYOUKAN/hagiwara/custom1e.html).

Many of the microbes that are important in aquaculture reside in companies that sell products to the aquaculture industry. These companies obviously have an important interest in maintaining the stocks of these microbes. This system also has the advantage of maintaining dispersed stocks on microbes in various locations.

6. RESEARCH, EDUCATION AND TRAINING

There is clearly a need for increased research in the area of genetic resources for microorganisms of current and potential use in aquaculture. Advances in molecular technologies in microbiology, including genomics and metagenomics, are not yet being extensively exploited for the benefit of the aquaculture industry. Aquaculture companies could find it to be beneficial in the mid to long- term to invest more heavily in research in microbiology to broaden their product base and maintain competitive advantages. Countries that want to be leaders in aquaculture into the future should provide competitive funding opportunities for academic researchers working in the area of genetic resources for microorganisms of use in aquaculture.

A challenge in education and training in genetic resources for microorganisms of current and potential use in aquaculture is that this area is highly interdisciplinary, requiring skills in microbiology of a wide range of microbial species as well as in molecular approaches and knowledge of aquaculture. Aquaculture microbiology is emerging as a new area of specialization and yet there are few training programmes dedicated to this speciality. This may be an area that warrants establishment of new graduate programmes for advanced training.

FAO has an important role to play in education of professionals involved in the aquaculture industry in the importance of microbial systems. One useful step here would be to include more microbial resources in the Cultured Aquatic Species Information Programme.

7. STAKEHOLDERS AND RESOURCES

Table 2 reports a list of relevant universities and research institutes working on microorganisms of current and potential use in aquaculture. List of important companies and culture collections are also provided at Table 3 and Table 4, respectively.

TABLE 2.
Universities and research institutes for microorganisms of current and potential use in aquaculture

Country or region	Contact details	Speciality
United Kingdom	Institute of Aquaculture, University of Stirling, Stirling, FK9 4LA, United Kingdom (www.aquaculture.stir.ac.uk/people//brian-austin)	Probiotic bacteria
Belgium	Laboratory of Aquaculture and Artemia Reference Center, Faculty of Bioscience Engineering, Blok F, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium (www.aquaculture.ugent.be/index.htm)	Probiotic bacteria, *Artemia*
South Africa	Department of Molecular and Cell Biology, University of Cape Town, Private Bag Rondebosch 7701, South Africa (www.mcb.uct.ac.za/mcb/people/staff/academic/coyne)	Probiotic bacteria
Belgium	Laboratory of Aquaculture and Artemia Reference Center, Faculty of Bioscience Engineering, Blok F, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium (www.aquaculture.ugent.be/index.htm)	*Artemia*, genomics
Belgium	Laboratory of Aquaculture and Artemia Reference Center, Faculty of Bioscience Engineering, Blok F, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium (www.aquaculture.ugent.be/index.htm)	*Artemia*
Japan	Graduate School of Fisheries and Environmental Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan (www.fe.nagasaki-u.ac.jp/english/index.html)	Rotifers
United States of America	Institute of Marine and Environmental Technology, 701 East Pratt Street, Baltimore, MD 21202 USA (http://imet.usmd.edu/people/hill.html)	Microalgae, marine bacteria
Viet Nam	College of Aquaculture and Fisheries, Can Tho University, Can Tho City, Viet Nam	*Artemia*
Israel	Biology, Technion – Israel Institute of Technology, Technion City, Haifa 3200003, Israel	Rotifers
Nigeria	Modibbo Adama University of Technology, Yola, Adamawa State, Nigeria	Probiotic bacteria
China	Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, 17 Chunhui Road, Laishan District, Yantai, China	Microalgae

(cont.)

Country or region	Contact details	Speciality
Uganda	Fisheries and Aquaculture, Mountain of the Moon University, School of Agricultural Sciences, P.O. Box 837, Fort Potal, Uganda	*Artemia*
Mexico	Departamento de Sistemas Biológicos, Universidad Autónoma Metropolitana-Xochimilco, Calzada del Hueso 1100, 04960 Mexico City, Mexico	Probiotic bacteria
United States of America	Institute of Marine and Environmental Technology, 701 East Pratt Street, Baltimore MD 21202 USA (http://imet.usmd.edu/people/saito.html)	Bacteria in recirculating aquaculture systems
United States of America	Institute of Marine and Environmental Technology, 701 East Pratt Street, Baltimore MD 21202 USA (http://imet.usmd.edu/people/schott.html)	Probiotic bacteria
United States of America	Institute of Marine and Environmental Technology, 701 East Pratt Street, Baltimore MD 21202 USA	Probiotic bacteria
Belgium	Laboratory of Aquaculture and Artemia Reference Center, Faculty of Bioscience Engineering, Blok F, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium (www.aquaculture.ugent.be/index.htm)	*Artemia*
United States of America	Institute of Marine and Environmental Technology, 701 East Pratt Street, Baltimore MD 21202 USA (http://imet.usmd.edu/people/sowers.html)	Bacteria and archaea in recirculating aquaculture systems, methanogenesis
Denmark	Technical University of Denmark, Charlottenlund Slot, Jægersborg Alle 1, 2920 Charlottenlund, Denmark	Copepods
China	Institute of Marine Biodiversity and Evolution, Darwin Building, Ocean University of China, 5 Yushan Road, Qingdao 266003, China(http://web.ouc.edu.cn/iemb/c8/2f/c7958a51247/page.htm)	*Artemia*
Taiwan Province of China	Tungkang Biotechnology Research Center, Fisheries Research Institute, Council of Agriculture, Taiwan	Rotifers
Belgium	Laboratory of Aquaculture and Artemia Reference Center, Faculty of Bioscience Engineering, Blok F, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium (www.aquaculture.ugent.be/index.htm)	*Artemia*
Viet Nam	Research Institute for Oil and Oil Plants, Ministry for Industry and Trade, 171–175 Ham Nghi St., Dist. 1, Ho Chi Minh City, Viet Nam	Microalgae
United States of America	Institute of Marine and Environmental Technology, 701 East Pratt Street, Baltimore MD 21202 USA	Microalgae, rotifers

TABLE 3.

Companies working on microorganisms of current and potential use in aquaculture

Name	Country or region	Contact details	Speciality
ALGAtechnologies Ltd	Israel	Kibbutz Ketura, D.N. Hevel Eilot, 8884000, Israel (www.algatech.com)	Microalgae (*Haematococcus*)
BIOMIN	Austria	BIOMIN Holding GmbH, Erber Campus 1 3131 Getzersdorf, Austria	Probiotic bacteria
Chlorella Industry Co. Ltd	Japan	1-18-16, Hamamatsucho Minato-Ku, Tokyo, 105-0013 Japan	Microalgae
INVE Aquaculture	United States of America, with branches globally	3528 W 500 S, Salt Lake City, UT 84104 USA (www.inveaquaculture.com) (see www.inveaquaculture.com/about-inve for global branches)	*Artemia*
Great Salt Lake Brine Shrimp Cooperative	United States of America	5859 North Cottonwood Canyon Road Mountain Green, UT 84050 USA (www.gsla.us/about-us.html)	*Artemia*
Keeton Industries, Inc.	United States of America	1520 Aquatic Dr., Wellington, CO 80549 USA (http://keetonaqua.com)	Probiotics
Necton Phytobloom	Portugal	Companhia Portuguesa de Culturas Marinhas, S.A., 8700-152 Olhão, Portugal (www.necton.pt/index.asp?idioma=EN&z=1)	Microalgae
Ocean Nutrition Europe	Belgium	Rijkmakerlaan 15, 2910 Essen, Belgium (www.oceannutrition.eu/en/default.aspx)	*Artemia*
Reed Mariculture Inc.	United States of America	Reed Mariculture Inc., 900 E Hamilton Ave, Suite 100, Campbell, CA 95008 USA (http://reedmariculture.com)	Microalgae, rotifers

TABLE 4.
Culture collections of microorganisms of current and potential use in aquaculture

Name	Country or region	Contact details	Speciality
Australian National Algae Supply Service	Australia	Australian National Algae Supply Service, CSIRO Hobart, Castray Esplanade, Battery Point Tas 7004, Australia (www.csiro.au/en/Research/Collections/ANACC/Australian-National-Algae-Supply-service)	Microalgae
Culture Collection of the National Institute for Environmental Studies	Japan	National Institute for Environmental Studies 16-2 Onogawa, Tsukuba-City, Ibaraki, 305-8506 Japan (www.nies.go.jp/kenkyu/yusyo/index.html)	Microalgae
Freshwater Algae Culture Collection at the Institute of Hydrobiology	China	Institute of Hydrobiology, No. 7 Donghu South Road, Wuchang District, Wuhan, Hubei Province, China (http://algae.ihb.ac.cn/english)	Microalgae
Korea Marine Microalgae Culture Center	Republic of Korea	Sung Bum Hur, Dept. of Marine Biomaterials and Aquaculture, Pukyong National University, Busan 608-723, Korea (www.kmmcc.re.kr)	Microalgae, cyanobacteria
Microalgae Collection at National Research Institute of Aquaculture as Food Organisms for Aquaculture Animals	Japan	National Research Institute of Aquaculture, Fisheries Research Agency, Minamiise 422-1, Watarai-gun, Mie 516-0193, Japan (http://nria.fra.affrc.go.jp/bank/index.html)	Microalgae
National Center for Marine Algae and Microbiota	United States of America	Bigelow Laboratory for Ocean Sciences, 60 Bigelow Drive P.O. Box 380, East Boothbay, Maine 04544 USA (https://ncma.bigelow.org)	Microalgae, bacteria
Research Culture Collection of Institute of Biotechnology	Viet Nam	Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Viet Nam	
TISTR Algal Culture Collection	Thailand	Thailand Institute of Scientific and Technological Research, TISTR Algal Culture Collection, Bioscience Department 35 Mu 3 Techno Polis, Khlong 5, Khlong Luang, Pathum Thani 12120 Thailand (www.tistr.or.th/tistr_culture/index.php)	Microalgae, bacteria
UTEX, the Culture Collection of Algae at the University of Texas at Austin	United States of America	205 W. 24th St, Biological Labs 218, the University of Texas at Austin (A6700), Austin, TX 78712 USA (https://utex.org)	Microalgae
Tungkang Biotechnology Research Center and Bioresource Collection and Research Center	Taiwan Province of China	Fisheries Research Institute, Council of Agriculture, 199 Hou-Ih Road, Keelung, Taiwan 20246 (https://www.tfrin.gov.tw/cp.aspx?n=368&s=51)	Microalgae, rotifers, bacteria

8. FUTURE PROSPECTS

For aquaculture to continue to diversify and expand to meet the challenge of providing high-quality seafood to a growing human population predicted to reach 9.7 billion by 2050, the contributions of microbiology are essential. Fortunately, this is an exciting time for the fields of microbial ecology and microbial genomics and metagenomics. By using molecular approaches to study the diversity and phylogeny of microbes, the range of microbes that can be of use in aquaculture can be rapidly extended. Existing and newly discovered microbes of importance in aquaculture can be characterized increasingly rapidly at a genomic level.

The use of metagenomic approaches makes it feasible to characterize complex microbial communities, including those found in natural green water systems and in biological filters in recirculating aquaculture systems. By monitoring the diversity of these microbial communities and maintaining desired microbial community structures, it may be possible to increase the productivity of aquaculture systems in the future.

The significant insights that have been made into the links between human microbiomes and health may have analogous benefits in species that are important in aquaculture. Perhaps by analysing the gut microbiomes of fish species grown in aquaculture, it will soon be possible to manipulate those microbiomes to optimize feed conversion ratios or to maintain better health. The technology is certainly in place to be able to rapidly and economically determine the diversity of fish gut microbiomes.

However, it is important to keep pace with traditional techniques in microbiology. The fundamental importance of maintaining diverse culture collections of all microbes of importance to aquaculture cannot be overemphasized. It is quite possible that changing environmental conditions, including those brought about by climate change, may mean that some key microbes can no longer be isolated from the environments in which they were once found and can only be recovered from culture collections.

Close integration of microbiology and aquaculture and the curation of key microbial species that are used in the aquaculture industry as well as the expansion of the diversity of microbes used in aquaculture, are critically important for the aquaculture industry to continue to flourish and prosper and to play its part in providing healthy food for the expanding world population.

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The future success and growth of aquaculture depends, among other things, on continued availability and more efficient management of cultured microorganisms, including their conservation, and on diversification of genetic resources of microbes used in aquaculture. Microorganisms fall into the microbial groups of (1) microalgae and fungal-like organisms, (2) bacteria, including cyanobacteria and (3) zooplankton. Important issues include the ability to achieve long-term storage of important organisms without them being subject to genetic drift, the role of commercial and public culture collections, and the need for increased use of genomics to characterize all key microbial species used in aquaculture.

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