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Autochthonous yeasts as growth factor in *Rhamdia quelen*: preliminary approaches

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Abstract

Guidoli, M.G.; Mendoza, J.A.; Cáceres, A.C.; Boehringer, S.I.; Sánchez, S.: ***Autochthonous yeasts as growth factor in Rhamdia quelen: first approaches.*** *Rev. Vet. 28: 1, 37-40, 2017. Rhamdia quelen* is an autochthonous fish used for aquaculture in the Northeast of Argentina. Antibiotics as growing factors in fish production had been criticized in the last years and the use of microorganisms emerged as a putative replacement. The aim of this study was to isolate autochthonous yeasts from *R. quelen* and to evaluate the effect of the administration over biometrical parameters of larvae under intensive culture system. Fungi were isolated from the digestive tract of wild specimens, phenotypically identified and evaluated on their ability to exert beneficial properties. One selected fungal isolate was administered, four times a day, dead or alive at 1 or 2% together with balanced feed to *R. quelen* larvae for 15 days. After treatment larvae were counted and weighted in order to obtain the values of survival, mean weight and total biomass. Only four *Candida tropicalis* isolates were obtained. None of them expressed beneficial properties. One fungal isolate was randomly selected for *in vivo* assays. Results indicated that only dead yeast at 1% induced a significant increment of biomass when compared with the control group ($p<0.05$). Survival rate was boosted significantly ($p<0.05$) with dead yeast and not significantly ($p>0.05$) with the fungal isolate. None of the treatments induced significant increments in mean weight ($p<0.05$). These results allowed us to propose the treatment with dead yeast at 1% as a growing factor for *R. quelen*, considering this microorganism as an effective prebiotic in the aquaculture of this specie.

Key words: fish *Rhamdia quelen*, *Candida tropicalis*, prebiotic, aquaculture.

Resumen

Guidoli, M.G.; Mendoza, J.A.; Cáceres, A.C.; Boehringer, S.I.; Sánchez, S.: ***Levaduras autóctonas como factores de crecimiento en Rhamdia quelen: primeras aproximaciones.*** *Rev. Vet. 28: 1, 37-40, 2017. Rhamdia quelen* es un pez autóctono cultivado en el nordeste argentino. El uso de antibióticos como factores de crecimiento en acuicultura fue criticado en los últimos años y la administración de microorganismos surgió como una opción posible. El objetivo de este trabajo fue aislar levaduras autóctonas de *R. quelen* y evaluar el efecto de su administración sobre parámetros biométricos de larvas en cultivo intensivo. Los hongos se aislaron del tracto digestivo de especímenes de vida silvestre, se identificaron fenotípicamente y se evaluaron en cuanto a la expresión de propiedades benéficas. Se seleccionó una de las cepas y se administró cuatro veces por día, muerta o viva al 1 o 2% junto con el alimento balanceado durante 15 días. Luego del tratamiento las larvas se contaron y pesaron para obtener los valores de sobrevida, peso medio y biomasa. Sólo se obtuvieron cuatro aislamientos de *Candida tropicalis*. Ninguno expresó propiedades benéficas y uno fue elegido al azar para los ensayos *in vivo*. Solo la levadura muerta al 1% incrementó significativamente la biomasa en comparación con el control ($p<0,05$). La sobrevida mejoró significativamente ($p<0,05$) con la levadura muerta y sin significancia ($p>0,05$) con el hongo vivo. Ninguno de los tratamientos induce incrementos significativos del peso medio ($p<0,05$). Estos resultados nos permiten proponer al tratamiento con levadura muerta al 1% como un factor de crecimiento para *R. quelen*, considerando al microorganismo como un prebiótico efectivo para su uso en esta especie.

Palabras clave: pez *Rhamdia quelen*, *Candida tropicalis*, prebiótico, acuicultura.

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INTRODUCTION

Rhamdia quelen (bagre, jundiá) is an autochthonous fish which inhabits in the Northeast of Argentina and Southeast of Brazil and Uruguay. It was previously considered of low commercial value. However, its production since 2012 increased to 1.39 tons, representing a 0.03% of the total Argentinian aquaculture production with prices *per* kg higher than those corresponding to *Leporinus obtusidens* (boga), *Prochilodus lineatus* (sábalo), *Oreochromis aureus (tilapia*), mussels and oysters and equal to that for the common carp [7-9].

In the last decades there was an increment in the use of chemical compounds to increase the growth rate in animal production. However, it was proved that their use in aquaculture could cause the appearance of residual drugs in treated fish that could affect consumers, the exposure to untargeted animals and the threat to the environment [13]. International organizations as the European Food Safety Authority prohibited the use of antibiotics as growth promoters in animals for human consumption [3].

Despite controls and regulations, antimicrobial resistance and toxicity are increasing in developing countries where the aquaculture industry is growing very fast [10]. Then, the search of novel and efficient strategies to increment productivity without the use of chemotherapeutic agents and other drugs are strongly required.

Several alternatives to increment health status, survival and growth rate have been proposed. Most of them are based on using extensive, instead of intensive, culture systems, and applying natural, novel and safe products to replace chemotherapeutic drugs. Based on the fact that the microbiota of the gastrointestinal tract (GIT) directly affects nutrition and health of the host, it has been pointed as a probable target of many new strategies.

The use of microorganisms to establish, improve or restore the native microbiota of fish as a mean to increment nutritional, immunological and biometrical factors emerged as a putative solution. There is a wide availability of commercial products containing microorganisms. However, they are relatively ineffective in fish culture, mainly because most of them include strains isolated from non-fish sources [11]. Then, it is essential to isolate and evaluate autochthonous microorganisms as components of microbiological formulae with aquaculture purposes [5].

The aim of this study was to isolate autochthonous yeasts of wild specimens, select one of them based in the expression of beneficial properties *in vitro* studies and determine if it could increment biometrical parameters in the aquaculture of *Rhamdia quelen*.

MATERIAL AND METHODS

A total of 25 wild specimens were collected from different locations in the province of Corrientes, Argentina and subjected to 24 h of fast. After desensitization with a 2% benzocaine solution, animals were slaughtered and the digestive tract was aseptically removed. The intestinal content was removed with sterile saline solution, separated by centrifugation, stroked in Agar Sabouraud and incubated for 24 h at 37 °C. Obtained isolates were conserved properly until use.

Phenotypic identification was performed by culture in Chromagar ® *Candida* for 48 h at 37 °C and observation of micromorphology in rice agar incubated for 72 h at 25 °C. Isolates were evaluated for the expression of beneficial properties: *Hydrogen peroxide* (H_2O_2) production was evaluated by culturing isolates for 24 h at 37 °C in plates containing Agar Sabouraud added with 1mM 3, 3', 5, 5'- Tetramethylbenzidine and 2 IU/ml horseradish peroxidase; the turn to blue color of obtained colonies after exposed to air indicated the presence of the oxidative metabolite.

Inhibition of pathogens and foodborne microorganisms was evaluated by the diffusion method in soft agar using fish pathogens and foodborne bacteria as indicators. The appearance of inhibition halos determined the capacity of the isolates to produce antagonistic substances. The identification of the selected isolate was confirmed by using the commercial identification test RapID™ YEAST PLUS System ® Remel.

For the *in vivo* assays, *Rhamdia quelen* larvae were obtained by controlled reproduction. Spawning was induced by injection of pituitary extract from *Prochilodus lineatus* [2]. The sexual gametes were obtained by stripping, mixed immediately, washed twice and translated to a hatchery device with a constant recirculation system until hatching [4].

Experimental units consisted of 100 larvae in 5 l plastic fishbowls with a constant recirculation system. Lyophilized selected yeast was administered dead (D) or alive (A) at 1 and 2% together with the commercial balanced feed used in aquaculture. Feeding was performed *ad libitum* four times a day. Control group was fed without the addition of microorganisms. Water

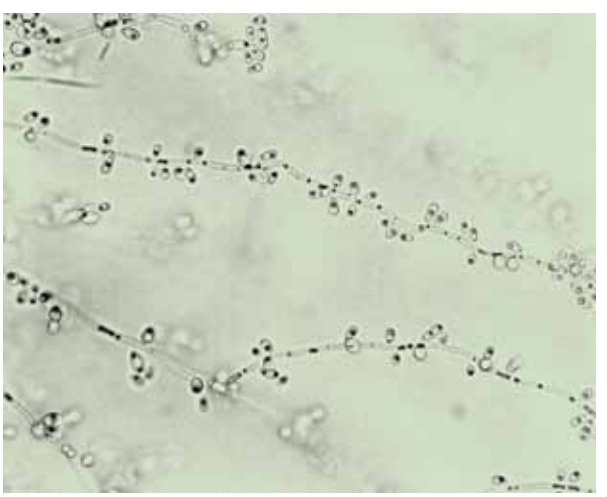

Figure 1. Micromorphology of the selected isolate cultivated in rice agar. The other results together with the presence and distribution of blastospores along the pseudomycelium allowed the classification as *Candida tropicalis*.

quality (pH, dissolved oxygen and temperature) was monitored daily. After 15 days of intensive larvaculture in laboratory conditions, juveniles of each experimental unit were counted and weighed in order to determine survival and mean weight.

Assays were performed by quintuplicate using a completely randomized design. Replicates become from different parents to exclude the genetic factor. Comparisons were performed, first, through one-way ANOVA including controls and treatments, then, by using a two-way factorial ANOVA with interactions including only the treatments with subsequent post hoc tests (Duncan). Analyses were carried out using Statistica 6.0 for Microsoft Windows with A significance of 0.05 (α=0.05).

RESULTS

Only four yeasts isolates were obtained from all samples. The development of blue colonies after 48 h of incubation at 37°C in Chromagar® Medium and the distribution of blastospores along pseudomycelium observed in the microcultures (Figure 1) determined that all isolates belong to *Candida tropicalis*. None of the isolates was able either to produce hydrogen peroxide neither to inhibit the development of fish pathogens or foodborne microorganisms by the production of other antagonistic compounds. As none of the *C. tropicalis* isolates presented beneficial properties, only one strain was randomly selected to be used in further studies.

The one-way ANOVA analyses of the *in vivo* assays indicated significant differences among groups for all the variables evaluated ($p < 0.05$) (Table 1). Treatments with dead yeast showed significant higher survival rates than the control group. On the other hand, larvae administered with live yeast showed higher but no significant survival values when compared with the control group.

Although higher, the mean weight of larvae treated with 1% yeast (either dead or alive) showed no significant differences with the control group. Treatments with yeast at 2% showed lower mean weight than the control group, but significant when administered dead microorganisms. Only the treatment administered with dead yeast at 1% showed significant higher values of biomass compared with all other treatments and the control group (Figure 2).

The two-way ANOVA showed interactions between the concentration and the state of the yeasts for mean weight and biomass and no interaction for survival (Table 2) (α = 0.05). The survival rate was not significantly affected by the percentage of yeast added. On the other hand, the use of dead yeasts induced a

Table 1. Mean weight, survival and biomass of *R. quelen* larvae administered with 1 and 2% of live or dead yeast.

variable	n	ss	df	ms	f	p-value	sig.
x̄ weight (mg)	25	3.30	4	0.82	4.98	0.0060	S
survival (%)	25	314.96	4	78.74	2.93	0.0467	S
biomass (mg)	25	6931.60	4	1732.90	5.33	0.0044	S

One way ANOVA. n: number of values, ss: sum of squares due to the source, df: degrees of freedom in the source, ms: mean sum of squares due to the source, f: F-statistic, sig.: significance, S: significant.

Table 2. Two way ANOVA results for interactions between concentration and state of the yeasts for mean weight, survival and biomass of *R. quelen* larvae administered with 1 and 2% of live or dead yeast.

variable	n	ss	df	ms	f	p-value	int.
x̄ weight (mg)	25	0.80	1	0.80	8.74	0.0093	i
survival (%)	25	22.05	1	22.05	0.99	0.3346	ni
biomass (mg)	25	2289.80	1	2289.80	10.25	0.0056	i

n: number of values, ss: sum of squares due to the source, df: degrees of freedom in the source, ms: mean sum of squares due to the source, f: F-statistic, int.: interaction, i: interaction, ni: no interaction.

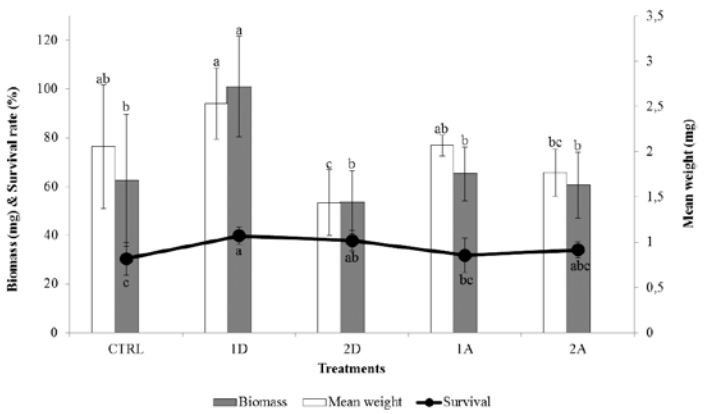

Figure 2. Biomass, mean weight and survival of *R. quelen* larvae after fifteen days of treatment. CTRL: control without the addition of microorganisms, 1D: dead yeast at 1%, 2D: dead yeast at 2%, 1A: alive yeast at 1%, 2A: alive yeast at 2%. Bars indicate standard errors. Different letters indicate significant differences.

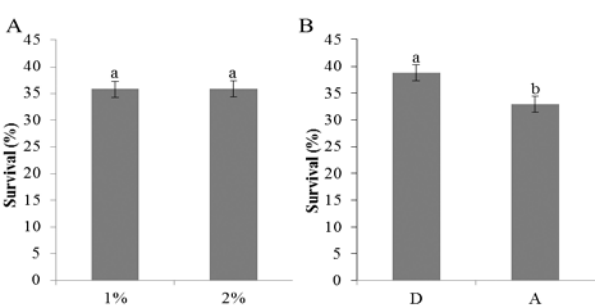

Figure 3. Survival percentages of *R. quelen* larvae after fifteen days of treatment analyzed separately for A: concentration (1% and 2%) and B: state of yeast (D: dead and A: alive). Bars indicate standard errors. Different letters indicate significant differences.

significant increment of survival in comparison with treatments using live yeasts (Figure 3).

DISCUSSION

Candida tropicalis is one of the most frequently fungi isolated from the GIT of marine and fresh water fish. Some authors described around 10^6 fungal cells *per* gram of material [5]. The low number of isolates obtained in this study could be improved in further assays by using animals without the fast of 24 h and sampling by gentile scraping the intestinal mucosa. These modifications would allow not only a higher number of isolates but also a greater diversity of microorganisms.

The administration of microorganisms to animals and humans tends to influence the immunomodulatory activity, boosting up the healthy benefits in aquatic animals [1]. Most of these microorganisms act as probiotics, defined for aquaculture as "a live microbial adjunct which has a beneficial effect on the host by modifying the host associated or ambient microbial community, by supporting an improved use of feed or enhancing its nutritional value, by stimulating the host response or by improving the quality of its ambient environment" [14].

However, due to the biochemical composition of their cell wall, yeasts can act also as prebiotics, defined as non-digestible forage additives that stimulate the activity or abundance of beneficial gastrointestinal bacteria [12]. Also, the high concentration of mannan oligosaccharides, the presence of polyamines and the stimulation of the digestive enzymatic activity promote toxins agglutination, cellular differentiation and a faster development of the gastrointestinal tract of larvae [10].

These characteristics explain the fact that the use of dead yeasts induces a significant increment of survival in comparison with treatments using live yeasts, indicating that in this particular case the strain of *Candida tropicalis* has the potentiality of being a prebiotic instead of a probiotic microorganism. The differences between the results obtained with different percentages of the dead yeast encourage further studies evaluating more concentrations.

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