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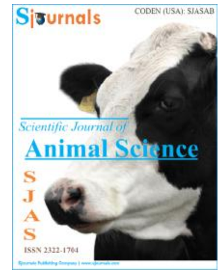
Sjournals
Scientific Journals

Scientific Journal of Animal Science (2016) 5(12) 370-375
ISSN 2322-1704
doi: 10.14196/sjas.v5i12.2341

CODEN (USA): SJASAB



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Scientific Journal of **Animal Science**

Journal homepage: www.Sjournals.com

Original article

Rhodococcus sp. as probiotic bacteria for increase the survival, growth and coloration of fish *Puntius conchonius*

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A R T I C L E I N F O

Article history,
Received 10 November 2016
Accepted 12 December 2016
Available online 17 December 2016
iThenticate screening 13 November 2016
English editing 10 December 2016
Quality control 14 December 2016

Keywords,
Bacteria probiotic
Carotenoids
Pigments
Rhodococcus

A B S T R A C T

An The aim of this study was to evaluate the effect of bacteria *Rhodococcus* sp. in growth, survival, and coloration of *Puntius conchonius.* It was obtained a batch of 300 larvae of seven days after hatching and were divided in six aquariums of 60 litters (50 org per aquarium) and fed with *Artemia franciscana* nauplii, enriched with *Rhodococcus* sp. at a concentration of 1 x 10^7 cells per milliliter. There was also a control treatment where larvae were only fed with *Artemia* nauplii without probiotic. Every fifteen days, biometric parameters were obtained such as weight, total length, high, wide. Also, physicochemical parameters were measured like pH, dissolved oxygen, nitrates, nitrites, and ammonium, and it was evaluated the increase in fish coloration following the method for pigment extraction. Feeding period was carried out for 60 days. Survival was 100% in both treatments, but in fish fed with *Rhodococcus* sp. presented better growth results in total length, wide and weight, than control group. Regarding to fish coloration, variance analysis showed significant differences between treatments (P=0.035), having a better coloration in tissue of fish fed with probiotic, proving that this genus present enormous benefits as it not only improves productive parameters, but also increases coloration of fish; fundamental aspect for ornamental species commercialization.

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1. Introduction

In Mexico, approximately 43 million of ornamental fish are sold, mainly of fresh water (SAGARPA, 2015), which has increased the search of diverse additives, chemicals, hormones, and antibiotics that improve production and product presentation. Nevertheless, many of these compounds are of high economic cost for producer, also, they have made a great environmental impact and bacterial resistance (Negrete et al., 2004). Therefore, in recent years it has been implemented the use of probiotics as a strategy to improve aquaculture production in a more natural way. Recent studies have demonstrated that probiotic microorganisms improve intestinal balance of fish, which allows greater assimilation of nutrients and hence higher growth rates; in addition to increasing immune response which gives them greater resistance to diseases (World Gastroenterology Organization, 2008; Castro et al., 2011). Among the most commonly used probiotics are bacteria of genus *Lactobacillus*, *Lactococcu*s and *Bacillus*, however, there are other genus that can be used such as *Rhodococcus* sp. which is part of intestinal microbiota of fish and naturally produce carotenoid pigments, so it can be use as probiotic *Puntius conchonius* diet, to increase its growth and coloration, without high economic and environmental cost that commercial additives have (Ahamed et al., 2012). Therefore, the aim of this study is to evaluate the effect of *Rhodococcus sp*. in survival, growth and coloration of fish *Puntius conchonius* under laboratory condition.

2. Materials and methods

2.1. *Puntius conchonius*

Fish were obtained from four pairs of brood stock from Live Food Laboratory for Aquaculture (UAM-X), and 300 larvae stage of seven days after hatching were used.

2.2. *Rhodococcus* sp.

The bacteria *Rhodococcus* sp. was previously isolated from the intestinal tract of rainbow trout in the laboratory, which was reactivated in MRS medium, until reaching wanted concentration of 10^7 ufc/mL.

2.3. *Artemia franciscana* enriched with probiotic

For *Rhodococcus* sp. supply it was used *Artemia franciscana* nauplii, which were decapsulated following Sorgeloos et al. (1997) technique and hatching in 10 L containers at 40 gL^{-1} salinity, with air supply and light continuous during 24 hours. After hatching, 3000 nauplii of *Artemia franciscana* were placed in 1 L of sterile water and it was added 1.5 mL of probiotic bacteria at a concentration of 1 x 10^7 CFU mL^{-1}, and were left during 30 minutes in a magnetic stirrer. After this period, mix was sieved and given to fishes (500 nauplii per aquarium). It is worth mentioning that as an experiment progressed and fish were growing, naupliar stage of *Artemia* changed by meta nauplii or preadult, considering 10% of total biomass per treatment.

2.4. Experimental design

Larvae were randomly distributed in six containers of 40 L of capacity, maintaining a temperature of 20°C, constant aeration and a photoperiod of 12 hours' light and 12 hours darkness, each container had 50 organisms, three of those containers were fed with *Artemia* enriched with *Rhodococcus* sp. and other three with *Artemia* without bacteria. Feeding period took place during 60 days. Solid wastes (feces and food) were removed with a net of 0.3 mm aperture and with plastic tube as siphon. Every week pH, dissolved oxygen (O_2), nitrates (NO_3), nitrites (NO_2) and ammonium (NH_4), were monitored to maintain water quality.

2.5. Survival and biometry

The survival was registered daily and after 30 days fish feeding, were began to get biometry every 15 days using a digital Vernier (Cienceware) considering total length, high and width of fish. Also, fishes were weighed with a digital balance (Adventure Pro Ohaus).

2.6. Fish coloration

To evaluate increases in fish coloration, it was used the method for pigment extraction described by Olson (1979) and quoted by Narayana et al. (2008). For which one gram of corporal tissue of fish was taken, except from head and digestive tract, and was placed in 10 mL vial, where it was added 2.5 g of anhydrous sodium sulfate. The sample was pushed against vial walls with a glass stirrer, it was added 5 mL of chloroform and could stand during one night at 0°C. When chloroform presented a clear layer of 2 cm above residue, lecture of optical density was made at 500 nm in a spectrophotometer (Spectronic 20 Genesys): For this, it was taken three aliquots of 0.03 mL of prepared samples and were contrasted with control. Total content of carotenoids was calculated as a µg per wet weight of the tissue with next formula:

Total content of carotenoids = [Absorption at the maximum wavelength / (0.25 x weight of sample (g))] x 10.

Where:
10 = Dilution factor
0.25 = Extinction coefficient

2.7. Statistical analysis

For all generated data, it was created a data base in Excel 2010 to perform parametric statistics. Subsequently to determine significant differences between treatments it was made a variance analysis (ANOVA), with SYSTAT 12.0 program.

3. Results and discussion

Survival in both treatments was of 100%. The evaluation of physicochemical parameters in experimentation, did not show significant variations because the parameters of dissolved oxygen, pH, NO_3, NO_2 and NO_4, were maintained constant during treatment (Table 1).

Table 1
Average values of physicochemical parameters.

Measurements	Control					*Rhodococcus*				
	pH	$O_{2\,(\%)}$	NO_3	NO_2	NO_4	pH	$O_{2(\%)}$	NO_3	NO_2	NO_4
1	8.44	99.93	0.37	0.67	0.18	8.30	99.2	0.90	0.22	0.26
2	8.26	99.93	0.62	0.77	0.09	8.20	99.16	0.87	0.15	0.26
3	7.93	99.03	3.90	0.12	0.1	8.10	99.50	3.93	0.16	0
4	8.10	97.66	3.90	0.17	0.006	7.67	98.16	3.42	0.11	0

Regarding to fish growth, in Fig. 1 and Table 2, it is observed that treatment with *Rhodococcus* sp. overcame control treatment in weight, length, and width, with values of 0.072 g, 8.07 mm and 1.03 mm. Nevertheless, control treatment exceeds in high values with respect *Rhodococcus* sp. treatment with a value of 4.59 mm.

Table 2
Average values of growth (weight, length, high and width) of *Puntius conchonius*.

Measurements	Control				*Rhodococcus*			
	Weight (g)	Length (mm)	High (mm)	Width (mm)	Weight (g)	Length (mm)	High (mm)	Width (mm)
1	0.24 ± 0.027	22.52 ± 1.277	6.60 ± 0.555	3.01 ± 0.146	0.33 ± 0.073	25.84 ± 1.419	7.98 ± 0.702	3.88 ± 0.197
2	0.74 ± 0.249	27.58 ± 2.149	7.55 ± 1.075	2.51 ± 0.218	0.99 ± 0.712	32.11 ± 1.083	10.63 ± 0.302	4.49 ± 0.137
3	0.49 ± 0.071	32.61 ± 0.444	10.93 ± 0.445	4.98 ± 0.096	1.18 ± 0.110	34.37 ± 1.637	11.60 ± 0.242	5.58 ± 0.369
4	0.69 ± 0.038	33.09 ± 1.471	12.71 ± 0.278	4.21 ± 0.142	1.09 ± 0.002	38.42 ± 0.987	13.12 ± 0.200	5.02 ± 0.330
Gain	0.006	2.81	4.59	0.002	0.072	8.07	1.14	1.03

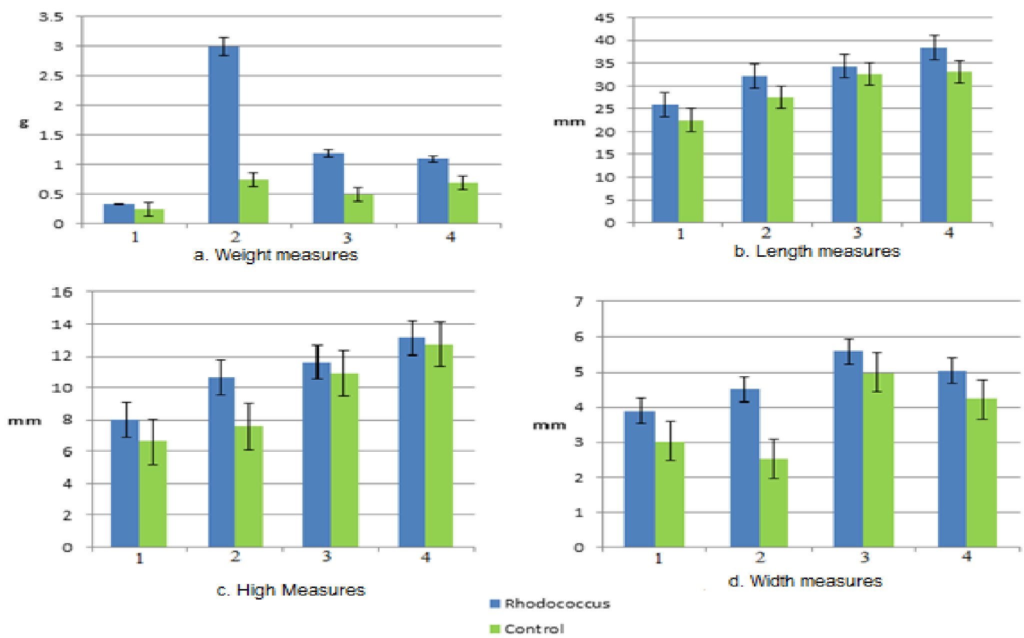

Fig. 1. Growth values of *Puntius conchonius* fed with *Rhodococcus* sp.

The variance analysis showed significant differences between treatment in weight and total length, with a value of P=0.01 and P=0.03 respectively, while in measurements of high there are not significant differences. Regarding to fish coloration, it was observed a clear difference in optical density and content of carotenoids, in fish fed with *Rhodococcus* sp. compared to control group (Table 3). Variance analysis indicated significant differences between treatments with a value of P=0.035 (Fig. 2).

Table 3
Optical density and carotenoids content in both treatments.

Treatments	Optical density (500 nm)	Carotenoids content
Rhodococcus sp	0.085	3.4
Rhodococcus sp	0.175	7.04
Rhodococcus sp	0.156	6.24
Control	0.051	2.04
Control	0.052	2.08
Control	0.53	2.12

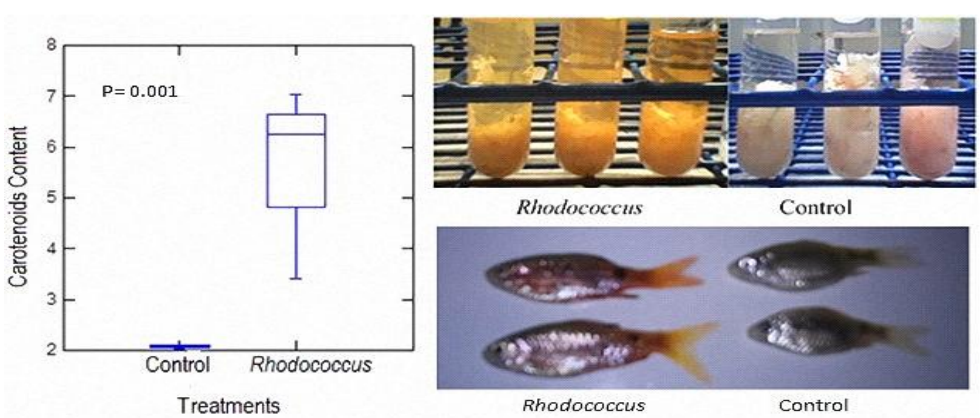

Fig. 2. Differences in carotenoids content between control group and treatment with *Rhodococcus* sp.

One of the characteristics that a microorganisms considered as probiotic has to have, is the capacity to increase the assimilation of nutrients and improve the digestive enzymatic activity, which is reflected in the increase of survival, height and weight, of cultured organisms (Balcazar et al., 2006; Boutin et al., 2013), which is observed in this study, because the obtained gain in weight, total length and width was higher in the treatment with *Rhodococcus* sp, these data are relevant for ornamental production as Indicated by Ramírez et al. (2010), that mentions that is better for producers to increase the height and weight of ornamental fish because they are more attractive to the consumers.

It should also be noted that there are no previous studies about the use of Rhodococcus sp as a probiotic, nevertheless, it has been reported that this microorganism is part of the intestinal microbiota of fish and crustaceans as the studies of Boutín et al. (2013), who obtained positive results to control pathogen bacteria *Flavobacterium psychrophilum* using *Rhodococus* sp. strain in *Salvelinus fontinalise* culture. Also, it was recognized that *Rhodococcus* have capacity to improve culture water quality of aquatic organisms. This is important because in past few years it has taken advantages use of diverse organisms like probiotics to improve nutrient assimilation, increase immune response and diseases resistance, which were important aspects to aquatic production (Monroy et al., 2012).

On the other hand, Carotenoids are lipid soluble pigments extracted from plant, microalgae, zooplankton, fungi and bacteria. The animals cannot have produced them and must be obtained by the food (Ako et al., 2001). The commercial production of carotenoids was made in a synthetic way, but chemical additive supply concern to aquaculture foods has sparked an interest to obtain nature carotenoids through biotechnology process, as the use of microorganisms.

In this study, the tissue pigmentation of fish fed with *Rhodococcus* sp. obtained a greater coloration compared to control group, because the bacteria produce carotenoid pigments (Cañizares-Villanueva et al., 1998; Villa et al., 1999), which could easily be integrated into the metabolism of fish. Because of this, the use of this bacteria in aquiculture is relevant because it not only improves the growth and survival of fish, but also pigment their tissue: important aspect for consumers that have preferences is products of orange coloration as salmonids or in ornamental fish where coloration, body and fins shape, are important criteria in the market (Ponce et al., 2016). On the other hand, the use of microorganisms as *Rhodococcus* sp. can contribute to the obtainment of natural pigments in a more economical way, because fish are unable to synthetize carotenoids and depend on the pigment supplement in diet that are very expensive if they are commercially acquire (Gouveia, 2003).

References

Ahamed, F., Ahmed, Z.F., Yeamin, M., Ohtomi, J., 2012. Growth study of the Pool *Barb Puntius sophore* (Cyprinidae*: Barbinae*) through multimodel inferences. Zool. Stud., 51(7), 1077-1085.

Ako, H., Tamaru, C.S., Asano, L., Yuen, B., Yamamoto, M., 2000. Achieving natural coloration in fish under culture. In: Tamaru, C.C.T., Tamaru, C.S., Mcvey, J.P., Ikuta, K., (eds) Spawning and maturation of aquatic species. United States-Japan cooperative program in natural resources technical report no 28. University of Hawaii, Sea Grant College Program, Honolulu, Hawaii.

Balcázar, J.L., De Blas, I., Ruiz-Zarzuela, I., Cunningham, D., Vendrell, D., Múzquiz, J.L., 2006. The role of probiotics in aquaculture. Vet. Microbiol., 114, 173-186.

Boutin, S., Audet, C., Derome, N., 2013. Probiotic treatment by indigenous bacteria decreases mortality without disturbing the natural microbiota of Salvelinusfontinalis. Can. J. Microbil., 59, 1-9.

Cañizares-Villanueva, R.O., Rios-Leal, E., Olvera, R.R., Ponce, N.T., Márquez, R.F., 1998. Fuentes microbianas de pigmentos. Rev. Lat-Am. Microbiot., 40, 87-107.

Castro, B.T., Monroy, D.M.C., Castro, M.J., De Lara, A.R., Castro, M.G., 2011. Efecto de cuatro probióticos en el crecimiento y la sobrevivencia de *Carassius auratus*. En Ciencia Pesquera., 19(1), 12-28.

Gouveia, L., 2003. Colouring ornamental fish (Cyprinuscarpio and Carassius auratus) with micro algal biomass. Aquaculture Nutr., 9, 123-129.

Monroy, D.M.C., Castro, B.T., Fernánde, Z.P.J., Mayorga, R.L., Herrera, G.H., Cortés, S.S., 2012. Bacteria with probiotic capabilities isolated from the digestive tract of the ornamental fish Pterophyllum scalare. En: Probiotics in animals. INTECH. Croacia, Chapter 10, 231-246.

Narayan, S., Kass, K.E., Thoma, S.E.A., 2007. Chronic haloperidol treatment results in a decrease in the expression of myelin/oligodendrocyte-related genes in the mouse brain. J. Neurosci. Res., 85, 757-765.

Negrete, P., Romero, J., Arredondo, J., 2004. Resistencia a antibióticos y presencia de plastmidos en: *Aeromonas hydropjila*, *Vibrio fluvialis* y *Vibrio furnissii*, aislados de *Carassius auratus auratus*. En veterianaria Meéxico. 35(1), 21-30.

Ponce, G.C.V., Monroy, D.M.C., Becerril, C.D., Castro, J.M., Ocampo, C.J.A., 2016. Potencial uso de *Rhodococcus* sp en la acuacultura. En prensa.

Ramírez, C.M., Mendoza, R.A., Aguilera, C.G., 2010. Estado actual y perspectivas de la producción y comercialización de peces de ornato de agua dulce en México. México, Monterrey, 66-75.

SAGARPA, 2015. Impulsa SAGARPA-CONAPESCA la acuacultura de peces ornamentales como alternativa de negocio en el país. México D.F., Comunicado de prensa. NUM. 162-15.

Sorgeloos, P., Bossuyt, E., Laviña, E., Baeza, M., Persoone, G., 1997. Decapsulation of *Artemia cysts*: Asimple technique for the improvement of the use of brine shrimp in aquaculture. En Aquaculture., 12, 311-315.

Villa, T.G., Calo, P., Sieiro, C., 1999. *Phaffia rhodozyma*: Primer microorganismo explotado para la producción de astaxantina. Biotecnología y aplicaciones de microorganismos pigmentados: IV Simposio científico en biología celular y molecular: Universidad de Coruña, 11-39.

World Gastroenterology Organisation, 2008. Guía práctica de la OMGE probiótico y prebióticos I., 128.

How to cite this article: Carol Vianey, P.G., María del Carmen, M.D., Ramirez Tórrez, J.A., Ocampo Cervantes, J.A., Mejía, J.C., 2016. *Rhodococcus* sp. as probiotic bacteria for increase the survival, growth and coloration of fish *Puntius conchonius*. Scientific Journal of Animal Science, 5(12), 370-375.

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