

Potential use in aquaculture of *Rhodococcus* sp. (Zopf, 1891).

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ABSTRACT

Aquaculture production has shown a great growth becoming an important food provider industry with high nutritional values and generating jobs and incomes. However, there are obstacles that limit considerably the productive success, such is the case of infection diseases, contaminant wastes generation and indiscriminate use of chemicals and antibiotics which has produced bacterial resistance in the principal diseases in cultured fishes and crustaceans. That is why today it exist great interest to obtain probiotic bacterial strains, which can help to minimize the infection process by pathogen exclusion. Likewise they were directly involved in host immunologic response, which it made them resistant to environmental variations. Also it was recognized that some bacteria have capacity to improve culture water quality of aquatic organisms. So the search of strains with probiotic potential, xenobiotic and nutritional, remains as aquaculture priority. One of this strains which was isolated last few years, from fishes, water and sediment in ponds aquaculture was *Rhodoccocus* sp. bacteria, so we consider necessary to make an analysis of their possible use in fish and crustacean culture, taking into consideration their degradation contaminant compounds capacity, their ability to competitive exclusion and carotenoids pigment production, which can improve better survival, growth, diseases resistance and coloring cultured, making them more attractive to consumer, without need for use chemicals and antibiotics which were harmful to aquaculture production.

Key words: Aquaculture, bacteria, probiotics, *Rhodococcus* sp.

convirtiéndose en una importante industria proveedora de alimentos de alto valor nutricional y generadora de empleos e ingresos. Sin embargo, a pesar de que se ha observado un avance en el desarrollo de esta actividad, existen obstáculos que limitan considerablemente el éxito productivo, tal es el caso de las enfermedades infecciosas, la generación de desechos contaminantes v el uso de químicos y antibióticos de forma indiscriminada que ha producido resistencia en las principales enfermedades bacterianas de peces y crustáceos en cultivo. Por lo que hoy en día existe un gran interés por la obtención de cepas bacterianas probióticas, las cuales pueden ayudar a minimizar los procesos infecciosos mediante la exclusión de patógenos. Asimismo participan de forma directa en la respuesta inmunológica del hospedero lo que los hace resistentes a las variaciones ambientales. También se ha reconocido que muchas cepas bacterianas tienen la capacidad de mejorar la calidad del agua de cultivo de organismos acuáticos. Por lo que la búsqueda de cepas con potencial probiótico, xenobiótico y alimenticio sigue siendo prioridad en acuicultura. Dado que en los últimos años uno de los microorganismos que se ha aislado de peces, agua y sedimento en los sistemas de cultivo acuícola es la bacteria Rhodococcus sp, consideramos hacer una análisis pertinente de su posible aprovechamiento en el cultivo de peces y crustáceos, tomando en cuenta su capacidad de degradación de compuestos contaminantes, su habilidad de exclusión competitiva y la producción de pigmentos carotenoides, lo que puede mejorar la supervivencia, crecimiento, resistencia a enfermedades y la coloración de las especies cultivadas haciéndolas más atractivas al consumidor sin la necesidad de utilizar compuestos químicos o antibióticos tan dañinos en la producción acuícola.

RESUMEN

La producción acuícola ha mostrado un fuerte crecimiento

Palabras clave: Acuicultura, bacterias, probióticos, *Rhodococcus* sp.

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INTRODUCTION

The *Rhodococcus* genus is a bacterial group which belongs to actinomycetes, aerobe, positive-Gram and immobile. They are widely distributed in environment, particularly in soils, water reservoirs, rivers, lakes and others aquatic environments (Sánchez et al. 2004), they even have been isolated in extreme places like deserts and Antarctic (Fernandez 2013). Due to its high degradative compounds capacity were ideal candidates for bioprocess use, both in industrial and environmental applications (Sánchez et al. 2004).

Studies like Aráis (2003), showed the *Rhodococcus* sp. capacity to remove sulphate organic compounds from hydrosulfidation of diesel with low sulfur and high recalcitrant contents. While López et al. (2006) makes reference to their great metabolic diversity which can transform, biodegrade and utilized hydrophobic compounds as carbon source, besides accumulating heavy metals, so its use for bioremediation was relevant. This studies makes us asking if *Rhodococcus* sp. can be used and applied in others areas like aquaculture, due to recent researchers which identified that this bacteria is part of fish and crustacean microbiota, as noted by Dagmar (2012), which reported that *Rhodococcus* sp. is part of benefic intestinal microbiota of salmon.

Boutín et al. (2013), obtained positive results control pathogen bacteria Flavobacterium to psychrophilum using Rhodococus sp. strain in Salvelinus fontinalise culture. 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). In this bacteria genus it also was reported pigment production like astaxanthin and cantaxanthin, compounds used as food additives (Ingle de la Mora 2006). In aquaculture, pigments were also used to increase coloring in fishes and there were synthesized obtained from diverse microalgae (Lorenz y Cysewski 2000). However, their commercial costs are expensive, so there must be economical alternatives to obtain nature dves which



can used in aquatic production. Therefore, *Rhodococcus* sp. use in aquaculture show promissory advantages but still needs studies that confirm the results. That is why the aim of this study is to give a general overview about characteristics of *Rhodoccocus* sp. genus and their possible benefit effects to use it in aquaculture.

Rhodococcus sp. GENUS

The *Rhodococcus* sp. genus belongs to nocardiform actinomycetes group and subgroup of bacteria which have mycolic acids in their wall cells. The gender was identified by Tsukamura (1974) with six species: *R. aurantiacus, R. bronchialis, R. rhodochrous, R. roseus, R. rubropertinctus* and *R. terrae.* Later, Goodfellow and Anderson (1977), included three species: *R. coprophilus, R. corallinus, R. equi.*

In recent years, due to molecular biology techniques four species were included and this gender was redefined. In terms of shape, this gender was characterized to cluster bacteri with basilar shape, which can form vegetative ramose filaments, septate and fragmented in basilar and coccoid shapes (Pavia et al. 2005). They are Gram-positive bacteria (variability may occur), partially alcohol-acid resistance and aerobic. They are motile, grow in 30-37°C temperature and grow in common used laboratory media. They are find widely distributed in nature, both soil as all aquatic environments. Some strains can be human and animal pathogen, but they are rare because they only act as opportunistic way in immunosuppressed organisms, the most important is R. equi (Rabagliati et al. 2005) (Fig.1).

THE IMPORTANCE OF *Rhodococcus* sp. IN BIOREMEDIATION

The inadequate materials and harmful wastes management has generated in a worldwide scale a contamination problem of soils, air and water. The bioremediation practices consists in plant, fungi, and natural bacteria or genetically modified to neutralize

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Fig. 1: Rhodococcus sp.

toxic substances, transforming them less toxic substances or innocuous to environment and living beings (Schmidt 2000). Bacteria group are the most used in this process, but also fungi, microalgae and cyanobacteria are used. In *Rhodococcus* sp. gender case, it has a high metabolic diversity potential to hydrophobic compounds, like chlorinated hydrocarbons, phenolic compounds, steroids, lignin, carbon and petroleum (Finnerty 1992).

In Mexico, Mexican Petroleum Institute researcher's isolated *Rhodococcus* sp. from petroleum contaminated, a bacteria capable of desulphurize diesel samples, so they are developing some strategies for the use of this microorganism which objective was to alleviate oil discharges and increase recuperation effects. time of environmental damages (Barrios and Escobar 2011). Another relevant aspect with this bacteria is that it can transform contaminant nitrogenous compounds in aerobic and anaerobic conditions from soil and water, improvement the environmental quality. Considering that nitrogenous compounds are a risk factor in aquatic organism culture due to sensibility increase on fishes and crustaceans from these compounds, the use of bacteria capable to transform nitrogenous compounds results fundamentally for aquaculture optimization. Sánchez et al. (2004) mentioned that metabolism in some microorganisms, among them, *Rhodococcus* sp. can transformed nitriles in amides and acids throughout nitriles enzymes.

Rhodococcus sp. AS PROBIOTIC

Probiotic bacteria were defined like live microorganisms which administrated like food supplement can cause microbiota modifications associated to host gastrointestinal tract and generate beneficial effects like increase of food conversion, diseases resistance and water quality (Balcázar 2006). During last decade, their supply in fishes and crustaceans culture has become common, because various commercial products created for this porpoise have emerged (Villami et al. 2010). At same time, some researches have been designed to obtain specific endogenous probiotics and improved profits with commercial probiotics that is why several studies were focused to characterize the intestinal microbiota of aquatic organisms, which allow stablished different representative bacterial groups between various cultivated species (Monroy et al. 2012).

Previously it was considered that in aquatic organisms, the intestinal microbiota was formed by common bacteria from aquatic environment, mainly by gender: Aeromonas, Vibrios and Pseudomonas. However, different studies allowed to recognize a great bacterial variety at intestinal microbiota among other studied aquatic species like Rhodococcus sp. which recently was studied and reported as beneficial fish microbiota. Dagmar (2012), informed that Rhodococcus sp. was part of salmon intestinal microbiota. Hassan et al. (2010), identified intestinal microbiota from some freshwater pond species, where it was established *Rhodococcus* sp. presence in fishes, on sediment and culture water. Likewise, Boutín et al. (2013), obtained positive results to pathogen bacteria Flavobacterium control psychrophilum when Rhodococus sp. was used in Salvelinus fontinalise cultivation.

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PIGMENTSPRODUCTIONBY*Rhodococcus* sp.

Carotenoids are lipid soluble pigments extracted from plant, microalgae, zooplankton, fungi and bacteria. This pigments were responsible of great diversity of appreciated colors in nature and associated to various yellow, orange and red shades. The animals cannot produced them and must be obtained by the food (Ako et al. 2001). Carotenoids were used like food additives, in pharmaceutical and cosmetic industries. Also were used by their provitamin A activity, to strengthen immunity system and decrease degenerative diseases (Valduga et al. 2010). 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 sense, the Rhodococcus sp. genus has an important when there are cultured in nutritive mediums, because their colonies showed mucoid, smooth or roughened shapes, pigmented with cream, red or orange color, indicating carotenoids presence (Fig. 2).

With respect to fish culture, a fundamental criteria for their acceptation is the visual impact of their meat with pink-red, orange-red colors, since consumers prefer products with orange color like salmonids. Likewise, in ornamental fishes, the skin pigmentation, both body and fins shapes are most important quality criteria in market. Fish and others animals cannot synthetized and depend about diet pigmentation supplements (Gouveia 2003). Some synthetic carotenoids like canthaxanthin, zeaxanthin and astaxanthin were used like additives in fish and crustacean diets (Kalinowski *et al.* 2005). But because commercial acquisition of these pigments is expensive, the use of microorganisms like *Rhodococcus* sp. can contributed to obtain cheaper nature pigments.

Because of this, *Rhodococcus* sp. genus potential is encouraging, as it can be utilized as probiotic in aquaculture, because it forms part of some fish beneficial microbiota as well as it can be proved their competitive exclusion capacity versus *Flavobacterius psycophilum*. Otherwise. their metabolic and xenobiotic capacity must improve water quality of aquatic organisms cultured while improving species coloring due to nature pigments production. It is important to mention that Rhodococcus sp. strain selection is fundamental, because it exist some opportunistic pathogen species like *R. equi*, while it has not been reported in aquatic organisms, relevant studies to assure their use and apply in aquaculture should be done.



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BIBLIOGRAPHY

- Ako H, Tamaru CS, Brown C, Bailey R, Cole B. (2001).
 A Manual for Commercial Production of the Swordtail, *Xiphohporus helleri*. Honolulu: University of Hawaii Sea Grant Extension Services: 3-16.
- Arais GM. (2003). Evaluación de la actividad desulfuradora de la cepa *Rhodococcus globerulus* en diferentes sustratos. Revista Chilena de Infectología 22(2): 155-160.
- Balcázar I, Blas I, Ruiz-Zarzuela I, Cunningham D, Vendrell D. (2006). The Role of the Probiotics in Aquaculture. Veterinary Microbiology 114(3): 173-186.
- Barrios GO, Escobar BV. (2011). Natural-Fiber-Reinforced Composite Treated with Polyamines Used as a Support for the Biodegradation and Adsorption of Toluene. Industrial & Engineering Chemistry Research 50(12): 7572–7578.
- Boutin S, Audet C, Derome N. (2013). Probiotic treatment by indigenous bacteria decreases mortality without disturbing the natural microbiota of *Salvelinus fontinalis*. Canadian Journal of Microbilogy 59: 1-9.
- Dagmar WB. (2012). Caracterización bacteriana de intestino de Salmón del atlántico adulto. Tesis de Licenciatura. Bioquímica, Universidad Austral de Chile. 82 p.
- Goodfelow M, Anderson G. (1977). The actinomycetegenus *Rhodococcus*: a home for the *Rhodochrous complex*. Journal of General Microbiology 100: 99-122.
- Gouveia L, Rema P, Pereira O, Empis J. (2003). Colouring ornamental fish (*Cyprinus carpio* and *Carassius auratus*) with microalgael biomass. Aquaculture Nutrition 9(3), 123–129.
- Fernández HL. (2013). Estudio genético y bioquímico de la ruta de degradación del colesterol en Rhodococcus spp. Universidad Complutense de Madrid. Tesis de doctorado: 136p.
- Finnerty WR. (1994). Biosurfactants in environmental biotechnology. Curr. Op. Biotechnol. 5: 291-295
- Hassan, IE, Viola, HZ, Abdallah ME, Dinna AE. (2010). Studies on the effects of bacterial diseases on skin and gill structure of *Clarias gariepinus* in Dakahlia Province, Egypt. Annals of Biological Research 1 (4): 106-118.



- Ingle de la Mora G. (2006). Comparison of red chilli (*Capsicum annuum*) oleoresin and astaxanthin on rainbow trout (*Oncorhynchus mykiss*) fillet pigmentation. Aquaculture 258: 487-495.
- Kalinowski TLE, Robaina H, Fernandez-Palacio, S Schuschardt D, Izquierdo MS. 2005. Effect of different carotenoid sources and their dietary levels on red porgy (*Pagrus pagrus*) growth and skin colour. Aquaculture 244: 223-231.
- López JB, Quintero G, Guevara AL, Jaimes DC, Gutiérrez SM, García JM. (2006). Bioremediación de suelos contaminados con hidrocarburos derivados del petróleo. Revista Nova- Publicación científica 4(5): 5-6.
- Lorenz RT, Cysewski GR. (2000). Commercial potential for *Haematococcus* microalgae as a natural source of astaxanthin. Trends Biotechnol 18: 160-167.
- Monroy DMC, Castro BT, Fernández PF, Mayorga RL, Herrera GH y Cortés SS. (2012). Bacteria with Probiotic Capabilities Isolated from the Digestive Tract of the Ornamental Fish *Pterophyllum scalare*. Capítulo 10. pp. 231-246. En: Probiotics in animals. INTECH. Croacia. DOI: 10.5772/3319 <u>http://www.intechopen.com/books/bookstat/probiotic-in-animals</u>.
- Pavía P, Calderon C, Puerta C. (2005). Diferenciación de especies de *Rhodococcus* mediante una prueba de PCR-RFLP basada en los genes codificantes para la subunidad 16S ribosomal. Revista Nova-Publicación científica 3(4): 14-15.
- Rabagliati B, Morales S, Baudrand AB, Jorquera RA, Oddó BJ, García CD, Carmona PP, Cisternas MC, Huete GÁ. (2005). Neumonía cavitada por *Rhodococcus equi* en paciente inmunocomprometido no infectado por virus de inmunodeficiencia humana: Caso clínico y revisión. Revista chilena de infectología 22(2): 155-160.
- Sánchez N, Sandoval AH, Díaz CF, Serrano JA. (2004). El género *Rhodococcus*. Una revisión didáctica. Revista de la Sociedad Venezolana de Microbiología 24: 1-2.
- Schmidt W. (2000). Suelos contaminados con hidrocarburos: la biorremediación como una solución ecológicamente compatible. Cooperación Técnica Alemana (GTZ). En: www.gtz.org.mx/sitioscontam/articulos/biorremed Mex2.pdf

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- Valduga E, Valerio A, Tatsch PO, Treichel, H, Furigo A, Luccio, MD. (2009). Assessment of Cell Disruption and carotenoids extraction from Sporidiobolus salmonicolor (CBS 2636). Food. Bioproc. Technol. 2:234-238.
- Villamil L, Figueras A, Planas M, Novoa B (2010). *Pediococcus acidilactici* in the culture of turbot (*Psetta maxima*) larvae: Administration pathways. Aquaculture 307:83–88.

Accepted: April 23rd 2016