Available online at www.sciencedirect.com
ScienceDirect
Aquacultural Engineering 36 (2007) 97–104
aquacultural engineering
www.elsevier.com/locate/aqua-online

Production of a diatom-bacteria biofilm in a photobioreactor for aquaculture applications

Ruben E. Avendaño-Herrera *, Carlos E. Riquelme

Laboratorio de Ecología Microbiana, Departamento de Acuicultura, Universidad de Antofagasta, Casilla 170, Antofagasta, Chile

Received 18 April 2006; accepted 3 August 2006

Abstract

Navicula veneta biofilm improves the settling of marine larval shellfish. We attempted to optimise the production of *N. veneta* biofilm by the addition of native bacteria. As a first step, the ability of six bacteria to grow in extracellular products of *N. veneta* was evaluated, the best growing strain was NC1 (*Halomonas* sp.). Subsequently, three culture cycles in the Tanaka photobioreactor confirmed that the diatom gave highest production values when cultured with this bacterium, with cell densities of 1.3–2.4 $\times 10^6$ cells ml^{-1}; without NC1 the cell production was about 65% less. Comparing microalgal growth, chlorophyll *a* concentration, and bacterial load showed a positive statistical correlation, verifying that these three variables increased simultaneously. The results suggested the feasibility of using the NC1 strain as a promoter of growth of *N. veneta* and the potential use of the Tanaka photobioreactor for the mass production of mixed biofilms consisting of diatoms and bacteria, which could be use in settlement of mass cultures of marine invertebrates of commercial importance and/or improving of food in aquaculture.

Keywords: Biofilms; *Navicula veneta*; Photobioreactor; Diatom-bacteria; Aquaculture

1. Introduction

In recent years the development and use of bioreactors for the mass culture of microalgae has increased, due to the limitations encountered in the use of open systems (Rusch and Malone, 1993; Molina et al., 1994). This is mainly due to the entry of contaminant microorganisms, often converting cultures into sources of unwanted infections (Becker, 1994), as well as satisfying the needs of aquaculture enterprise to shorten the time and lower the costs involved in mass production of microalgal foods.

Information on closed systems for mass production of benthic diatoms is scarce (Lebeau and Robert, 2003), since most related studies have been directed towards improvement of the production of small sized, planktonic microalgae useful for feeding invertebrate larvae (James and Al-Khans, 1990; Smith et al., 1993; Becker, 1994). The benthic diatoms are also of interest, however, as they not only serve as food for the more advanced stages of larval marine invertebrates (Becker, 1994; Takami et al., 1997), but also, with bacteria and other microorganisms, form attractive settlement sites for the fixation of advanced larvae in the process of metamorphosis. Here, numerous studies have been carried out in determining characteristics of substrata which make them optimal for settlement, and the effects of diverse biofilms which control the steps in larval settlement (Pearce and Bourget, 1996; Harvey et al.,

* Corresponding author. Tel.: +56 55637447; fax: +56 55637804.
E-mail address: reavendano@yahoo.com (R.E. Avendaño-Herrera).

0144-8609/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaeng.2006.08.001

1997; Daume et al., 1999; Avendaño-Herrera et al., 2003). In the natural environment, the development of a biofilm formed of diatoms and other microorganisms is preceded by primary colonization by bacteria (Allison and Gilberg, 1992) aided by the production of extracellular polysaccharides which act as ''glue'' and function at cellular and intercellular levels to establish strong, irreversible bonds with given substrata (Wetherbee et al., 1998). This succession of microorganisms often precedes subsequent stages of succession on a substratum, on which the macroorganisms eventually become dominant (Crisp, 1974; Wahl, 1989).

Diverse ecological relations between various bacteria and diatom species can include competition, commensalism, parasitism and other microbiologically important processes (Salvensen et al., 2000). The microalgae can promote and/or inhibit bacterial growth by the production of organic exudates or toxic metabolites. Conversely, the bacteria can have stimulatory or inhibitory effects on the microalgae through the production or lack of nutrients and/or of stimulatory or inhibitory growth substances affecting the microalgae (Riquelme and Ishida, 1988; Munro et al., 1995; Avendaño and Riquelme, 1999).

On the other hand, recently we investigated the feasibility of improving shellfish post-larval settlement using substrata pre-treated with biofilms of native diatoms or bacterial cells, finding that the addition of selected microorganisms increased spatfall and produced larger settlement (Avendaño-Herrera et al., 2002, 2003). Therefore, the purpose of the present study was to optimize the production of biofilms formed by the diatom *Navicula veneta* and bacteria by using an innovative type of microalgal culture system, in order to utilize the findings in future development and/or improving of food in aquaculture.

2. Materials and methods

2.1. Culture of the diatom

The diatom *N. veneta* (Kutzing) used in this study was isolated from the surface of ''netlon''® polystyrene substratum upon which had occurred a dense settlement of larval *Argopecten purpuratus* (Lamarck 1819) under hatchery conditions at Caldera, Chile (27°03′24″S–70°51′30″W) by Avendaño-Herrera et al. (2003). This diatom was cultured in 100 ml Erlenmeyer flasks in F/2 culture medium (Fritz Aquaculture Inc.,) with added sodium metasilicate (F/2M, Guillard and Ryther, 1962) in a culture chamber at a constant temperature of 20 ± 1 °C and 12:12 h (light:dark) photoperiod. An axenic culture was obtained with the diatom, eliminating the accompanying bacterial flora by exposure to a wide range of anti-microbial drugs (Hoshaw and Rosowski, 1979).

2.2. Selection of the bacterial strain

A number of bacterial strains, which had been shown to be effective in inducing scallop larval settlement, were isolated from naturally microfouled ''netlon''® scallop collectors (>2500 postlarvae per collector), using the methodology given by Avendaño-Herrera et al. (2002). Seventy surface portions of microfouled netlon were cut into 100 cm^2 pieces, washed thoroughly several times with marine salt solution (SSM, Austin, 1988) to remove any weakly associated bacteria, and distributed in Schott bottles with 50 ml of sterile seawater. The netlon pieces were then sonicated for 60 s in a ultrasonic homogenizer (Cole Parmer Corp. model #4710) to dislodge firmly attached bacteria. Dilutions of the material obtained from the scallop collector surfaces were seeded onto Tryptone Soya Agar (Oxoid Ltd., Basingstoke, Hampshire, England) supplemented with 2% NaCl (TSA2) and Thiosulphate–Citrate–Bile-Salt–Sucrose Agar (TCBS, Oxoid) and incubated at 20 °C for 1 week.

After incubation, predominant morphotypes were isolated from the cultures by dominance, phenotype, and morphology (Avendaño-Herrera et al., 2001). The strains were Gram stained for examination by light microscopy, and identified to genus level using miniaturized test systems (Hansen and Sorheim, 1991). Six dominant bacterial strains were chosen for further study for the production of the biofilm, and for the purposes of the present study these were termed NC1, NC2, NV1, A, B and C.

2.3. Assays for bacterial growth using extracellular products of the diatom

Extracellular products were obtained from axenic cultures of *N. veneta* from pre-stationary phase cultures by separating the cells from the culture medium on Whatman GF/F filters; this fluid was then filtered twice through Millipore GS (0.22 μm) membrane filters to remove organic contaminants and possible bacterial cells. Each of the six selected bacterial strains were cultivated overnight in Zobell 2216 Marine Broth (Difco Laboratories, Madrid, Spain) and then centrifuged at 10,000 rpm for 15 min. The pellet was washed twice with sterile seawater and then resuspended in SSM. A volume of this suspension was then inoculated