IMBC 2005 CIBM

CIA INTERNACIONAL DE
IA MARINA CANADA 2005

BIOPROCESOS MARINOS

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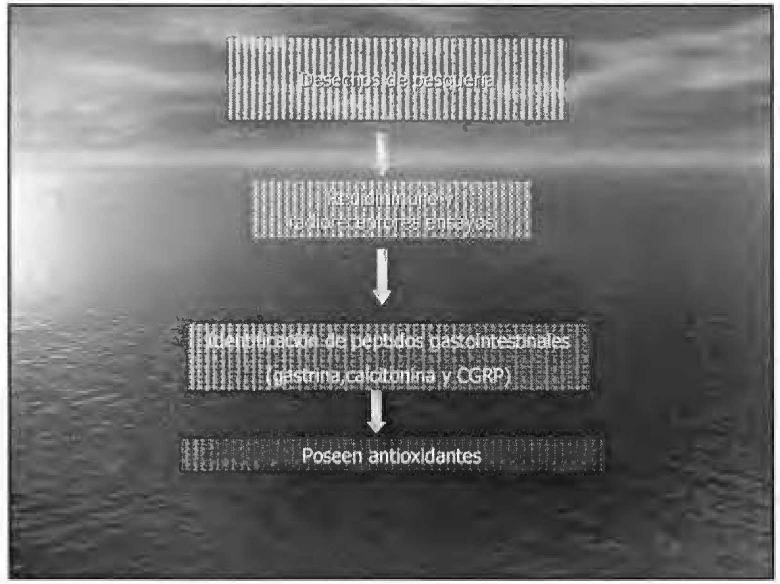
- **Bioprocesos o subproductos**: Es la utilización de los desechos marinos o de acuicultura para su aplicación en procesos industriales, mediante compuestos biológicos que intervienen en los procesos de fabricación

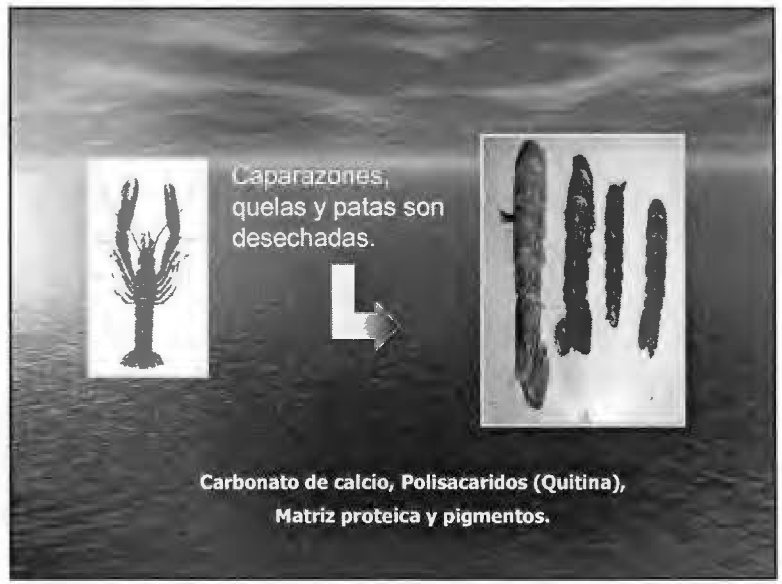
- Sub productos
  - Vísceras
  - Cabezas
  - Cortes
  - Huesos
  - Piel
  - Caparazón

Hoy en dia sub productos son todos aquella materia prima que deriva de los desechos de la pesquería y acuicultura.


PROCESAMIENTO DE DESECHOS MARINOS: PRODUCCIÓN DE HYDROLIZADOS ESPECÍFICOS
Université deLa Rochelle, Francia


Desechos de pesquería
Identificación de peptidos gastointestinales (gastrina,calcitonina y CGRP)
Poseen antioxidantes


BIOPROCESOS DE CAPARAZÓN CRUSTACEOS MARINOS
Universiy of Belfast, UK


Caparazones, quelas y patas son desechadas.
Carbonato de calcio, Polisacaridos (Quitina), Matriz proteica y pigmentos.

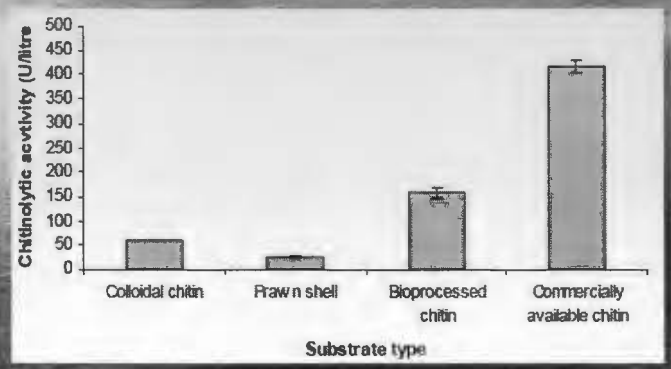
Caparazones de *Nephrops* sp.

Fermentación in situ
producción acido láctico
por bacterias

Pigmentos carotenoides ← Caldo → Licor rico en proteínas

Caldo ↓ Quitina

Efecto de diferentes sustratos sobre la actividad enzimática quitina



Usos

- Utilidad industrias farmacéuticas
- Alimento
- Cosméticas
- Derivado Quitosan

Actividad antibacterial de quitooligosacaridos producido por hidrólisis enzimáticas usando quitisanasa de origen Indonés

Yonsei University, Seoul, Korea

- Quitosan tiene un amplio rango de aplicaciones tanto medicinales, como en agricultura y aplicaciones industriales.
- La hidrólisis enzimática del quitosan es utilizado para la producción quitooligosacaridos .
- Quitosanasas → catalizadores

reduce la fracción acetilada

4 cepas *Bacilus* sp.

↓

Purificación y caracterización del quitosanasa

↓

La estabilidad enzimática

se produjo a 60º C y pH 5 - 9.

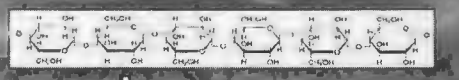
↓

Pseudomonas aeruginosa, Bacillus cereus, Staphylococus aureus y *Escherichia coli.*

DEGRADACION DE CELULOSA POR CELULASAS DE ABALON

Hokkaido University, Hokkaido, Japan

Haliotis discus hannai



La celulosa es una enzima empleada en varios procesos que involucra la degradación de materiales celulósicos

Protoplastos en plantas

Biomasa de celulasa de sacarificación

Fluido digestivo de *H. discus hannai*

HdEG66 **HdEG54**

?

Existe diferencia estructural y funcional en la

degradación de productos alimenticios

⬇

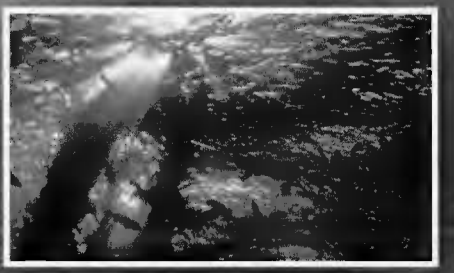
Secuenciación de aa

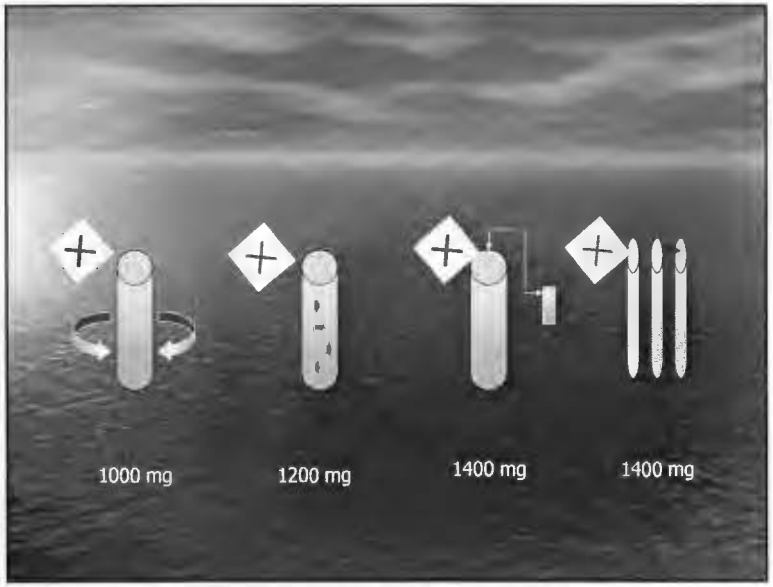
- **HdEG54** con **HdEG66** tienen una común configuración química en su constitución catalítica.
- Pero, HdEG54 no posee un hidrato de carbono en el N terminal. (isoenzima).
- No hay diferencia significativa en su actividad específica, T°, pH cuando es utlizado CBC como sustrato.

- Sin embargo, **HdEG66** actúa sobre la fracción cristalina de la celulosa, degradándola en fragmentos mas pequeños.

Comparación de biorreactores para cultivo de gametofitos de kelp transgenico

Instituto Oceanológico de China




1000 mg
1200 mg
1400 mg
1400 mg


Tomás Mella
Directora: Pamela Chávez
Alejandra Muñoz
Carlos Hidalgo
Lorena Rojas
Angela Cavour
Eduardo Álvarez
Cristián Nuñez
Andrea Contreras

Bulking and Foaming control in water treatment plant of Antofagasta, Chile.

Contreras Andrea [1], Signorelli Janetti [1], Alvarez Eduardo [1], Perez Danitza [2] and Chavez-Crooker[1] Pamela. 2005.

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2. Empresa Cascal S.A., Antofagasta, Chile.

ABSTRACT

Permanent problems of bulking and foaming during last years in the water treatment plant of Antofagasta city lead us to conduct research on causes and control of activated sludge.

A microbiological and operational monitoring was carried out during one year. In addition, screening for extracellular enzymes producing bacteria was carried out. A total of 13 strains showed high extracellular activity for proteases, amylases, lipases and celulases were isolated and grew under control conditions.

A new active sludge was produced "in situ" with daily addition of these strains. The effect of these strains on biodiversity, biologic stabilization, filaments bacterial growth and plant operation was analyzed.

Bacteria addition or Bioaugmentation showed a regulatory effect on filamentous bacteria growth, therefore bulking and foaming was controlled. Bioaugmentation also affected sludge biodiversity and it did produce a faster biological stabilization of the sludge.

This study allowed a good performance of the treatment changing from 40% to 100% of operation capability.

INTRODUCTION

The activated sludge process is the most widely used technology for biological waste water treatment. The diversity of the biological community is very large and composed of many species of viruses, bacteria, protozoa, fungi , metazoan and algae. In this complex ecosystem bacteria are about 95% of the total microbial population and they play an important buy role since they kept the activated sludge process under certain environmental conditions removing the organic material and nutrients from waste-water (Martins et al. 2004). The protozoa and other life forms may constitute approximately 5% of the estimated sludge biomass and are represented by about 2,000 species (Curd 1973, 1975). These organisms perform several important functions in activated sludge: the most important is the removal of non flocculated and loosely flocculated bacteria from waste water to produce a clarified effluent. In addition these organisms indicate changes in the performance of specific activated-sludge plants (Al-Shahwani and Horan 1991, Esteban et al. 1991). Another group of microorganism present in activated sludge are filamentous bacteria which over proliferation produce main biological problems in activated sludge sewage treatment plants: bulking and foaming. However filamentous microorganism are an essential part of flock population in the process because they form a back-bone which flock-forming bacteria adhere and form flocks with optimal parameters (size distribution, structure and density) to permit the solid-liquid separation in the clarified tank.

This study was conducted in an activated sludge plant of Cascal S.A. in the Northeast of Antofagasta in Chile (See Figure 1). The specific objectives were:

- To incorporate biological control in the water treatment plants.
- Isolation of bacteria with high degrading properties from the activated sludge, and evaluate the effect of the inoculation of this bacteria (Bioaugmentation) for bulking and foaming control.

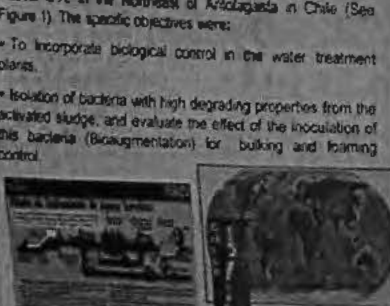


Figure 1.- Diagram of waste water treatment plant Cascal S.A. Chile

MATERIALS AND METHODS

Sample collection

Activated sludge samples were taken from the aeration basin of the Cascal S.A's waste-water plant and collected in plastic bottles. Bottles was one third filled with sludge to maintain aerobic conditions in the sludge as long as possible during transportation. The samples were stored at 4°C during transportation from the plant to the laboratory and analyzed after 1-2 hours.

Examination and filament identification

Microscopic observation of filament microorganism and protozoa counting were carried out under phase contrast and optic microscope at 400x and 1000x magnifications. The filamentous microorganism and protozoa were identified according to Eikelboom, 2000 and Jenkins et al. 2003. All samples were made in duplicate for microorganism density and identification of protozoa and filament microorganisms.

Sludge biotic index (SBI)

The sludge biotic index (SBI) was calculated to estimate biological quality of sludge in aeration tank of activated-sludge plant. Also to estimate diversity of microorganism using the criteria suggested by Madoni, 1994.

Filamentous microorganism index (FI)

Filamentous microorganism abundance and dominance were estimated using the criteria suggested by Jenkins et al. 2003. Filamentous organism were for overall abundance on a scale from 0 (none) to 6 (excessive). Individual filamentous microorganism are considered dominant if they are scored 4 (very common) or greater.

Screening of high extracellular activity for bacteria (HEAB)

Activated sludge sample were diluted with sterile water in 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}. 1 ml from every dilution were inoculate in a solid plated count media at room temperature. After 48h incubation plates with colonies (10^{-2}) was replicated in plates with specific substrate (milk cornstarch, yolk and egg). This replication technique was performed according Kelderberg and Liderberg (1952) method. Bacteria with degrading capacity were assayed for different degradation tests. Positive colonies were those with an halo around the colony.

Cocktail (mix) inoculation of degrading bacteria

2 lt of nutrient both were incubated with a cocktail of degrading bacteria at room temperature for 48h after that these media were inoculated in the aeration basin of the Bayesa S.A's plant. Additionally, a pellet of 200g of bacteria was prepared and inoculated to the aeration basin of the plant. Pellets were obtained by using a Beckman coulter Avanti J-25I. samples were centrifuge at 10,000 rpm for 15 minute at 4°C. Glycerol was include for freezing at −20°C.

Operational parameters

Operational parameters of the plant were made according to Standards Methods, 1995. Evaluated parameters were: Total solids dried (MLSS) (2540B), DBO_5 Test (5219B), Settled sludge volume Test (2710C), Sludge volume index (SVI) (2710D) and F/M Ratio (food/microorganism).

REFERENCES

Al-Shahwani S. and Horan N. 1991. [illegible]

Curds C. 1973. [illegible]

Curds C. 1975. [illegible]

Eikelboom D. 2000. [illegible]

Esteban G., Tellez C. and Bautista L. 1991. [illegible]

Jenkins D., Richard M. and Daigger G. 2003. [illegible]

Madoni P. 1994. [illegible]

Martins A. [illegible] 2004. [illegible]

RESULTS

In this investigation we isolated 13 strain showing high degrading activity for proteins, starch, celulases and lipids.

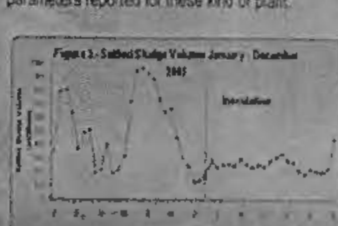
Figure 2.- Different media showing isolated bacteria

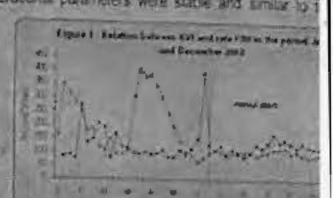
A.- Strain with high lipid extracelullar activity

B.- Strain with high extracelullar activity for starch

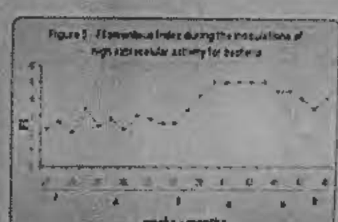
C.- Strain with high proteolytic extracelullar activity

Figures 3 and 4 show the effect of direct inoculation of the isolated bacteria on operational parameters (Settled Sludge Volume Test, SVI, F/M ratio), of the activated sludge from the wastewater plant. Before inoculation parameter were irregular, during inoculation period operational parameters were stable and similar to the parameters reported for these kind of plant.

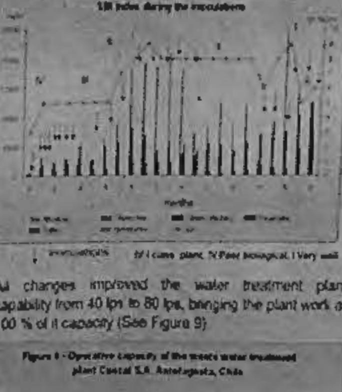




The FI observed during weeks 26 – 39 were 2 and 3 (some – common), after this period the FI change to 5 (abundant), but these FI did not produce Bulking (See Figure 5).



Inoculation of isolated strains showed a positive effect on bioaugmentation of protozoa and metazoan, causing a final biological stabilization in the system. The SBI index rise values of 9 meaning that the activated sludge were Class I, well colonized and stable sludge, excellent biological activity, very good performance (See Figure 8).



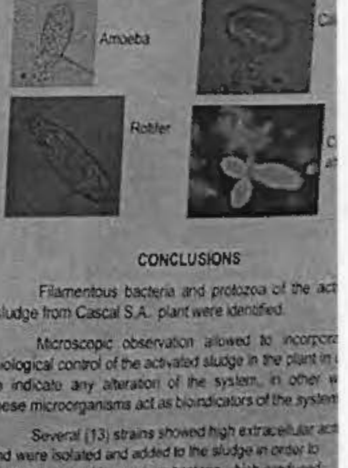
All changes improved the water treatment plant capability from 40 lps to 80 lps, bringing the plant work at 100 % of it capacity (See Figure 9).

Biological control before inoculation demonstrated filamentous microorganism causing bulking and foaming were ***Microtrix parvicella*** and ***Nocardia* sp.** (See Figure 6). Inoculations then did control filamentous microorganism growth and this growth was focused only inside the flock (See Figure 7).

Figure 6.- A.- Flock with *M. parvicella*. B.- Flock with *Nocardia* sp.

Figure 7.- Filamentous microorganism inside flock.

Representative microorganisms of the activated sludge of the waste water treatment plant



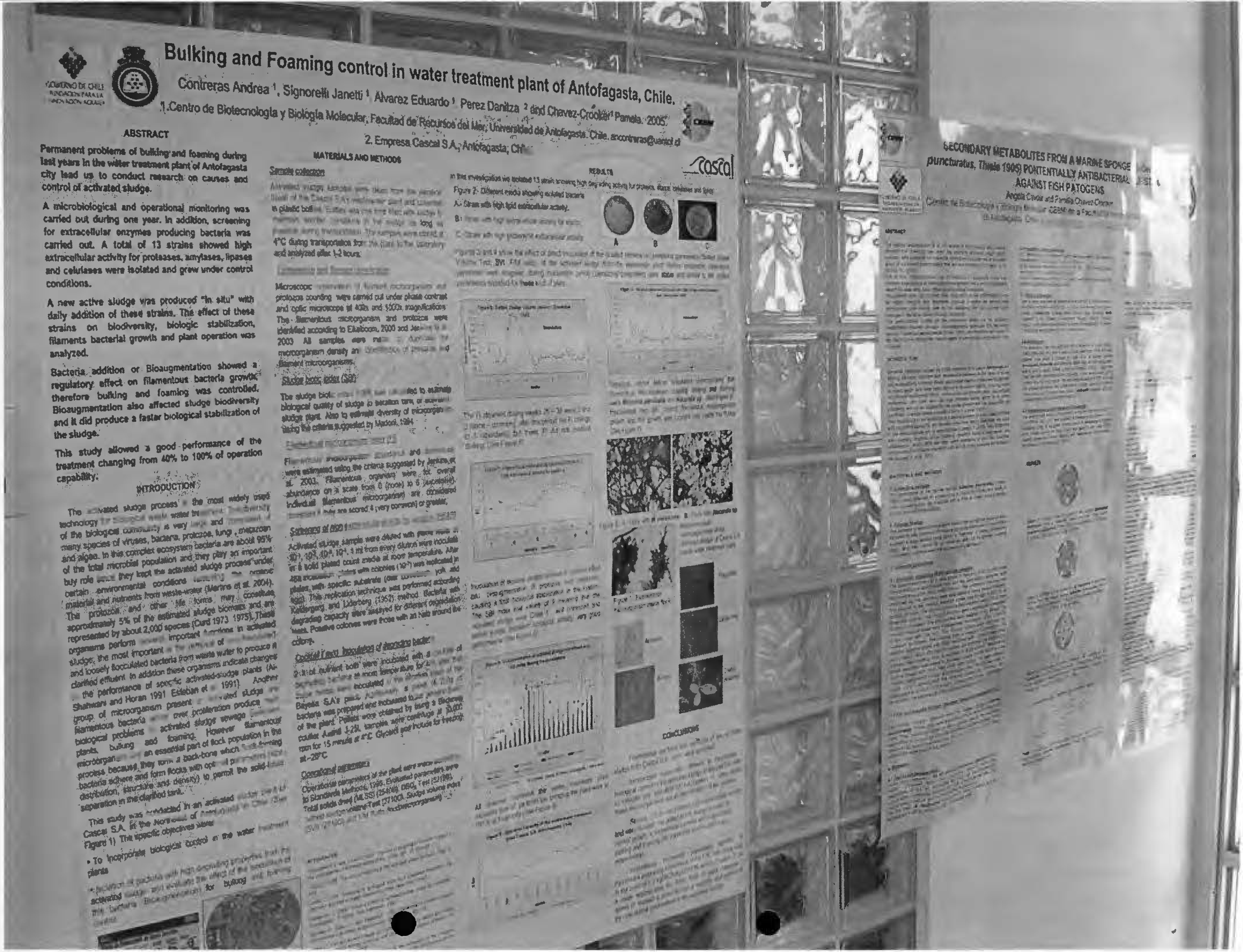
CONCLUSIONS

Filamentous bacteria and protozoa of the activated sludge from Cascal S.A. plant were identified.

Microscopic observation allowed to incorporate biological control of the activated sludge in the plant in order to indicate any alteration of the system, in other words these microorganisms act as bioindicators of the system.

Several (13) strains showed high extracellular activity and were isolated and added to the sludge in order to control growth of filamentous bacteria which produced bulking and foaming (M. parvicella and Nocardia sp., respectively).

Inoculations increased population density of microfauna producing a decrease in the F/M rate being it in the order of 0.2 kgDBO5/kgSSTA*d, which it seems to be a value appropriated for these kind of plant, because it allows to support a good biological stability and control of the operational parameters of the activated sludge.


Bulking and Foaming control in water treatment plant of Antofagasta, Chile.
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INTRODUCTION
MATERIALS AND METHODS
RESULTS
CONCLUSIONS
SECONDARY METABOLITES FROM A MARINE SPONGE
AGAINST FISH PATOGENS

SECONDARY METABOLITES FROM A MARINE SPONGE (*Suberites puncturatus. Thiele 1905)* PONTENTIALLY ANTIBACTERIAL SUBSTANCES AGAINST FISH PATOGENS.

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ABSTRACT

The marine environment is a rich source of both biological and chemical diversity. This diversity has been the source of structurally unique natural products with potential for industrial development. Sponges are an abundant group of [illegible] invertebrates that are very chemically rich (Rajeev, K. & Zirong, X. 2004).
Due to the indiscriminated use of antibiotics in aquaculture it has lead resistant populations of pathogen microorganisms, that is why is imperative to search for new and more effective antimicrobial compounds.
The present work aims to find new bioproducts as new antimicrobials, since an urgent need to find alternative products to replace the actually used compounds, this mainly due to the resistance that has acquired many pathogenic bacterial strains through mutations.
The antimicrobial activity of the methanolic extract and the secondary metabolites obtained through chromatographic techniques from the marine sponge **Suberites puncturatus** (Thiele 1905) was evaluated over cultures of the Aeromonas hydrophila, causal agent of haemorrhagic septicaemia in fish cultures.

INTRODUCTION

Bacterial infections caused by motile members of the genus ***Aeromonas***, are among the most common and troublesome diseases of fish raised in ponds and recirculating systems. Motile aeromonad infections have been recognized for many years and have been referred to by various names, including motile aeromonad septicemia (MAS), motile aeromonad infection (MAI), hemorrhagic septicemia, red pest, and red sore. Whether acting alone or in mixed infections with other organisms, the motile aeromonads are responsible for significant financial losses annually (Camus A.C. et al. 1998).
The resistance to the antibiotics is a serious problem of public health in Chile with important economic and sanitary projections, which have been the result of the indiscriminate use of antimicrobials (Cabello, F. 2003). New antibiotics are urgently required since multidrug resistant bacterial pathogens are increasing. Much effort has been spent attempting to find new bioactive compounds from marine organisms such as soft corals, sponges and molluscs (Armstrong, E. et al. 2001).

MATERIALS AND METHODS

1. Collecting samples
The specimens of the marine sponge ***Suberites puncturatus*** (Thiele 1905), were collected by scuba-diving in Coquimbo, Chile, at a depth of [illegible] removing from the substrate with a knife or chisel, using protective gloves and protective clothing.

2. Sample fixation
The samples of sponge tissue were fixed in the appropriate media as soon as possible after collection, for natural products identification or quantitative analysis a large sample were stored in methanol, in strong polyethylene bags, and then stored in a container with ice to prevent bacterial proliferation, and then were transported to the laboratory.

3. Chemical procedures

3.1 Alcoholic extraction of the sponge samples.
The sponge samples ± 50 g were stored in methanol ± 500 mL, then were filtered through a ground-glass filter and the solid residue extracted twice with methanol, both times with 250mL. The methanolic fractions were combined and the Methanol were evaporated in vacuo using a rotavapor. The volume of the residual aqueous phase was brought to 250 mL by addition of water. The aqueous phase is then extracted with CH2Cl2, three times with 250 mL. The CH2Cl2 fractions were combined and evaporated in vacuo to dryness. The solid residue were weighted (= EA extract). The residual aqueous phase were extracted with the mixture CH2Cl2 /EtOH 3:2 (three times with 250 mL). The organic phases were combined and evaporated to dryness. The resulting solid is weighted (= EB extract). The residual aqueous phase was finally extracted with 1-butanol (three times with 250mL). Again, the organic phases are combined and evaporated to dryness. The resulting solid is weighted (= EBut extract). The aqueous phase were then evaporated to dryness in vacuo and the resulting solid residue treated with EtOH (two times with 100mL). The heterogeneous mixtures were filtered through a ground-glass filter. The solid was dried in vacuo (= EC extract), and the ethanolic phases are combined and evaporated to dryness (= ED extract). During all the process, it is important to maintain the temperature of the solutions at maximum 30°C.

3.2 Extract analysis through Thin-layer liquid chromatography (TLC)
Fractionated extracts obtained from the marine sponge ***Suberites puncturatus*** Thiele 1905 were analyzed by TLC at Catholic University of Chile. This technique allows to get a general idea of the secondary metabolites in the extract. From this examination by chromatography were obtained five secondary metabolites: H-1, H-2, PYSA, NE, and AE, those metabolites were used in this research.

4. Bioassays

4.1 Test strain preparation
Freeze dried cultures of the fish pathogen ***Aeromonas hydrophila*** LMG 13683 [illegible] Gram Laboratory Collection were used [illegible] several times [illegible] of trypticase soy broth, and then transferred to [illegible] trypticase soy broth to rehydrate the cells. The [illegible] A. hydrophila were grown in tubes containing 3ml of [illegible] 30°C (optimum growth temperature) [illegible] overnight for bioassay tests.

4.2 Preparation of test compounds.
Four dilutions from the methanolic extract were prepared in the following concentrations: 1μg/μl, 0.5μg/μl, 0.33μg/μl, 0.25μg/μl.
All the secondary metabolites (H-1, H-2, PYSA, NE, y AE) for the bioassay were prepared in a concentration of 48 μg/ml.
DMSO, Dimethylsulfoxid (MERCK p.a. grade) were used to prepare all the dilutions of purified compounds and extract.

4.3 Control preparation
Disks of paper were used as negative controls with 10μl of DMSO previously esterilized by filtration (MILLEX-GV, a 0.22μm pore diameter), as positive control, Oxitetraciclina (forticina retard, 200μg/μl, Syva laboratory) were prepared in the following concentrations: 200μg/μl, 100μg/μl, 50μg/μl, 20μg/μl, using sterile deionized water as solvent for resuspension of extract. The dilutions were prepared as way to minimize errors.

4.4 Antibiogram.
The antibiogram test were made according to Bauer A. W., Kirby W.M.M. (1966). Whatman N°1 discs of paper of 6 mm were used. From the broth cultures were plated in tripticase soy agar 100μl of growing bacteria ***Aeromonas hydrophila*** with a glass loop, paper discs of 6mm, impregnated with a 10ul of extract dilutions (0.25μg/μl, 0.33μg/μl, 0.5μg/μl, 1μg/μl) and other set with metabolites dilutions (48μg/μl), then were placed on the surface of a TSA solid medium. The plates were incubated at 30°C for 24-48 hrs, antibacterial activity were verified when a circular region of no bacterial growth develops, in positive cases inhibition zone were measured, and then compared with Ericsson and Sherris table (1971).

4.5 Antimicrobial activity evaluation of compounds.
The antimicrobial activity of the methanolic extract and the metabolites (H-1, H-2, PYSA, NE, y AE), were evaluated by measuring the diameter (D), in milimetric units, of the growth inhibition zones of ***Aeromonas hydrophila*** LMG 13683, around the disks, control tests were for each assay. In the positive cases, the inhibition diameter were measured and then checked by de Ericsson, H. & Sherris, J.C. (1971).

RESULTS

Figure 1. Inhibition zones of growth of fish patogen ***Aeromonas hydrophila*** using a methanolic extract from the marine sponge ***Subiretus puncturatus*** (Thiele, 1905) in concentrations of 1. 0.25μg/μl, 2. 0.33μg/μl, 3. 0.5μg/μl y 4. 1μg/μl, 5. control

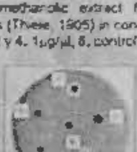

Figure 2. Inhibition growth zones over ***A. hydrophila*** of metabolites purified from the marine sponge ***Suberites puncturatus*** (Thiele, 1905), using a concentration of 48μg/μl of the following metabolites: 1. AE, 2. H-2, 3. H-1, 4. PYSA, 5. NE, 6. Control

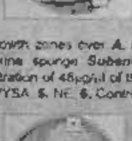

Figure 3. Inhibition growing zones obtained over cultures of ***A. hydrophila*** using the reference antibiotic Oxitetraciclin (200mg/ml Syva laboratory) using the following concentrations: 1. 20μg/μl, 2. 50μg/μl, 3. 100μg/μl, 4. 200μg/μl y 5. control

Concentration μg/μl	diameter mm	Inhibition zone
1	11	(+++)
0.5	8	(++)
0,33	7	(++)
0.25	**	**
Control (DMSO)	**	**

Table 1. Antibiogram test results using methanolic extract from the marine sponge ***Suberites puncturatus*** (Thiele, 1905) over growth patogen ***A. hydrophila***.

Compound	Concentration μg/μl	diameter mm	Inhibition zone
H-1	48	7	(++)
H-2	48	**	**
PYSA	48	14	(+++)
NE	48	**	**
AE	48	11	(+++)
Control	DMSO	**	**

Table 2. Antibiogram test results using the secondary metabolites from the marine sponge ***Suberites puncturatus*** (Thiele, 19[illegible] over growth patogen ***Aeromonas hydrophila***.

Concentration μg/μl	diameter mm	Inhibition zone
200	38	(+++)
100	30	(+++)
50	27	(+++)
20	25	(+++)
Sterile deionizated water	**	**

Table 3. Antibiogram test results for the positive control using [illegible] antibiotic Oxitetraciclin over the growth of ***A. hydrophila***.

Note:
(+ inhibition zone less than 5mm)
(++ inhibition zone from 5 to 10mm)
(+++ inhibition zone grater than 10mm)
(** no inhibition zone)

DISCUSION

The ocean as a rich source of natural products with pharmac[illegible] activity, its vital for de discover of new bioactive compounds [illegible] the treatment of diseases, while othe products have been de[illegible] as analgesic, antimicrobials, etc. The life saving drugs are found abundantly in microorganisms, algae and invertebrates [illegible] scarce in vertebrates (Rajeev, K. & Zirong, X. 2004). A cons[illegible] future screening programs is the search for new metaboli[illegible] farmacological potential from non explorated environments (M[illegible] C. 2003).
Result of aquaculture intensification, stress of the organisms, appearance of diseases, caused by the proliferation of gram n[illegible] bacteria as ***Aeromones salmonicida*** and ***Aeromonas hydr***[illegible] those are under control by the use of antibiotics, a[illegible] indiscriminated use of those antibiotics cause resistance [illegible] ecosystem destruction too. That is why the natural products [illegible] viable alternative to mitigate those damage (Nuñez, M. et al. 20[illegible]
In the case of sponge genus ***Suberites***, organic extracts [illegible] ***Suberites domuncula*** showed strong antibacterial activ[illegible] ***domuncula*** produce a neurotoxin called suberitin, that probabl[illegible] responsible of the antimicrobial activity observed. Sec[illegible] metabolites obtained through thin-layer cromatography, s[illegible] antibacterial activity (Narsinh, L.T., et al 2003). In experiments re[illegible] by Kelman D. et al (2001), shows that extracts of ***S. clavatu***[illegible] ***Amphimedon viridis*** tested against fish patogens like [illegible] ***alginolyticus*** shows strong antimicrobial activity in agar dif[illegible] assays.
Different studies realized by Newbold, R., et al (1999), on cari[illegible] sponges, shown of 33 studied species, 16 (48%) of those pr[illegible] secondary metabolites with antibacterial activity over m[illegible] pathogens like ***Vibrio parahemoliticus***.
Marine sponge ***Suberites puncturatus*** (Thiele, 1905) is a sou[illegible] secondary metabolites with antimicrobial activity with huge pot[illegible] application in aquaculture systems.
Methanolic extract obtained from ***Suberites puncturatus*** (Th[illegible] 1905), inhibit the growth of the test pathogen ***A. hydrophila***.
Exists inhibitional activity in two of five purified secondary metabo[illegible] from ***Suberites puncturatus*** (Thiele, 1905) over cultures o[illegible] ***hydrophila***.
The mayor inhibitory activity over growth of ***A. hydrophila*** [illegible] obtainded at a concentration of 1μg/μl of methanolic extract, ge[illegible] inhibitory diameter zones of 11mm.
The highest inhibitory activity over growth of ***A. hydrophila*** [illegible] obtained when are used 48μg/μl of secondary metabolites PYSA [illegible] AE in antibiogram tests getting inhibitory zones of 14 and [illegible] diameters respectly.
The purified compounds PYSA and AE are candidates for being u[illegible] as new antimicrobials for preventing and control of ***A. hydrophila*** fish cultures.

[illegible]

Armstrong, E., [illegible]
Cabello, F. [illegible]
Camus, A.C., [illegible]
Ericsson, H. & Sherris, J.C. [illegible]
[illegible]
Newbold, R., [illegible]
[illegible]
Rajeev, K. & Zirong, X. (2004) [illegible]

DESIGN AND CONS[illegible]TION OF DIMERIC RECOMBINANT ANTIMICROBIAL PEPTIDES (drAMPs) WITH EN[illegible]ANCED SPECIFIC ACTIVITY

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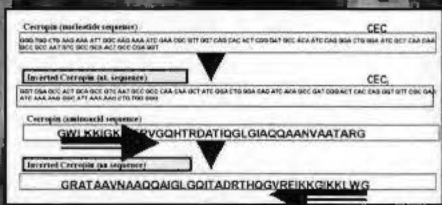
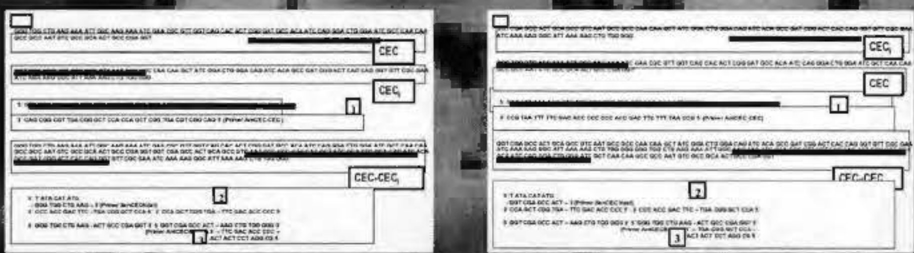
ABSTRACT

Most antimicrobial peptides (AMPs) act by penetrating bacterial me[illegible] and pr[illegible] cell lysis "from without". Although th[illegible] of action is known to critically depend o[illegible] cationic and amphipathic nature, it has also [illegible] that it depends on a dimeriz[illegible] process occurring in the target membrane. On the oth[illegible] also been observed that a [illegible] of AMP classes do potentate each other in a synergi[illegible]ng their specific activity. Base[illegible] these facts we have designed and constructed a n[illegible] hoping to ac[illegible] a single molec[illegible] the best efficiency of their parental counterparts. Using the known DNA seque[illegible] different natural AMPs ORFs, we have generated functional hybrids between either two identical an[illegible] different peptides. Among th[illegible] we have selected those with the following conf[illegible] tail-head ▼ head-tail and [illegible] ▼ tail-head. heterodimer: head-[illegible] ▼ head-[illegible] rec[illegible] mol[illegible] were generated [illegible] novel three-primer driven PCR [illegible] were a hybrid mole[illegible] the [illegible] sequence of pep[illegible] linked in fr[illegible] with the [illegible] of peptide "B" was initially [illegible] using a hybrid [illegible] especially designed for th[illegible] and C terminal flanking primer[illegible] for a second [illegible] definitive selection. It is wo[illegible] that for homodimer constructions [illegible] inverted peptide-co[illegible] sequence was [illegible] the expected polarity [illegible] coding triplets. All drAMPs were cloned [illegible] vectors, expressed in *E. coli*, [illegible] cell extracts and their specific activ[illegible] comparative way with their monome[illegible] counterparts against [illegible] of di[illegible] Gram po[illegible], Gram negative and fungi. Results will be [illegible] in the frame of [illegible] [illegible] activity as well as those cases [illegible] were designed "tail[illegible]" to inac[illegible] given pathogen.



F[illegible] [illegible]rted peptide-co[illegible] design. Polarity of sequence is indicated by a[illegible] arrow (triplet).



[illegible] (left) and CEC_I-CEC (right) dime[illegible] three-prime[illegible] reaction with [illegible] (1) and [illegible] primers (2,3) using CE[illegible] and CEC_I (in [illegible]). Sequence of [illegible] plates used [illegible] in r[illegible]

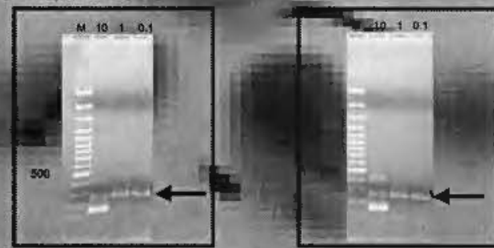


Fig. 3. PCR profile of CEC-CEC_I (left) and CEC_I-CEC (right) generated by three-primer driven PCR reaction at different (10, 1, 0.1 μM) hybrid primer concentrations resolved through agarose (2%) gel electrophoresis. M= DNA size marker (pb)

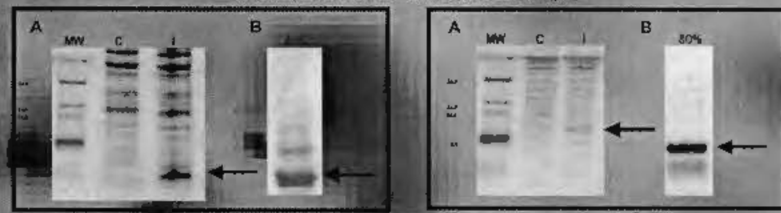
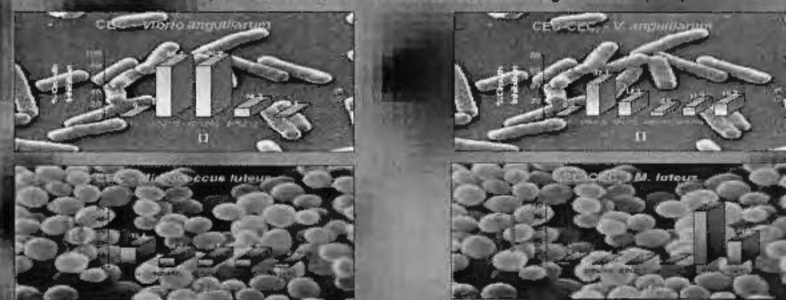


Fig. 4. Protein [illegible] of CEC (left) and CEC-CEC_I (right). A) crude extract B) 40% and 80% ACN fraction [illegible]esolved through Tris-tricine / Urea (15%) gel electrophoresis. [illegible]ontrol, I: Induced with IPTG. MW= Molecular Weight marker (kDa).



[illegible] 5. Antibacterial a[illegible] of CEC (left) and CEC-CEC_I (right) from [illegible] fractions [illegible] with 5%, 40% and 80% ACN. *Vibrio ang[illegible]* [illegible] *Micrococ[illegible] luteus* (down).

CONCLUSIONS

- [illegible]sign and construction of inverted coding sequence genes [illegible] cecropin as a model [illegible]tem)
- [illegible]esign, construction, synthesis and cloning of two novel dimeric peptides derived from cecropin: CEC-CEC_I and CEC_I-CEC.
- Expression and purification of CEC-CEC_I from *E.coli*.
- Expressed CEC-CEC_I displayed enhanced antimicrobial activity than its parental monomeric form.

REFERENCES

[illegible]

[illegible] [illegible]chnology [illegible] CHILE Funding: [illegible] COPEC-UC [illegible]