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# Presumptive probiotic isolated from *Oncorhynchus mykiss* (Walbaum, 1792), cultivated in Mexico

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**Abstract:** The intestinal microbial community of fish is determining by biotic and abiotic factors, and depending on the niche they occupy, we can distinguish between transient and permanent microorganisms. The last ones, are important for aquaculture, due to its probiotic potential. Three adult of *Oncorhynchus mykiss* were used to extract their intestine to isolate the bacteria present and tested to determine probiotic ability: resistance to acidic pH and bile, adhesion, hemolytic activity, antibiotic susceptibility, and *in vitro* antagonism. Bacteria groups were identified by sequencing the 16S rRNA. Fourteen bacterial strains were obtained, of which the strains *Bacillus pumilus*, *Shewanella xiamenensis*, *Bacillus* sp. and *Bacillus methylotrophicus* were able to grow at acidic pH in the presence of bile and only *S. xiamenensis* showed α-hemolytic activity. *B. pumilus* and *Bacillus* sp. had better adhesion and antagonism *in vitro*. We can conclude that *B. pumilus* and *Bacillus* sp. presented potential probiotics *in vitro* and is recommendable to evaluate them in rainbow trout to determine what benefits they can offer to this fish.

**Keywords:** *Oncorhynchus mykiss*, *Bacillus* spp., probiotics

## Introduction

The microbial community of the gastrointestinal tract (GIT) of fish is determined by biotic and abiotic factors, environment conditions, interaction with other organisms, and consumed food (Pereira *et al*., 2011; Sullam *et al*. Ingerslev *et al*., 2014). There are two types of bacteria present in fish: transient and permanent. Permanent bacteria are directly related to body section in which they are located and the stage of development. In the case of healthy adult fish, the bacteria present in GIT are considered as permanent. Transient bacteria are founded, mostly, in the skin and gills (Cahill, 1990).

Permanent microorganisms colonize the fish GIT in their initial stages and are maintained during the host live cycle, so they must be found in wild and cultured organisms. These microorganisms were located principally at intestinal mucosa, and proximal or distal part of GIT (Ringø and Birkbeck, 1999). Studies on the intestinal microbiota of fish have focused on the evaluation of bacteria as bioindicators of water bodies (Geldreich and Clarke, 1966) and principally to identified pathogenic microorganisms that seriously affect aquaculture production (Sharifiyazdi *et al*., 2010; Huang *et al*., 2013). However, in recent years, the interest to analyze microbiota communities located in fish GIT, was to develop probiotic sources (Spanggaard *et al*., 2000; Gómez and Balcázar, 2008), because they have a significant role to maintain fish health conditions (Sugita *et al.,* 1996; Brunt *et al.,* 2007; Gómez and Balcázar, 2008); by competing with pathogenic bacteria, which provide molecules that improve host nutrition (Verschuere *et al*., 2000, Balcázar *et al*., 2006). The relationship between resident bacteria and the host, suggests an association history over the fish life cycle (Ringø and Gatesoupe, 1998; Hong *et al*., 2005; Gómez and Balcázar, 2008). All previous about microorganism's research with probiotic potential in adult fish GIT, involves the possibility of obtaining them (Ringø and Gatesoupe, 1998; Hong *et al*., 2005; Gómez and Balcázar, 2008).

The development of probiotics in aquaculture has gained importance because due to benefits that these microorganisms can offer to aquaculture activity (Akhter *et al*., 2015; Hai, 2015). However, in Mexico there are few studies that have been carried out on

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probiotics development, obtained from the fish autochthonous microbiota. Based on the importance of *Oncorhynchus mykiss* culture in our country, and the need to develop and implement sustainable biotechnologies, this research aimed to obtain bacteria with probiotic characteristics present in the intestine of rainbow trout locally cultivated, and the possibility to apply them on Mexican trout culture.

## Materials and methods

### Obtained fishes and euthanasia

The trout's (average of ~25 cm and 300 g) were obtained from a stock belongs to FES-Iztacala-UNAM Aquaculture Production Laboratory, and maintained in fast during 24 h (Monroy-Dosta, 2010; Woynarovich *et al*., 2011). Fish euthanasia was made according the American Association of Veterinary Medicine (2013), using a lethal dose of benzocaine (>250 $mgL^{-1}$). Thereafter, all procedures were made under aseptic conditions.

### Isolation of bacteria

Fish ventral zone was cleaned with ethanol (70%). After aseptically GIT removing, it was rinse with sterile saline solution (0.89%) to remove residual food and feces. The tissue was placed in sterile saline solution to homogenize it at 1:10 (w/v) proportion. From the homogenate, three decimal sequential dilutions were made, and aliquots were inoculated in three culture media: BHI, MRS and TCBS (DifcoTM and BBLTM Manual, New Jersey). Agar plates were incubated at 32 °C for 24 to 48h. Each differentiated bacteria colony was subculture onto culture plates. Once isolation was achieved, the cell morphology was determined by Gram stain (Madigan *et al*., 2012), and was used for subsequent identification.

### Bacterial identification

The identification of isolated bacteria was done by sequencing the gene encoding of 16S of rRNA (Han, 2006; Mignard and Flandrois, 2006; Janda and Abbott, 2007), and amplified by the polymerase chain reaction (PCR), based in the methodology described by Hamdan (2004) and Sambrook and Russel (2011). The DNA was extracted using the Wizard® Genomic DNA Purification Kit (PROMEGA®, Wisonsin, USA), following manufacturer instructions.

The PCR was made with the Master Mix® PCR kit (PROMEGA®, Wisonsin, USA). The amplification reaction had a total volume of 25µL, 0.1µL of *Taq* polymerase, 0.75µL of MgCl2, 0.4µL of the deoxynucleoside triphosphate mixture, 0.8 µL of the 8for primer. (5'-AGACTTTGATCMTGGCTCAG-3'), 0.8 µL of the 1492rev primer. (5'-TACGGYTACCTTGTTACGACTT-3'), 2.0 µL 10x colorless buffer, 14.15 µL molecular water and 1µL genomic DNA (Hamdan, 2004). The reaction was induced in the thermocycler (Bio-Rad® My Cycler, California, USA), with the following profile, a pre-incubation cycle at 94°C for 5 min; 40 cycles of denaturation at 94°C for 38 s, hybridization at 52°C for 40 s and pre extension at 72°C for 40 s; followed by an extension cycle at 72°C for 7 min and finally, a final cooling cycle at 4°C. PCR amplicons were visualized on agarose 1.5% and stained with ethidium bromide (0.5 $µgmL^{-1}$), exited under ultraviolet illumination (300 nm).

PCR products were purified with the Illustra® Exoprostar® kit (GE®, Connectivut, UEA); sequencing was made on Macrogen® Inc. and the information obtained was analyzed using the NCBI Blast algorithm and compared with the sequences available in the GenBAnk data base (https://www.ncbi.nlm.nih.gov/genbank/).

### Initial Selection Criteria

The initial bacteria selection from 14 strains identified, was carried out on the following criteria: a) strains that have not been reported as pathogenic (FAO and WHO, 2001), for aquatic animals and for humans (Borch *et al*., 2015), and b) bacteria isolated from aquatic systems or belonging to the microbiota of fish or aquatic animals (Verschuere *et al*., 2000, Vine *et al*., 2004, Balcázar *et al*., 2006).

### Tests for probiotic characterization of bacteria

Tolerance to acidic pH and bile

The pH of the BHI broth was adjusted at 1.5, 2.5 and 3.5 with hydrochloric acid (HCl). The tolerance to bile salts was made with bile of rainbow trout, which was extracted with a sterile syringe under aseptic conditions from the gallbladder, and stored at -20°C until use (Pérez-Sánchez *et* al., 2011; Burbank *et al*., 2012). Three final concentrations were prepared 1%, 2% and 3%. In both tests, 20 µL of the cell suspension was inoculated at an absorbance (ABS) of 0.5, and the exposure in the two conditions was carried out at 30 °C for 24 h. The initial and final ABS were measured at 0 h and at 24 h; from them the change of ABS in percentage was calculated.

*In vitro* adhesion

The adhesion capacity of the bacteria to fish intestinal epithelium was determined by adhesion to toluene

(Vinderola and Reinheimer, 2003, Vine *et al*., 2004). In this case, the method described by Pérez-Sánchez *et al*. (2011), with some modifications was used. Cells were collected by centrifugation at 10,000 rpm at 25°C for 5 min from a broth culture in BHI and an inoculum with an ABS 0.5 suspended in 50mM K2HPO4 (pH 7.4) was used. The mixture of the toluene and the cell suspension was made in a ratio of 1:5 and vortexed (Vortex Maxi Mix II, Thermo Scientific) for 2 min at maximum speed. The samples were incubated at 30°C for one hour and phases separation was allowed. The ABS of the aqueous phase was measured and the adhesion percentage (H%) was determined by the formula: $H\%=[(ABS_0-ABS)/ABS_0]X100$;

Where ABS and $ABS_0$ are the initial and final optical densities, respectively. The decrease in ABS of the aqueous phase was interpreted as a greater adhesion capacity (Collado and Salminen, 2009).

Hemolytic activity

BHI agar petri dishes were prepared with 5% lamb defibrinated blood and 100 µL of a density of 1 x $10^7$ CFU $mL^{-1}$ of bacteria suspension was seeded. The plates were incubated at 30 °C, and were analyzed after 24 h and 48 h. Results were classified in three types of hemolytic activity, α, β or γ (Buxton *et al*., 2006; Leboffe and Pierce, 2011; Madigan *et al*., 2012), the strains with last type were selected.

Susceptibility to antibiotics

This assay was performed by agar diffusion technique and the Polidiscos system (PBM S. A de C. V., Mexico City). The kit has 12 antibiotics for Gram positive bacteria (Cephalotina30, ciprofloxacin5, clindamycin2, erythromycin15, fosfomycin50, gentamicin10, nitrofurans 300, oxacillin1, penicillin G10, tetracycline10, trimethoprim sulfamethoxasol25 and vancomycin30) and Gram negative (Cephalotin30, ciprofloxacin5, fosfomycin50, gentamicin10, nitrofurans300, tetracycline10, trimethoprim sulphamethoxasol25, amikacin30, ampicillin10, ceftazidime30, netilmicin30 and norfloxacin10), at different concentrations in micrograms. The plates were incubated at 30°C for 24 h. The criteria proposed by the manufacturer were used to determine the sensitivity or resistance of antibiotics used on evaluated strains.

*In vitro* antagonism

Two bacterial strains of aquatic importance, *Aeromonas hydrophila* (Fattahi *et al*., 2015) and *Enterobacter sakazakii* (Monroy-Dosta *et al*., 2015), isolated from infected *Carassius auratus*, were separately inoculated into BHI petri dishes, at 1 x $10^7$ CFU $mL^{-1}$. The agar diffusion method was used (Madigan *et al*., 2012). Filter paper (Whatman No. 5), 7 mm Ø, which were immersed during 5 min in a cell suspension at 1 x $10^7$ CFU $mL^{-1}$ of presumptively probiotic bacteria, discs were extracted and then were placed in the center of the agar plates. The incubation was performed at 30°C after 24h and the zones of inhibition were measured. In some cases, the incubation time was prolonged to 48h. Presumptively probiotic strains that generated zones of inhibition were selected.

**Statistical analysis**

All experiments were carried out by triplicate. The results are expressed as means with their standard error. The data were checked for normal distribution with Kolmogorov-Smirnov test. The statistical differences between strains inside of a test were assessed by the one-way analysis of variance ($P<0.05$), with *Tukey* ($P<0.05$) *post hoc* test (Montgomery, 2011; Zar, 2010). All statistics was made using SYSTAT® ver. 12.0 for Windows®.

## Results

**Bacteria identification**

Fourteen strains were identified, of which 93% had a bacillary form and the rest were coccus. The 71% of them were aerobic, 14% anaerobic and the rest anaerobic facultative (15%). Most of the bacteria strains (79%), belong to Phylum Firmicutes, 7% to Gamaproteobacteria, 7% to Actinobacterias, and an unknown clone. Based on the initial selection criteria (FAO and WHO, 2006; Vine *et al*., 2004), six strains (1, 2, 4, 8, 9) of Table 1 were selected for *in vitro* characterization as probiotics.

**Probiotic characterization of bacteria**

Under acidic conditions, all strains were able to survive and growth. However, three were selected, *Bacillus pumilus*, *Bacillus* sp. and only *Bacillus methylotrophicus* was significantly different ($P<0.05$) from the rest of the strains (Fig. 1). From the four strains that could growth under acidic conditions, was *Shewanella xiamenensis* that showed α-type hemolytic activity.

After acidic pH test, only three strains were select for the bile tolerance. They were able to survive and

**Tab. 1: Bacteria isolated from the intestine of *O. mykiss*.**

| Description | Metabolism | Form | Gram | Taxonomic group |
|---|---|---|---|---|
| *Bacillus pumilus* SG2 | A | BM | + ó V | Firmicutes |
| *Shewanella xiamenensis* BC01 | AF | BM | - | Gamaproteobacteria |
| *Bacillus* sp. NIOT-3 | A | B | - | Firmicutes |
| *Bacillus* sp. CNJ732PL04 | A | B | - | Firmicutes |
| *Bacillus subtilis* BN1 | A | BM | + | Firmicutes |
| Clone bacteria not cultured nbw1184b05c1 | A | C | - | - |
| *Clostridium* sp. K27 | An, AF | B | + | Firmicutes |
| *Bacillus subtilis* yxw4 | A | BM | + | Firmicutes |
| *Bacillus methylotrophicus* IS04 | A | BM | + | Firmicutes |
| *Bacillus subtilis* CYBS-5 | A | BM | + | Firmicutes |
| *Clostridium sporogenes* 39NIG1 ana4-1 | AF | BM | + | Firmicutes |
| *Bacillus subtilis* F121112 | A | BM | + | Firmicutes |
| *Bacillus* sp. ACH-14L-88 | A | B | - | Firmicutes |
| *Rhodococcus* sp. JSM 2215131 | A | BR | + ó V | Actinobacteria |

Note: A: aerobic. An: anaerobic. F: facultative. B: bacillus. C: coccus. N: no. M: mobile. V: variable.

growth in the three concentrations used and *B. methylotrophicus* displayed the smallest increase in ABS (Fig. 2).

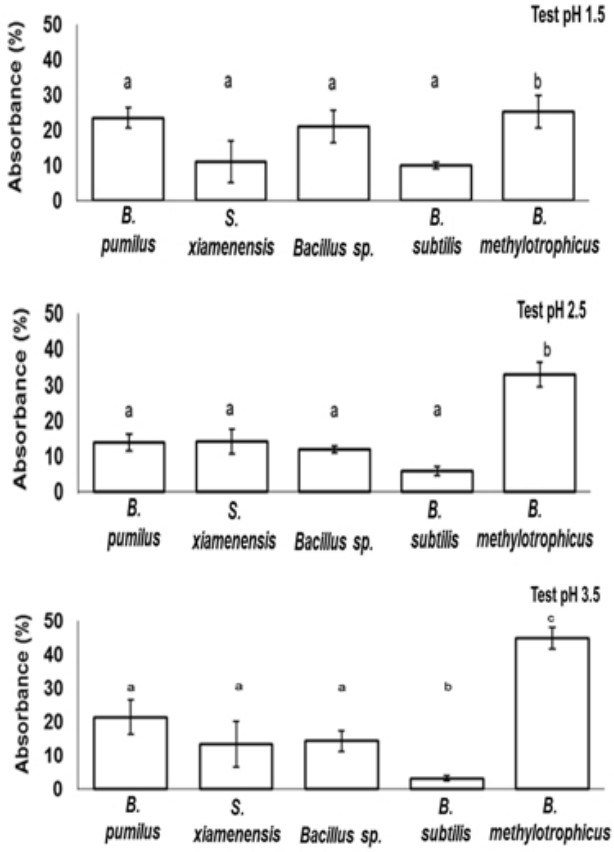


**Fig. 1: Change in absorbance of five bacterial strains isolated from *O. mykiss* intestine, in BHI at three pH´s. Different letters between column bars show significant differences (p<0.05).**

The same three strains were tested to determinate it adhesion capacity and were *B. pumilus* and *Bacillus* sp. that performed better, with -42.3% ± 6.4% and -12.1% ± 2.1% decreases in ABS, respectively; while *B. methylotrophicus* had -4.4% ± 1.4% (Fig. 3).

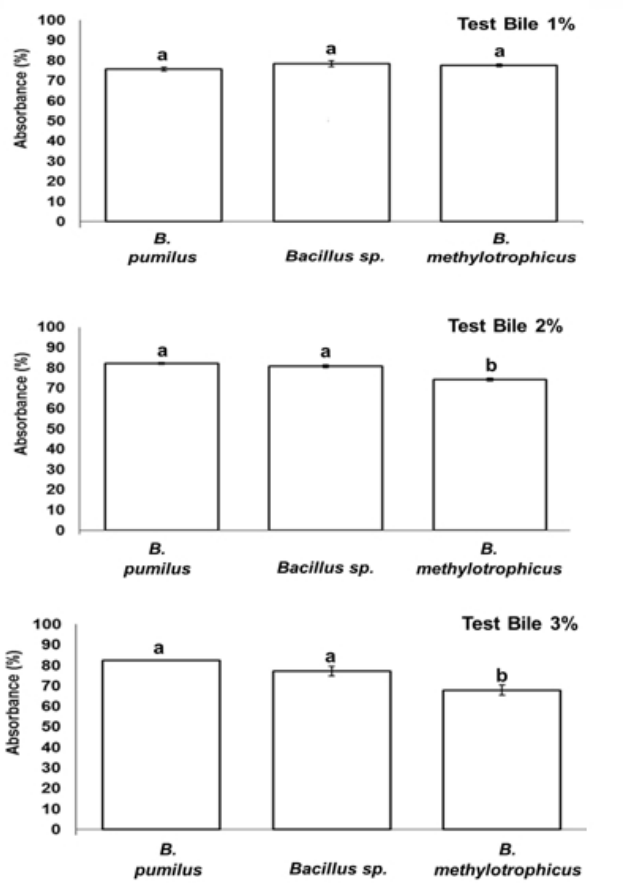


**Fig. 2: Change in absorbance of three bacterial strains isolated from *O. mykiss* intestine, in BHI at three bile concentrations. Different letters between column bars show significant differences (P<0.05).**

The antagonism evaluation *in vitro* show that the same strains generated zones of inhibition against *A. hydrophila* and *E. sakazakii. Bacillus* sp. strain produce bigger zones (17 mm ± 1.13 mm and 16 mm ± 1.73 mm respectively). *B. methylotrophicus* did not generate zones of inhibition (Fig. 4).

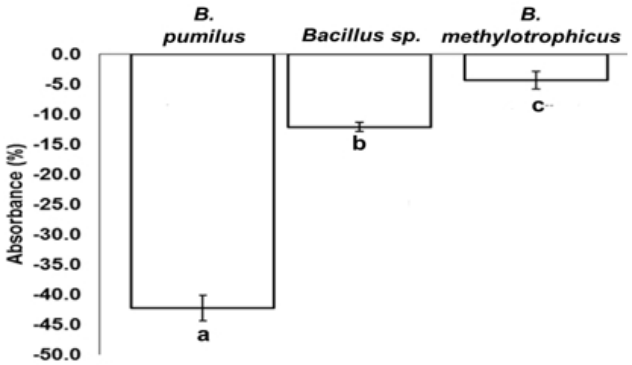


**Fig. 3: Change in absorbance of three bacterial strains isolated from *O. mykiss* intestine exposed to toluene. Different letters between column bars show significant differences (P<0.05).**

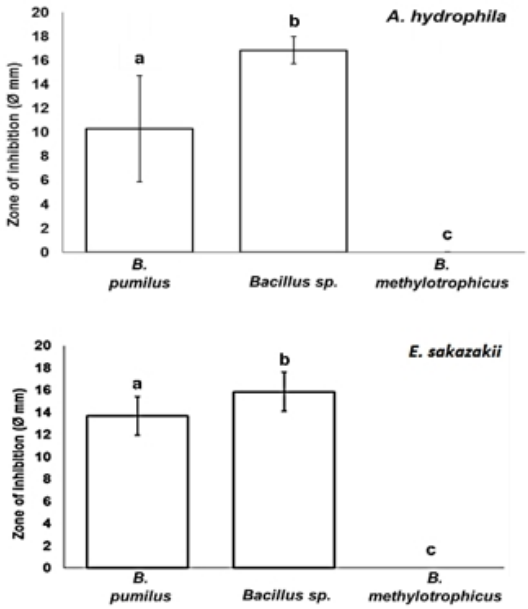


**Fig. 4: *In vitro* antagonistic activity of three bacterial strains isolated from the intestine of *O. mykiss* against of two pathogens in aquaculture. Different letters between columns bar show significant differences (P<0.05).**

The antibiotic sensitivity test show that *Bacillus* sp. was sensitive to 50% of antimicrobials; while *B. pumilus* and *B. methylotrophicus* were sensitive to 75% and 83% respectively of antibiotics used (Tab. 2).

## Discussion

The bacterial groups identified in this work correspond mainly to Phylum Firmicutes (Gamaproteobacteria and Actinobacteria), which agrees with that reported by Spanggaard *et al*. (2000) and Ingerslev *et al*. (2014), which mentioned that bacillary forms were dominant. In general terms, this bacteria form corresponds to bacterial community of carnivorous freshwater fishes, mainly salmonids (Sullam *et al*., 2012). The presence of *Clostridium* sp. in the intestine of rainbow trout can be explained by the fact that the microbial community present in GIT, is influenced by the microorganisms present in the environment (Cahill, 1990, Ringø *et al*., 1995, Novotny *et al*., 2004). This genus is commensal for humans (Ivanov and Honda, 2012; Lopetuso *et al*., 2013), and is usual found in GIT of rainbow trout (Etyemez and Balcázar, 2015). It is possible that their presence in GIT, may be due to organism's management in culture systems (Spanggaard *et al*., 2000).

**Tab. 2: Antibiotics sensitivity of three bacteria strains isolated from O. mykiss intestine with probiotic potential.**

| Antibiotic (µg) | Gram + | | Gram - |
|---|---|---|---|
| | BPU | BME | BSP |
| Cephalotin 30 | S | S | R |
| Ciprofloxacin 5 | S | S | S |
| Clindamycin 2 | S | S | NA |
| Erithromycin 15 | S | S | NA |
| Phosphomicyn 50 | R | S | S |
| Gentamicin 10 | S | S | S |
| Nitrophuran 300 | S | S | S |
| Oxacillin 1 | R | S | NA |
| Penicillin G10 | R | R | NA |
| Tetracycline 10 | S | R | R |
| Trimethoprim-Sulfamethoxasol I25 | S | S | R |
| Vancomycin 30 | S | S | NA |
| Amikacin 30 | NA | NA | S |
| Ampicillin 10 | NA | NA | R |
| Ceftazidime 30 | NA | NA | R |
| Netilmicin 30 | NA | NA | R |
| Norfloxacin 10 | NA | NA | S |

Note: BPU: *B. pumilus*, BME: *B. methylotrophicus*, BSP: *Bacillus sp*., S: sensitive, R: resistant, Na: not apply

The results obtained show that *B. pumilus*, *Bacillus* sp. and *B. methylotrophicus* can survive and growth in stomach pH range that *O. mykiss* has. The pH values used in the test correspond to those of rainbow trout during digestion (Kapoor *et al*. 1975; Hidalgo *et al*., 1999, Furné *et al*., 2005). Krogdahl *et al*. (2015), mentioned that pH of carnivorous fishes can be the same to those trout fishes show. Although there are no reports on bile concentrations during the digestion of this fish, three concentrations (1%, 2% and 3%) were used, in which all three strains were able to survive and reproduce, regardless of the concentration used. When *B. pumilus* was exposed at pH 2.2 and bile 0.3% - 2% tests *in vitro* to evaluate it as a probiotic in pigs, show the same behavior as that obtained in this study (Prieto *et al*., 2014). This has also been observed in *B. methylotrophicus* exposed to pH of 2.5 and bile of 0.5%, simulating human GIT conditions (Sim *et al*., 2014).

In the case of the bile test, the behavior observed may be due to bile concentrations used, because it not generates bacteria stress. Bile can be used as enricher of selective culture media (such as TCBS), where the concentration is ~ 2% (DifcoTM and BBL™, 2009). For example, when some lactic acid bacteria were evaluated with various concentrations of bile, 0% - 1% showed tolerance, and only significant differences existed in two of the five concentrations used (Pérez-Sánchez *et al.,* 2011). Although, when concentrations of 10% of bile have been used, several bacterial strains survived and growth, as Burnabk *et al*. (2012) reported; considering that bile concentration in fish GIT was 0.4% to 1.3% (Balcázar *et al*., 2008). However, it is important to highlight that it is necessary to know the specific concentrations of bile *in situ*, during digestion, as specific manner, to made *in vitro* test, as well as the biological process occur.

Regarding the biosecurity of evaluated strains, only *S. xiamenensis* presented α-hemolytic activity, and was discarded in the subsequent tests. Although there are no reports on any type of pathogenicity. However, the α-hemolytic may indicate hemolytic toxins productions (Santos *et al*., 1999; Gomathi *et al*., 2013) and represent a risk for humans and animals. For this case, no hemolytic activity was detected in the strains of *Bacillus* genus used. Luis-Villaseñor *et al*. (2011), reported that strains belonging to this genus showed the three types of hemolysis, since the absence of this phenomenon in bacteria is an indication of non-virulence (De Vuyst *et al*., 2003).

The two strains with better adhesion were *B. pumilus* and *Bacillus* sp., Which would involve that they can be established in the intestinal mucosa of rainbow trout. In any case, these strains were isolated from healthy adult fish, indicative that they can be established and proliferate at rainbow trout GIT. Guidoli *et al*. (2015) reported that strains of the genus *Bacillus* can adhere and had *in vitro* self-aggregation. Although it has been observed that adhesion is not a constant behavior in strains belonging to *Bacillus* genus. Mahdhi *et al*. (2011), only found that two of three strains had *in vitro* adhesion capacity, according with this work. The importance of evaluating this feature in potential probiotic strains differs greatly from bacterial adherence from the clinical point of view. In the latter case, the ability of adhesion to biological and non-biological materials defines its potential to develop an infection (Boland *et al*., 2000). In contrast, microorganisms considered as probiotics, must have the ability to adhere to biological materials (Ibrahim *et al*., 2004), *ergo*, intestinal epithelium, since this is associated with the benefits that probiotics can offer to the host (Verschuere *et al*., 2000, Pandiyan *et al*., 2013, Balcázar *et al*., 2008). However, a difficulty exists of evaluating it *in vivo* makes the *in vitro* tests a good option as a preliminary measure to evaluate this variable (Duary *et al*., 2011).

In addition, the growth of these strains was also determined in the presence of two pathogens for fish, *A. hydrophila* and *E. sakazakii*, in culture systems; acting as opportunistic, although they represent a latent problem in commercial aquaculture (Bruno *et al*., 2013; Monroy-Dosta *et al*., 2015). *B. pumilus* and *Bacillus* sp. showed *in vitro* antagonism, but not *B. methylotrophicus*. Many of the probiotics used in aquaculture belong to *Bacillus* genus (Panigrahi *et al*., 2007, Bagheri *et al*., 2008, Olmos and Paniagua-Michel, 2014). Sugita *et al*. (1996) and Brunt *et al*. (2007), emphasize the ability of this genus to compete against pathogens *in vitro* and *in vivo*. Particularly, Lalloo *et al*. (2007), found that *Bacillus* spp. had antagonistic activity against *A. hydrophila*. Aly *et al*. (2008), reported antagonistic activity *in vitro* by *B. pumilus*; Hill *et al*. (2009), obtained that this strain had the greatest capacity of inhibition against several species of *Vibro* spp. genus, contrary to that observed in this case, where *Bacillus* sp. was better. On the other hand, Liu *et al*. (2015), found that *B. pumilus* B16 strain had antagonism against *V. parahaemolyticus*; although the proven benefit of this strain has been on host growth as part of a bacterial consortium (Avella *et al*., 2010). Regarding *B. methylotrophicus*, there are no reports on its behavior antagonistic activity *in vitro*.

There are no references that the strains used have antibiotic resistance plasmids. However, all three had resistances to some of antibiotics used. This feature represents an important characteristic for probiotics development, since the use of these compounds in aquaculture is a common practice. This type of chemotherapy significantly alters the fish GIT microbiota, increasing the possibility of infection (Merrifield *et al*., 2010; Romero *et al*., 2012). Theoretically, bacteria (Johnsborg *et al*., 2007) and yeasts such as *Saccharomyces* sp. (Kawai *et al*., 2015) can suffer transformations, a phenomenon that has important implications in aquaculture, due to the presence in the environment and in fish GIT, of pathogenic strains resistant to antimicrobials. In this context, the resistance observed in strains with probiotic traits would mean a problem for the

development of this type of products.

Few evidence exist in relation to obtaining probiotic, application and observed benefits in the same host (Kumar *et al*., 2008). However, we can infer that the development of autochthonous probiotics is the best option (Waché *et al*., 2006; Vieira and Tavares, 2012), because there is an adaptation process between them (Kesarcodi- Watson *et al*., 2008; Sullam *et al*., 2012). Nevertheless, probiotics used in humans (Nikoskelainen *et al*., 2003) and swine (Sealey *et al*., 2009), tested in fish with good efficiency. Nevertheless, some author recommend that the probiotics must be autochthonous (Cross 2002, Vine *et al*., 2004, Ringø *et al*., 2010).

The obtaining of different bacteria from rainbow trout GIT, as well as its determination of their possible probiotic capacity, not only confirms the wide diversity of microorganisms in different organisms studied (Austin, 2011; Ingerslev *et al*., 2014; Etyemez and Balcázar, 2015), of which, few, can be considered beneficial (Hill *et al*., 2009, Pérez-Sánchez *et al*., 2011, Burbank *et al*., 2012). The strains found in this study, allows to increase the database of bacteria with probiotic capacity, which can be used in aquaculture.

## Conclusion

The information obtained in this study indicates that not all bacteria isolated from the intestine of rainbow trout, have the capacity to tolerate *in vitro* conditions like the GIT environment of this fish. We can conclude that there are bacteria in the intestine of *O. mykiss* has probiotics characteristics. This highlights the importance of this type of studies, before considering the use of these products.

The next phase in the development of a probiotic, after the *in vitro* tests, indicates its evaluation on a host to determine which are benefits that the presumptively probiotic strains, *B. pumilus* and *Bacillus* sp., can offer to rainbow trout.

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