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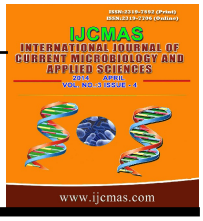

Original Research Article

Growth enhancement of micro algae, *Chaetoceros calcitrans* and *Nannochloropsis oculata*, using selected bacterial strains

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Keywords

Microalgae, Probiotics, *Bacillus*, *Pseudomonas*

A B S T R A C T

In natural systems phytoplankton interact with planktonic (free living) and attached epiphytic bacteria both synergistically and antagonistically. The specificity of the association with micro algae and bacteria differs in terms of adhesion mechanisms and metabolic cooperation. Present research was carried out to study the effect of bacterial isolates namely *Bacillus* sp. and *Pseudomonas* sp. from algal culture systems on the growth of micro algae such as *Chaetoceros calcitrans* and *Nannochloropsis oculata*. *C. calcitrans* (F= 15.34; P<0.05) and *N. oculata* (F=12.52; P<0.05) showed significantly higher growth, in treatments with *Bacillus* sp. and *Pseudomonas* sp when compared to control.

Introduction

Associations between algae and bacteria are common, and studies have generally focused on the benefits provided to the bacteria, such as support of bacterial growth by dissolved organic carbon released by algal cells (Rier and Stevenson, 2002). Certain microorganisms have been shown to provide their host algae with growth factors, nutrients, or protection. In aquaculture, micro algae are widely used as an indispensable food source in the commercial rearing of all growth stages of bivalve molluscs, larval stages of crustaceans and early growth stages of fishes (FAO, 1996; Norman et al., 2010). An extensive review of the nutritional aspects of micro-algae used in mariculture of bivalve molluscs, crustaceans, and fish is presented in Brown et al. (1989) and this micro alga is believed to play a role in stabilizing the water quality, nutrition of the larvae, and microbial control (Riquelme et al., 1997). Algae can be produced using a wide variety of methods, ranging from closely controlled laboratory methods to less predictable methods in outdoor tanks.

However, due to the nutrient enrichment for micro algal development, the possibility of developing microorganisms, like bacteria are high in various systems.

They are common inhabitants of micro algae cultures and may even contribute to the success of these cultures by leaking essential vitamins into the medium (Mason, 1963). Since many bacteria and micro algae are demonstrated to have close interactions, the bacterial population developed in the culture interacts with the micro algae. The numbers of bacteria in a micro algae culture is usually small during exponential growth and increases as algal cells die and release organic compounds to the medium. *Chaetoceros calcitrans* and *Nannochloropsis oculata* are widely used in aquaculture industries, as it is comprised of nutritional value suitable for most marine filter feeders especially for larval rearing of penaeid shrimp and bivalves (FAO, 1996; Ju et al., 2009). The present study is aimed at establishing the variation in growth of the algae when cultured in association with bacteria viz. *Bacillus* and *Pseudomonas* that are isolated from the mass culture systems of micro algae.

Materials and Methods

Pure cultures of these micro algae *C. calcitrans* and *N. oculata*, were obtained from the Central Marine Fisheries Research Institute, Cochin, India and are maintained at 25 ^{o}C with 2000 lux fluorescent light with 24 h light period, using Guillard's F/2 medium (Guillard, 1975) and axenic cultures were developed (Hoff and Snell, 1987; Gopinathan, 1996). The stock cultures of algae were maintained to a cell density approximately 3.0 x 10^8 cells ml^{-1} and transferred to the test flask to attain initial cell density of 6.0 x 10^4 cells ml^{-1}. Bacterial strains (*Bacillus* and *Pseudomonas*) used in the present study were isolated from the algal culture systems. Water from the algal culture systems were filtered through 40 µ mesh and inoculated to Zobell's marine agar plates. Most frequently occurring colonies were selected, isolated, purified and characterized following Buchanan and Gibbons (1974). *Bacillus* and *Pseudomonas* cultures thus isolated were selected for the present study because of its proven role as probiotics (Intriago and Jones, 1993; Gorospe et al., 1996).

A completely randomized experimental design was followed for assessing the effect of two bacterial strains on the growth of microalgae. Selected bacterial isolates were inoculated aseptically into Zobell's marine broth and incubated for 48 h. at 37 ^{o}C. The grown cultures were centrifuged at 3075 g to harvest the cells. The cell pellet was then re-suspended in 10 ml sterile neutral buffer and serially diluted to achieve the required cell density before inoculating the treatments. Two ml each of the diluted bacterial suspension was used to inoculate the treatments. Soon after the inoculation, initial micro algal cell density (cells ml^{-1}) and bacterial counts (cfu ml^{-1}) in control and treatments were determined by haemocytometer counts and spread plate method on nutrient agar plates respectively. Samples were drawn aseptically from the control and treatments using sterile micropipettes at designated time intervals, serially diluted and the cell numbers of micro algae and bacteria were determined. Micro algal cell density was assessed on a daily basis and bacterial count was taken in every third day. All the experiments were done in 1000 ml Erlenmeyer flask with four replicates. The mean algal density in treatments and control on 12th day of culture, when the cultures showed maximum growth, were compared using one-way ANOVA. Tukey's test was employed to detect significant differences among treatments at the 0.05 significance

level. The data are expressed as mean ± standard deviation of four observations.

Results and Discussion

Growth of *C. calcitrans* and *Bacillus* sp. when grown separately as control and in mixed cultures is given in Fig. 1. *C. calcitrans* attained first peak of growth on 8^{th} day both in control and with inoculum of *Bacillus.* Maximum cell density of *C. calcitrans* recorded in control (1.58 x 10^6 cells ml^{-1} on 8^{th} day) was much lower than those achieved in the treatment with *Bacillus* (2.19 x 10^6 cells ml^{-1} on 11^{th} day). *C. calcitrans* attained a maximum cell density of 2.09 x 10^6 cells ml^{-1} in 9^{th} day and the peak extended up to 12^{th} day when cultured with *Pseudomonas* sp (Fig. 2). The growth of *C. calcitrans* is found to be significantly high in treatments with *Bacillus* and *Pseudomonas* ($F= 15.34$; $P<0.05$) when compared to control.

Growth pattern of *N. oculata*, when cultured with *Bacillus* sp. is given in Fig 3. *N. occulata* attained a cell density of 6.0 x 10^6 cells ml^{-1} in control on 8^{th} day and stationary phase prolonged for two days. Whereas, *N. occulata* grown with *Bacillus* and *Pseudomonas* attained peak cell density on 13^{th} and 11^{th} day with 1.34 x 10^7 cells ml^{-1} and 1.28 x 10^7 cells ml^{-1} respectively (Fig. 3 and 4). The cell density of *N. oculata* was significantly higher in both the treatment groups, though the peak was achieved bit later than in the control. Significantly higher growth of *N. oculata*, when compared to control could be observed in treatments with *Bacillus* and *Pseudomonas* ($F=12.52$; $P<0.05$).

In control, both *Bacillus* sp. and *Pseudomonas* sp. attained maximum growth by 6^{th} and 9^{th} day respectively and showed a meager decline in the population still 12^{th} day. Stationary phase of the micro algae, *N. occulata* was found to be less prominent when grown with bacteria as we observed by a sudden decline in biomass after reaching the peak. However in *C. calcitrans* the stationary phase prolongs upto 4 days when grown with bacteria (2.00 x 10^6 cells ml^{-1}, 8^{th} to 12^{th} day) and retain its cell density of 1.50 x 10^6 cells ml^{-1} up to the end of the experiment (14^{th} day).

In nature the bacteria and algae probably co-exit under most conditions, as they may be shown to do in continuous culture where only slight inverse fluctuations between algal and bacterial biomasses can be observed (Daft et al., 1975). Bacteria may stimulate algal growth in various ways. These range from the very specific effects caused by the production of extracellular essential growth factors to more general effects such as those in which the bacterial flora may alter the partial pressure of CO_2 and partial pressure of O_2 and in this way affect the algal photosynthesis, photorespiration and ultimately growth (Tolbert, 1974). The relationship between algae and heterotrophic bacteria develops primarily when bacteria assimilate dissolved organic matter (DOM) that is generated from algae via processes including rupture and degradation of cells during grazing, viral lysis, direct extracellular release of dissolved photosynthate, and algal production of exopolymeric substances (Krembs et al., 2002). Co-variation between algae and bacteria is often thought to reflect the reliance of bacteria on algae for their organic carbon requirements (Gasol and Duarte, 2000) given that up to 50% of algal primary production is released as DOC and algal-released DOC may support up to 95% of

Figure.1 Growth of micro algae, Chaetoceros calcitrans and bacteria, Bacillus sp. in control and mixed culture

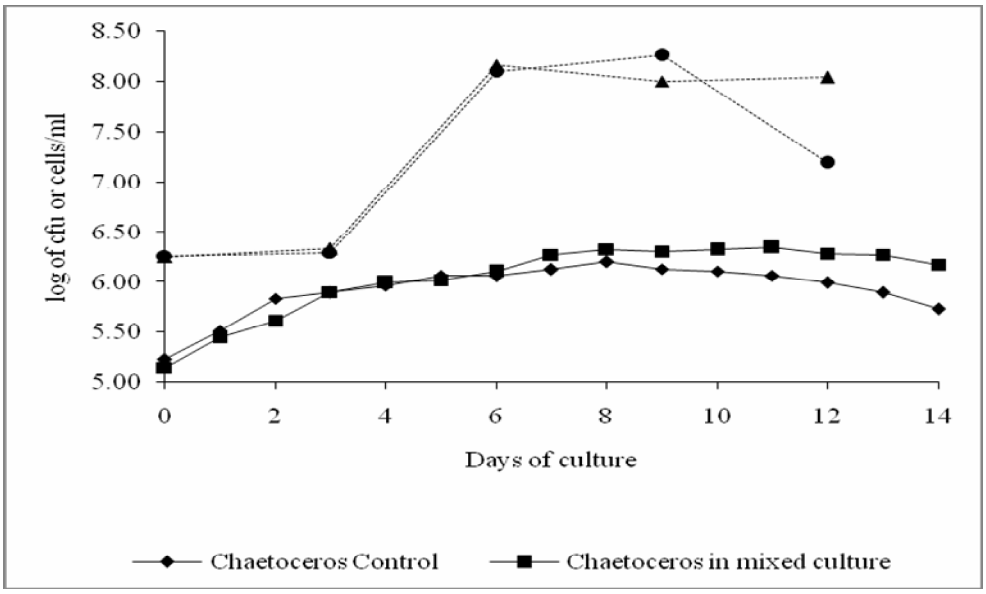


Figure.2 Growth of micro algae, *Chaetoceros calcitrans* and bacteria Pseudomonas sp. in control and mixed culture

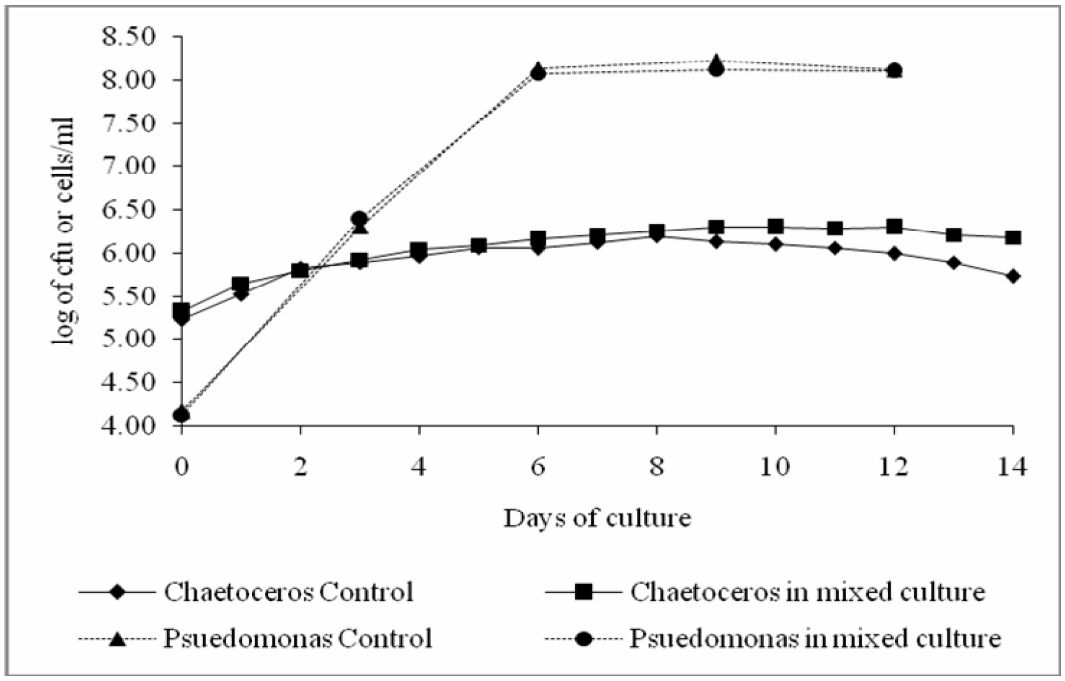


Figure.3 Growth of micro algae, *Nannochloropsis occulata* and bacteria, *Bacillus* sp. in control and mixed culture

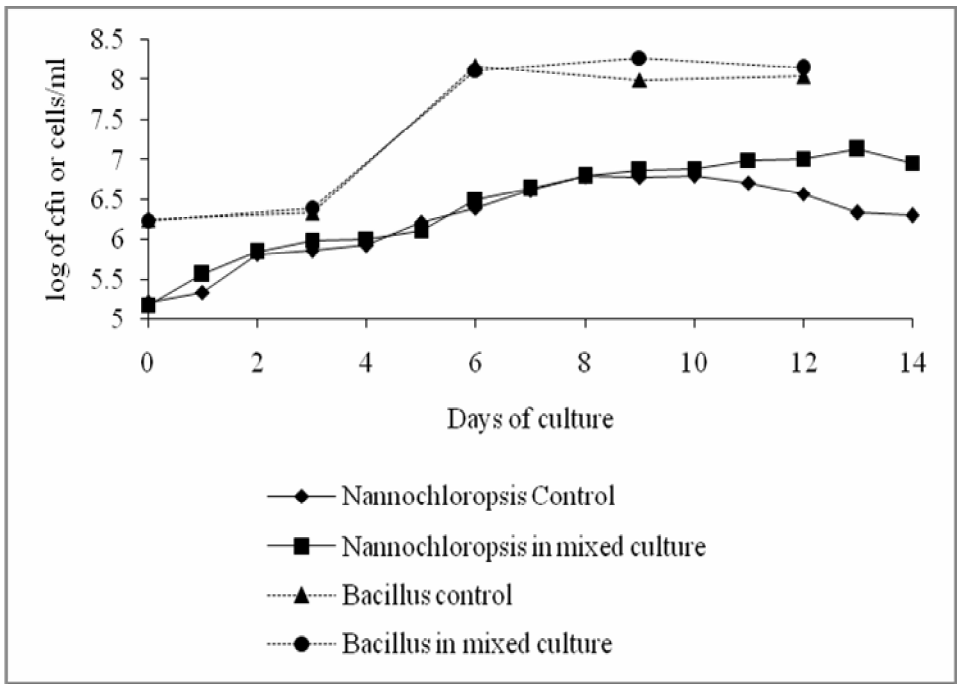
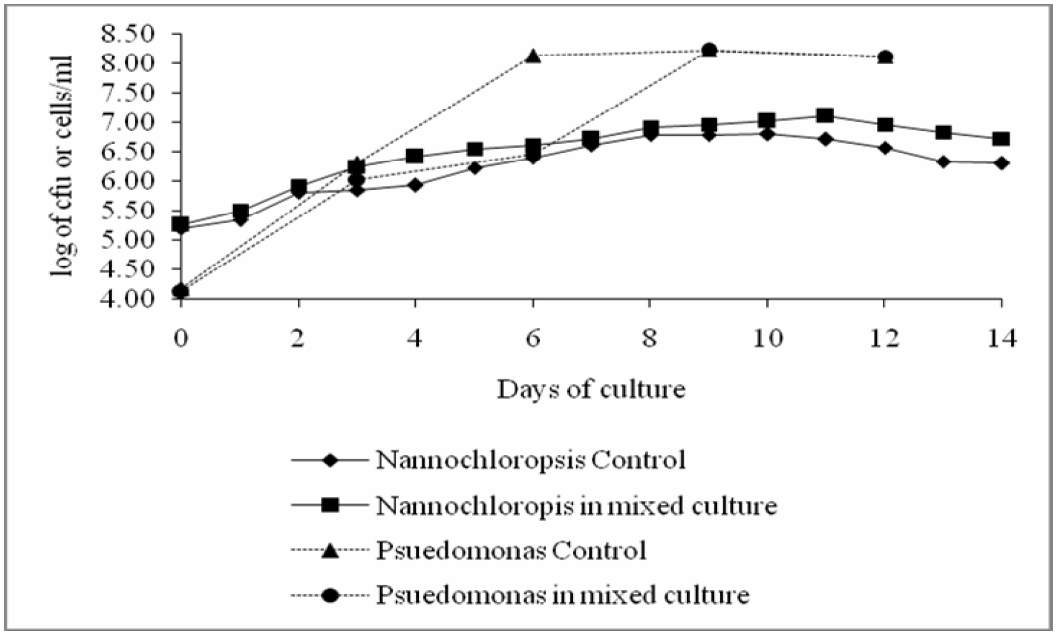


Fig.4 Growth of micro algae, *Nannochloropsis occulata* and bacteria, *Pseudomonas* sp. in control and mixed culture.



bacterial production (Lyche et al., 1996). Algal-bacterial co-variation could also stem from similar responses of both groups of organisms to common regulating factors such as the supply of inorganic nutrients (Coveney and Wetzel, 1995; Rier and Stevenson, 2001).

The results of the present experiment revealed that the addition of bacteria in algal culture media, in the case of two micro algae such as *C. calcitrans* and *N. oculata* showed marked increase in cell density when compared to the control. Mouget (1995) reported that algal growth enhancement by bacteria is mainly by consumption of photosynthetic oxygen. Bacteria, such as *Pseudomonas diminuta* and *P. vesicularis* are found to stimulate the growth of algae. Microbiologically pure cultures of green and blue green algae consumed amino acid from growth medium without any protein degradation. The test microorganisms such as *Pseudomonas* sp., and *Bacillus* sp. were found to exhibit good amylolytic, proteolytic and lipolytic activity (results not shown). Substantial proteolytic activity was detected in bacterial, mixed algal and bacterial culture and in natural reservoirs.

It was reported that bacterial activities in terms of exoenzymatic rates and secondary production were two folds higher in the water within macroalgal beds, than in the open water. These preliminary results suggest that high macroalgal biomass represents a 'hot spot' of bacterial density (Sadchikov and Marakov, 2000) and activity that may affect microbiological quality of water (Bartoli et al., 2005). However there are report between bacteria and algae for inorganic nutrients and potentially inhibitory compound to bacterial growth (Fisher et al., 1998), the present study did not report any bacterial inhibition in mixed culture. Hasanniya (2002) reported a positive effect of *Pseudomonas fluorescence* bacteria on the growth rates of *Chaetoceros sp, Skeletonema sp, Tetraselmis sp,* and *Chlorella sp.* Our experiments revealed that there was significant improvement in the number of algal cells when cultured in association with *Bacillus* sp. and *Pseudomonas* sp. The increase in the relative concentration of micro algae contributed towards improving the global efficiency of the system. The antibacterial activity achieved by the enhanced growth of micro algae (O'Farrill et al., 2003) against shrimp pathogens such as luminous vibrios could be an added advantage in developing such systems. A survey conducted by Martin et al. (2005) revealed that out of 326 algal species, 171 species require exogenous vitamin B_{12} for growth, implying that more than half of the algal kingdom are cobalamin auxotrophs and algae acquire vitamin B_{12} through a symbiotic relationship with bacteria and Yu et al. (1988) reported some *Pseudomonas* synthesize vitamin B_{12}, which could be used by the algae for their growth.

Pearl (1992) reported the interaction between bacteria and blue green algal cells involves a complex interchange of materials, which are important, both for the establishment (chemotaxis) and continued nutrition of both organisms. A bacterial consortium which normally act in natural environment for the break down and release of the nutrients from dead algae may also play a role in nutrient recycling and subsequent growth of promotion of the micro algae. Similarly, the test microorganisms being heterotrophic with good exoenzyme potential could help in the release of locked up nutrients and thereby enhancing the growth of micro algae.

The extra cellular proteolytic activity of *Bacillus subtilis* cultivated together with the algae increased by 1.5 to 2 times as compared to monoculture, suggesting a stimulating effect of the autotrophs on the proteolytic activity of bacteria. The stimulating effect is believed to be exerted by polysaccharide naturally excreted by the algae (Sadchikov and Marakov, 2000). In the present study it is concluded that *Pseudomonas* and *Bacillus* have a positive interaction in culture of *Chaetoceros* and *Nannochloropsis* and further study is needed to know the actual symbiotic relationship between these micro algae and bacteria.

Acknowledgement

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References

Bartoli, M., Nizzoli, D., Fanciulli, G., Viaroli, P., Fabiano, M., 2005. Relationships between macroalgal biomass and microbiological quality of water in a phytotreatment pond. Hydrobiologia 550(1), 211-219.

Brown, M.R., Jeffrey, S.W., Garland, C.D., 1989. Nutritional aspects of microalgae used in mariculture; a literature review. CSIRO Marine Laboratories Report 205, pp 44.

Buchanan, R.E., Gibbon, N.E., 1974. Bergeys Manuals of Determinative Bacteriology, eight ed. The Williams and Wilkins Co, Baltimore, pp.

Coveney, M. F., Wetzel, R. G., 1995. Biomass, production, and specific growth rate of bacterioplankton and coupling to phytoplankton in an oligotrophic lake. Limnol. Oceanogr. 40, 1187–1200.

Daft, M.J., Mccord, S., Stewart, W.D.P., 1975. Ecological studies on algal lysing bacteria in fresh waters. Freshwater Biol. 5, 577-596.

FAO, 1996. Manual on the Production and Use of Live Food for Aquaculture. Patrick Lavens and Patrick Sorgeloos (Eds), FAO Fisheries technical paper 361.

Fisher, M., Wilcox, L.W., Graham, L.E., 1998. Molecular characterization of epiphytic bacterial communities on charophycean green algae. Applied Environ. Microbiol. 64(11), 4384-4389.

Gasol, J.M., Duarte, C.M., 2000. Comparative analyses in aquatic microbial ecology: how far do they go? FEMS Microbiol. Ecol. 31, 99-106.

Gopinathan, C.P., 1996. Live feed culture micro algae. Bulletin of the Central Marine Fisheries Research Institute, Cochin 48, 110-116.

Gorospe, J.N., Nakamura, K., Abe, M., Higashi, S., 1996. Nutritional Contribution of *Pseudomonas* sp. in Artemia Culture. Fisheries Sci. 62 (6), 914-918.

Guillard, R.R.L., 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith, W.L. Chanley, M.H. (Eds.), Culture of Marine Invertebrate Animals. Plenum Press, New York, USA, pp 26-60.

Hasanniya, M.R., 2002. The role of *Pseudomonas fluorescence* Bacteria in developing of algal culture. Indian J. Fish. Sci. 2, 1-16.

Hoff, F. H., Snell, T.W., 1987. Plankton culture manual, first ed. Florida Aqua farms, Inc., Florida USA, pp 126.

Intriago, P., Jones, D.A., 1993. Bacteria as food for Artemia. Aquaculture 113, 115-127.

Ju, Z.Y., Forster, I.P., Dominy, W.G. 2005. Effects of supplementing two

species of marine algae or their fractions to a formulated diet on growth, survival and composition of shrimp (*Litopenaeus vannamei*). Aquaculture 292, 237-243.

Krembs, C., Eicken, H., Junge, K., Deming, J.W., 2002. High concentrations of exopolymeric substances in Arctic winter sea ice: implications for the polar ocean carbon cycle and cryoprotection of diatoms. DeepSea Res. I(49), 2163-2181.

Lyche, A., Andersen, T., Christoffersen, K., Hessen, D. O., Hansen, P. H. B., Klysner, A., (1996). Mesocosm tracer studies 2. The fate of primary production and the role of consumers in the pelagic carbon cycle of a mesotrophic lake. Limnol. Oceanogr. 41, 475-487.

Martin, T., Andrew, D.L., Evelyne, R., Martin, J.W., Alison, G.S., 2005. Algae acquire vitamin B_{12} through a symbiotic relationship with bacteria. Nature 438, 90-93.

Mason, D., 1963. The growth response of *Artemia salina* (L) to various feeding regimes. Crustaceana 5, 138-150.

Mouget, J.L., Dakhma, A., Lavoie, M.C., and De La Noue, J., 1995. Algal growth enhancement by bacteria: Is consumption of photosynthetic oxygen involved? FEMS Microbiol. Ecol. 18(1), 35- 44.

Norman L.C.R., Nick, K., Ellie, W., Jonathan, M., 2010. Optimising the delivery of the key dietary diatom *Chaetoceros calcitrans* to intensively cultured Greenshell mussel larvae, *Perna canaliculus*. Aquaculture in Press. *doi:10.1016/ j.aquaculture. 2010.05.010*.

O'Farrill, N.E., Travieso, L., Benitez, F., Becares, E., Romo, S., Borja, R., Weiland, P., Sanchez, E., 2003. Population dynamic of algae and bacteria in an oxidation channel. J. Environ. Sci. Health 38(4), 697-709.

Pearl, H.W., 1992. Epi and endo biotic interactions of cyanobacteria in algae and symbiosis. Plant, animals, fungi, viruses, interaction explored. J. General Microbiol. 43, 23-45.

Rier, S., Stevenson, R.J., 2001. Relation of environmental factors to density of epilithic lotic bacteria in 2 ecoregions. J. N. Amer. Benthol. Soc. 20, 520-532.

Rier, S.T., Stevenson, R.J., 2002. Effects of light, dissolved organic carbon and inorganic nutrients on the relationship between algae and heterotrophic bacteria in stream periphyton. Hydrobiologia 489, 179-184.

Riquelme, C., Araya, D., Vergara, N., Rojas, A., Guaita, M., Candia, M., 1997. Potential probiotic strains in the culture of the Chilean scallop *Argopecten purpuratus* Lamarck 1819. Aquaculture 154, 17-26.

Sadchikov, A.P., Marakov, A.A., 2000. Consumption and transformation of low-molecular dissolved organic matter by phyto- and bacterioplankton in two water bodies of different trophic status**.** Water Resources 27(1), 64-66

Tolbert, N.E., 1974. Photorespiration. In: Algal Physiology and biochemistry, ed. Stewart, W.D.P. oxford: Black well scientific publication.

Yu, J.P., Hino, A., Hiirano, R., Hirayamma, K., 1988. Vitamin B_{12} - producing bacteria a nutritive complement for a culture of the rotifer *Brachionus plicatilis.* Nippon Suisan Gakkaishi 54(11), 1873-1880.