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Microbiota of freshwater catfish species, Filhote (*Brachyplatystoma filamentosum*) and Dourada (*Brachyplatystoma rousseauxii*) from the Amazon Region (Belém-Pará-Brazil)

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This paper identified the bacterial flora composition and its influence on the microbiological spoilage process in economically important fish species: Filhote (*Brachyplatystoma filamentosum*) and Dourada (*Brachyplatystoma rousseauxii*) in the Amazon region. Microbiological characterization was performed: counts of total mesophilic aerobic bacteria, psychrotrophic bacteria and coliforms at 35 and 45°C, respectively. Bacteria were also isolated through seeding in agar surface using Violet Red Bile Glucose (VRBG) for enterobacteria strains and Baird-Parker Agar with Egg-Yolk Tellurite for *Staphylococcus* species, both with incubation at 36°C for 48 h. The bacteria isolated were identified using the API 20E kit (Enterobacteria), and Gram-positive bacteria with API Staph (Staphylococci). Finally, the lag phase for strain growth was measured using spectrophotometry readings (620 nm) at different temperatures (10, 15 and 37°C). The mesophilic aerobic bacteria counts for fresh fish samples ranged from 5.21 to 7.64. The count ranges of psychrotrophic aerobic bacteria found were 5.11 – 6.91 log CFU/g. They also had an average score above 10^3 MPN/g for total coliforms. The most predominant were *Hafnia alvei*, *Pseudomonas luteola*, *Staphylococcus xylosus* and *Staphylococcus lugdunensis.* When subjected to temperature of 10°C, the strains achieved growth after 6 h ($p<0.05$).

Key words: Spoilage, catfish, *Brachyplatystoma filamentosum, Brachyplatystoma rousseauxii.*

INTRODUCTION

Fish is one of the most nutritionally complete foods due to the availability of large amounts of essential nutrients such as high-biological-value protein, vitamins- especially A and D, lipid fraction, high unsaturated fatty acids content and low cholesterol concentration (Córser et al., 2000; Koffi-Nevri et al., 2011). These nutrients present in fish provide a good medium for microbial growth responsible for the overt spoilage and thereby establishes

product shelf life (Oliveira et al., 2008; Gram, 1995; Gram and Huss, 1996).

The spoilage of fresh fish by microbial activity is usually due to its microbiota located mainly in the outer surfaces (skin and gills) and in the intestines of live and newly caught fish (Amaral and Freitas, 2013). It can also be the consequence of fish cross-contamination associated with inappropriate handling and storage (Cruz-Romero et al., 2008). It is known that the flora in tropical fish often carries a slightly higher load of Gram-positive and enteric bacteria than fish from temperate waters. But also, it can be similar to that flora dominated by psychrotrophic Gram-negative, rod-shaped bacteria belonging to the genera Pseudomonas, Moraxella, Acinetobacter, Shewanella, Flavobacterium, Vibrionaceae, Aeroemonadaceae and to a lesser degree, Bacillus, Micrococcus, Clostridium, Lactobacillus and Corynebacterium (Liston, 1980; Apun et al., 1999; Austin, 2002; ICMSF, 2005).

In the Amazon River (Brazil), fish species represent one of the most important resources and significantly contribute to the local economy, playing a vital role in the local diet as one of the primary sources of protein for the majority of the population (Angelini et al., 2006). The catfish including filhote and dourada constitute a valuable resource and are exported to several countries (Angelini et al., 2006).

Filhote or piraiba (*Brachyplatystoma filamentosum*) is one of the most consumed fish species in the Amazon region. It holds great commercial value for its good taste and high yield in the filleting process. It can reach up to 2.50 m in length and 300 kg in weight. It is also considered the largest species of catfish from South America and one of the world's largest (Petrere Junior et al., 2004).

Brachyplatystoma rousseauxii is a large (>1.5 m) migratory catfish of the family Pimelodidae, commonly known as dorado in Bolivia and dourada in Brazil (Carvajal-Vallejos et al., 2014). It is one of the most emblematic species of the Amazon basin, owing to its economic importance and exceptional life cycle, which involves the largest known freshwater migration (Barthem and Goulding, 1997; Alonso, 2002).

The specific microbiota of dourada and filhote are not yet known. The objective of this paper was to identify microbiota and to determine the lag phase of isolated microorganisms to improve the refrigeration temperature conditions in the fish species filhote (B*rachyplatystoma filamentosum*) and dourada (B*rachyplatystoma rousseauxii*) from the Amazon region.

MATERIALS AND METHODS

Fish samples

Successive commercial-sized fish samples (n=4) (Filhote and Dourada) were collected between March and May 2014. At the time of collection, the samples were placed in sterile bags kept under refrigeration (around 10°C) and transported to the Laboratory of Food Microbiology (Federal University of Pará - UFPA) for further analysis.

Microbiological analysis

To analysis, 25 g of each sample (ventral part of the filet) were aseptically collected and added to 225 mL of 0.1% sterile peptone water (SPW), thus obtaining 1:10 dilution, which were homogenized in a stomacher (STOMACHER 400 CIRCULATOR SEWARD) at 2,300 rpm for 30 s. Next, counts of total mesophilic aerobic bacteria, psychrotrophic bacteria and coliform at 35 and 45°C were performed according to Brazil (2003). The total mesophilic aerobic and psychrotrophic bacteria counts were carried out in pour plate using plate count agar followed by incubation at 35°C/48 h for mesophilic and 7°C/10 days for psychrotrophic bacteria. Coliforms at 35 and 45°C were counted through the most probable number (MPN), with three sets of three tubes. Lauryl sulfate tryptose broth (LST) was used as a presumptive medium and incubated at 35°C for 24-48 h. The positive tubes were transferred to brilliant green bile broth 2% (GB) and *Escherichia coli* (EC) broth. The former was incubated at 35°C/24 - 48 h for confirmation of total coliforms and EC broth tubes were incubated in a water bath at 45.5°C/24 h for confirmation of thermo-tolerant coliforms.

Bacteria isolation

The homogenized matter used for microbiological characterization was subsequently used for bacteria isolation. Colonies were isolated from VRBG and Baird-Parker with egg-yolk Tellurite plates after incubation for 48 h at 36°C. Next, one plate was selected for each medium and 5-10 colonies per plate were randomly chosen. The selected colonies were sub-cultured in VRBG or Baird-Parker agar plates. After incubation at 36°C/48 h, one colony was transferred from each plate to brain heart infusion (BHI) with 10% glycerol and stored in a freezer to be used for further tests.

Bacterial strain identification

The bacteria isolated were previously identified with Gram stain tests. Next, Gram-negative strains were identified using the API20E kit (Enterobacteria), and Gram-positive strains with API Staph

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Table 1. Mean values of microbiological characterization in fresh fish.

Samples	Mesophilic aerobic bacteria (log CFU/g)	Psychrotrophic bacteria (log CFU/g)	Total coliforms (MPN/g)	Thermotolerant Coliforms (MPN/g)
Filhote 01	7.49 ± 0.02[a]	6.91 ± 0.02[a]	1.100[a]	1.100[d]
Filhote 02	5.27 ± 0.13[c]	5.69 ± 0.05[b]	1.100[a]	150[b]
Filhote 03	5.21 ± 0.09[c]	5.54 ± 0.02[b]	1.100[a]	1.100[a]
Filhote 04	5.43 ± 0.07[c]	5.11 ± 0.05[d]	1.100[a]	23[e]
Dourada 01	6.02± 0.11[b]	5.53 ± 0.04[b]	1.100[a]	43[a]
Dourada 02	5.18 ± 0.10[c]	5.65 ± 0.03[b]	1.100[a]	210[c]
Dourada 03	5.14 ± 0.09[c]	5.33 ± 0.01[c]	1.100[a]	1.100[a]
Dourada 04	7.64 ± 0.04[a]	5.17 ± 0.08[c, d]	1.100[a]	93f

*Different letters in each column indicate difference at 95% level of significance.

(Staphylococci). The procedure was in accordance with the manufacturer's recommendations (Biomérieux, France) (Harrigan, 1998).

Lag phase determination

Strains were reactivated in nutrient broth for 24 h at 36°C. After that, the isolates identified were transferred to a new nutrient broth (1:15 mL) and maintained at different temperatures: 37, 10 and 15°C during different times: 0, 2, 4, 6, 7, 8 and 9 h (15 and 37°C) and 0, 3, 6, 9 and 10 h (10°C). Spectrophotometric readings were performed in triplicate (Spectrophotometer Model Nova 2000 UV) at 620 nm (Adapted from Damasceno et al., 2015).

Statistical analysis

Tukey's test was applied to evaluate the difference of means among microorganism groups (mesophilic and psychrotrophic bacteria, total and thermotolerant coliforms) found in different fishes. The optical density (lag phase determination) data were subjected to ANOVA considering different groups of isolated microorganisms. The software Statistica 8.0 was applied considering a 95% level of significance.

RESULTS AND DISCUSSION

Microbiological characterization

The mesophilic and psychrotrophic aerobic bacteria count, as well as total and thermotolerant coliform values, showed variation considering 95% significance level. The mesophilic aerobic bacteria counts for fresh fish samples ranged from 5.21 to 7.49 log CFU/g for filhote and 5.14 to 7.64 log CFU/g for dourada (Table 1).

Brazil (2001) does not establish microbiological standards for mesophilic bacteria count in fresh fish. However, the International Commission on Microbiological Specifications for Foods (ICMSF, 1986) recommends the limits for mesophilic aerobic should not exceed values of 10^7 CFU/g or cm^2 in chilled fish samples for human consumption. Considering this limit, most of the samples were within the standards (except sample 1 for Filhote and sample 4 for Dourada).

Previous studies by Shewan (1977), Guizani et al. (2005) and Ercolini et al. (2009) indicated that mesophilic microorganisms are dominant in tropical fish species. Oku and Amakoromo (2013) found total mesophilic values of 10^8 to 10^{10} log CFU/g in *Clarias angularis*, *Channa obscura* and *Chrysicthtys auratus*. Thong et al. (2013) found total mesophilic counts on raw pangasius fish of about 5.1 log CFU/g. Vishwanath et al. (1998) observed a total mesophilic bacteria count ranging from 10^6 to 10^7 CFU/g for *Muscodor albus* (Manipur, India). Damasceno et al. (2015) found mesophilic bacteria values ranging from 4.52 to 8.23 CFU/g in Piramutaba (*Brachyplatystoma vailantii*) and Butterfly peacock bass (*Cichla ocellaris*), respectively, in tropical water. The high count of this microorganism in food may result from unsatisfactory storage conditions, with potential danger to health (Morton, 2001; Coelho et al., 2010; Franco and Landgraf, 2005).

Thus, it appears that mesophilic bacteria are relevant in characterizing the food handling conditions, so it is very important that the current legislation set limits for these microorganisms in fresh fish in order to ensure higher quality (Lopes et al., 2012).

The count of psychrotrophic aerobic bacteria ranged from 5.11 to 6.91 log CFU/g for filhote and from 5.17 to 5.65 log CFU/g for dourada (Table 1). The Brazilian legislation establishes no maximum limit for psychrotrophic microorganisms in fish for human consumption. But considering the ICMSF (1986), filhote and dourada species showed values above the maximum limit (10^7 CFU/g).

In refrigerated fish, the psychrophilic and psychrotrophic bacteria play direct roles in fish deterioration because they

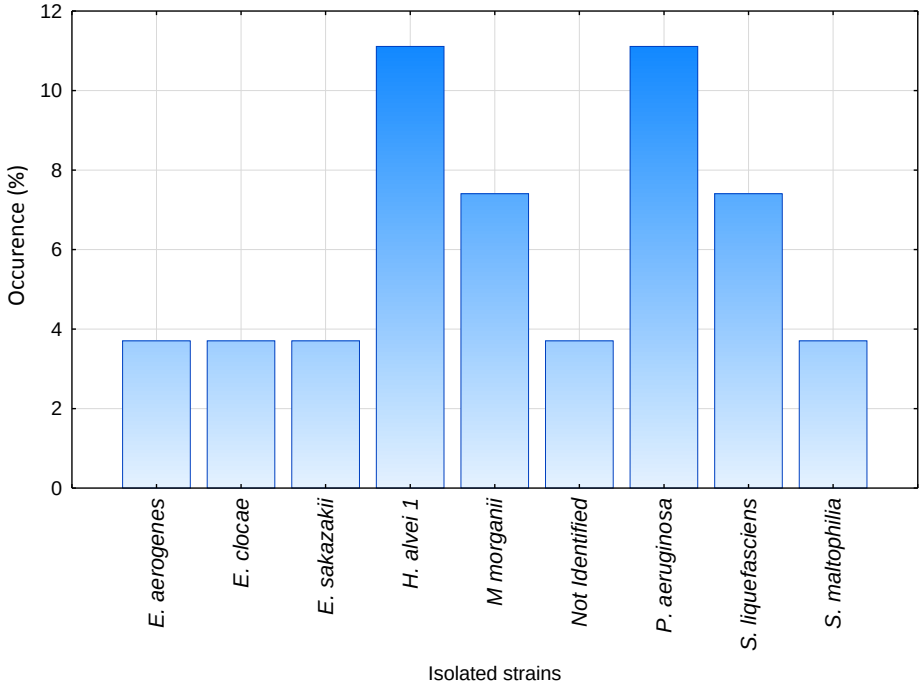

Figure 1. Percentage of occurrence (%) of gram-negative strains isolated from Dourada.

multiply well in these conditions (Franco et al., 1996). Bal'a et al. (2000) found psychrotrophic counts of about 10^3 – 10^7 in fresh fillets of channel catfish (*Ictalurus punctatus*).

Lazarin et al. (2011) found psychrotrophic values of 6.54 log CFU/g in pintado (*P. coruscans*) fillets. Rodrigues et al. (2008), while studying the quality of tilapia (*Oreochromis niloticus*), observed heterotrophic aerobic psychrotrophic microorganism counts between 0 and 7.90 log CFU/g in skinned muscle. Although, the current legislation does not establish limits, the ability of these microorganisms to deteriorate fish through proteolytic processes, even at freezing temperatures, is widely known, which would reduce the product's shelf life (Santos et al., 2008).

The Filhote and Dourada collected had an average score above 1,100 MPN/g for total coliforms (Table 1). Although, this does not indicate the presence of pathogens, total coliforms are important indicators of potential product deterioration and its mean shelf life (Agnese et al., 2001). Brazil (1997) and Brazil (2001) set the value of 10^2 MPN/g as the maximum acceptable standard for thermotolerant coliforms in fish and fishery products. Except for samples 4 for Filhote and 1 and 4 for Dourada, the others showed values that exceeded those established by the Brazilian legislation. Lopes et al. (2012) found total and thermotolerant coliform values ranging from 3.0 to 93 MPN/g in brackish-water grey snapper (*Cynoscion acoupa*). Araújo et al. (2012) observed values of 2,400 MPN/g and 11 to 150 MPN/g for total and fecal coliforms, respectively, in freshwater tambaqui (*Colossoma macropomum*). Oku and Amakoromo (2013) found values of 15 to 43 MNP/g for thermo-tolerant coliforms in the freshwater fish species *C. angularis, C. obscura* and *C. auratus.*

Total and thermo-tolerant coliforms are indicators of hygienic quality, not representing direct contact of the product with human or animal feces, but showing the degree of microbial pollution to which the food has been exposed. This score thus indirectly reflects the quality of production practices.

Bacterial strain identification

Among the 51 isolates, it was observed that 52.94% were Gram negative and the other 47.06% were Gram positive. From the results of the Gram stain test, the kits were selected to identify the strains. The data show that Gram-negative bacteria belonged to 15 different species, described in Figures 1 and 2. The most predominant were *Hafnia alvei* (11.1%) for Dourada and *Pseudomonas*

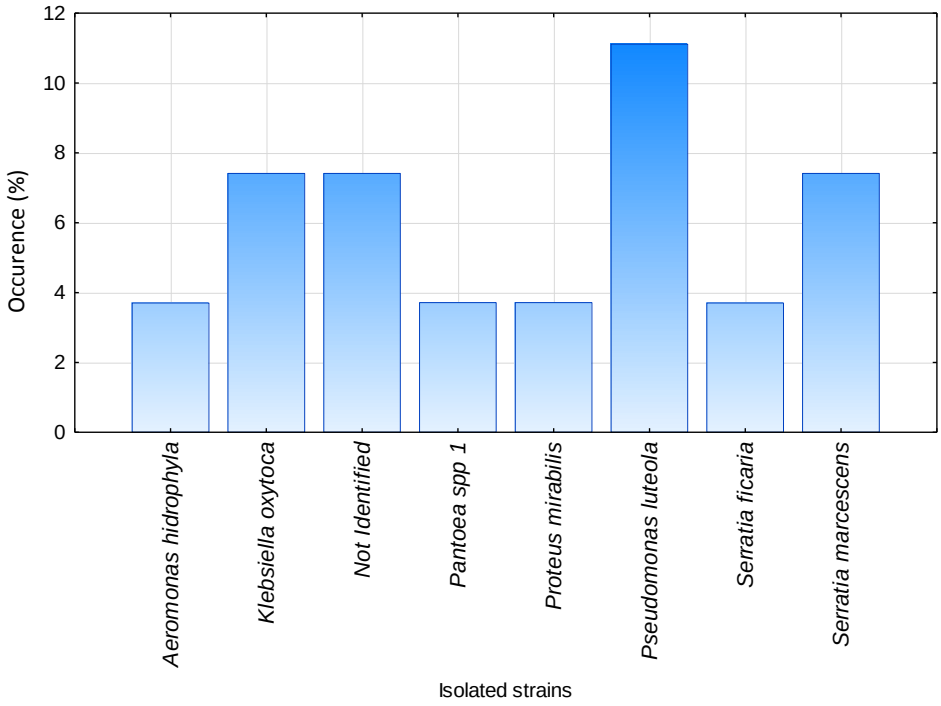

Figure 2. Percentage of occurrence (%) of Gram-negative strains isolated from Filhote.

luteola for Filhote (11.1%). In addition, the incidence of *Serratia marcencens*, *Klebsiella oxytoca*, *Stenotrophomonas maltophilia* and *Aeromonas hidrophyla* was observed, which are opportunistic pathogens but can also cause fish spoilage (Holt et al., 1994).

H. alvei is widely distributed in nature and has been found in a variety of mammals, fish and birds, and also in soil, sewage, freshwater and a number of foods such as meat and dairy products (Lindberg et al., 1998, Vivas et al., 2008). The pathogenicity of *H. alvei* is important in the food industry and infection outbreaks have been reported leading to septicemia in commercial laying hens, pullets and rainbow trout (Janda and Abbott, 2006; Liu et al., 2007; Crandall et al., 2006). In humans, *H. alvei* has been shown to be predominantly associated with several intestinal disorders, including gastroenteritis. Outbreaks or case reports of Hafnia associated with enteric infections have been chiefly reported (Hernandez-Milan and Nenendez-Rivas, 1998; Laguna et al., 1992; Orden and Franco, 1994; Reina et al., 1993; Reina and Borrell, 1991; Seral et al., 2001).

The genus Pseudomonas is known to change many protein-rich foods such as milk, eggs, meat, marine-based foods such as fish and shrimp, and vegetables. According to Franco and Landgraf (2006), pseudomonas are important in food due to their intense metabolic activity, being able to use a wide variety of organic compounds and produce water-soluble pigment and proteolytic and lipolytic enzymes.

P. luteola has been identified as a cause of infection in patients with underlying medical disorders (Otto et al., 2013; Anzai et al., 1997; Kiska and Gilligan, 1999, Connor et al., 1987; Hawkins et al., 1991; Rohav et al., 1995). The normal habitat of *P. luteola* is unclear; it is frequently found in soil, on plants, and in aqueous and damp environments (Freney et al., 1988; Silver et al., 1985; Hawkins et al., 1991). *Pseudomonas aeruginosa* is recognized as belonging to the normal flora of plant surfaces, human skin and animals, and can form biofilms on some surfaces or substrates (Maia et al., 2009). The *P. aeruginosa* species is the most important opportunistic pathogen in humans (Massaguer, 2006; Maia et al., 2009).

The family Enterobacteriaceae has been frequently isolated from the digestive tracts and flesh of freshwater fish (Austin, 2002; Yagoub, 2009; Gonzalez-Rodriguez et al., 2002; Paludan-Müller et al., 1998). Apun et al. (1999) showed some species of Enterobacteriaceae family such as *K. pneumoniae*, *E. aerogenes* and *Escherichia coli* have been isolated from the intestines of tropical freshwater fish. *Serratia* spp. have also been found in

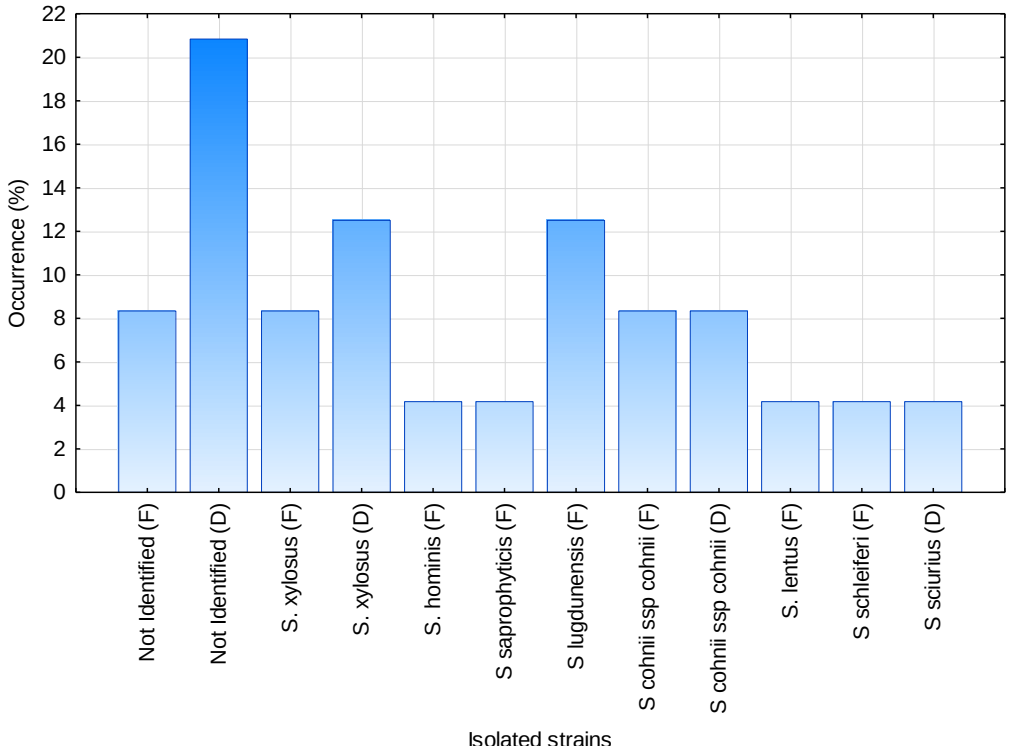

Figure 3. Percentage of occurrence (%) of Gram-positive strains isolated from Filhote (F) and Dourada (D).

Pangasius fillets (Thong et al., 2013). At room temperature (25°C), the microbiota is dominated by mesophilic *Vibrionaceae* (Gorczyca and Len, 1985; Gram et al., 1990) and, particularly if the fish are caught in polluted waters, mesophilic Enterobacteriaceae become dominant (Gram, 1992).

The Gram-positive bacteria found belong to eight different species of staphylococcus as shown in Figure 3. The most prevalent were *Staphylococcus xylosus* (12.50%) and *Staphylococcus lugdunensis* (12.50%) in dourada and filhote, respectively. Damasceno et al. (2015) found predominantly, *S. hominis*, *S. aureus* (Gram-positive), *S. maltophilia* and *E. intermedius* (Gram-negative) in Piramutaba (*Brachyplatystoma vailantii*) and Butterfly peacock bass (*Cichla ocellaris*).

The genus Staphylococcus contains 41 validly described species (DSMZ, 2008) that are traditionally grouped into coagulase-positive (CPS) and coagulase-negative staphylococci (CNS). The CNS *S. xylosus* strains play a significant role in food production. They can be used as starter cultures for the production of fermented meat products such as fermented sausages (Hammes and Hertel, 1998; Chajecka-Wierzchowska et al., 2015). They can also degrade biogenic amines *in vitro* (Martuscelli et al., 2000). *S. xylosus* have been identified in high numbers in dried salted cod (Vilhelmsson et al., 1997; Doe and Heruwati, 1988) and are rarely associated with human or animal infections (Kloos and Schleifer, 1986). *S. lugdunensis* is a common human skin commensal (Bellamy and Barkham, 2002; Vandenesch et al., 1995; Van der Mee-Marquet, 2003). These bacteria display pathogen characteristics, although they do not belong to this group, and exhibit pathogenicity similar to *Staphylococcus aureus*, with high associated morbidity and mortality (Cercenado, 2009; Frank and Patel, 2008; Poutanen and Baron, 2001). Considering that *S. lugdunensis* is not part of the normal fish microbiota, its presence in fish means there have been contamination from human sources.

Lag phase

Among the different 23 strains isolated, 13 bacteria were selected to determine the lag phase under different temperature conditions (Figures 4, 5 and 6). There was a significant variation ($p<0.05$) in optical density of the species studied at temperatures of 37, 15 and 10°C (Tables 2, 3 and 4).

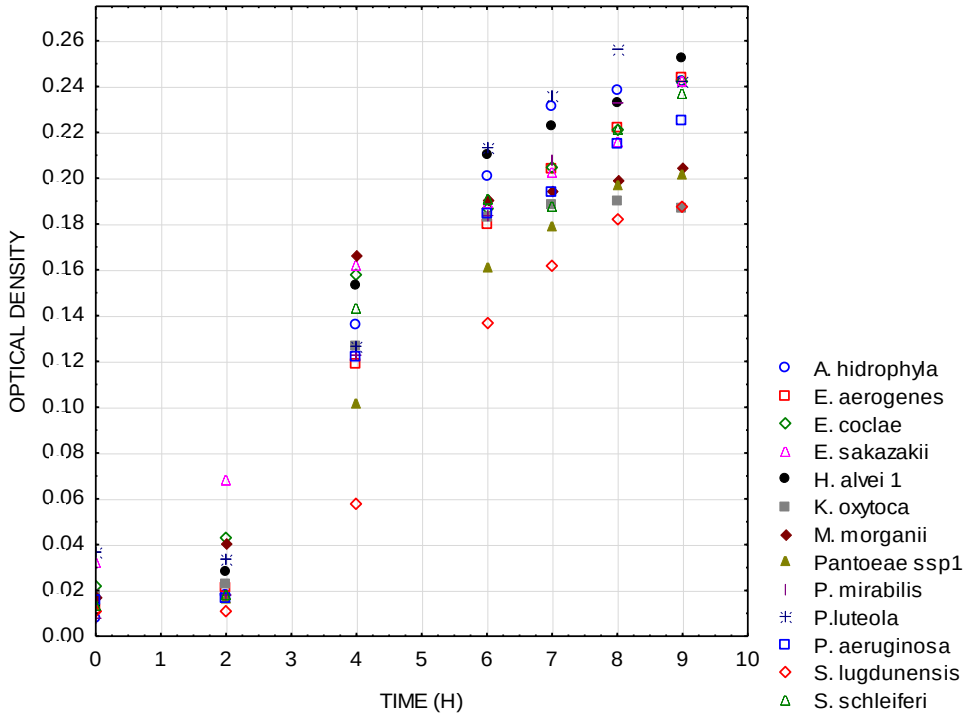

Figure 4. Lag phase measured through spectrophotometry at 37°C.

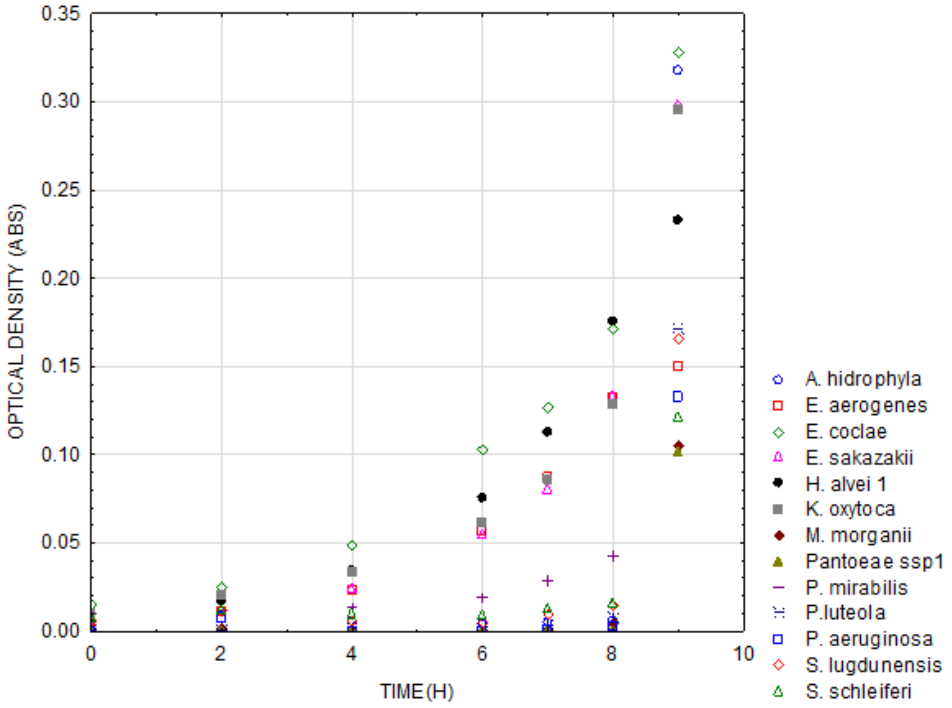

Figure 5. Lag phase measured through spectrophotometry at 15°C.

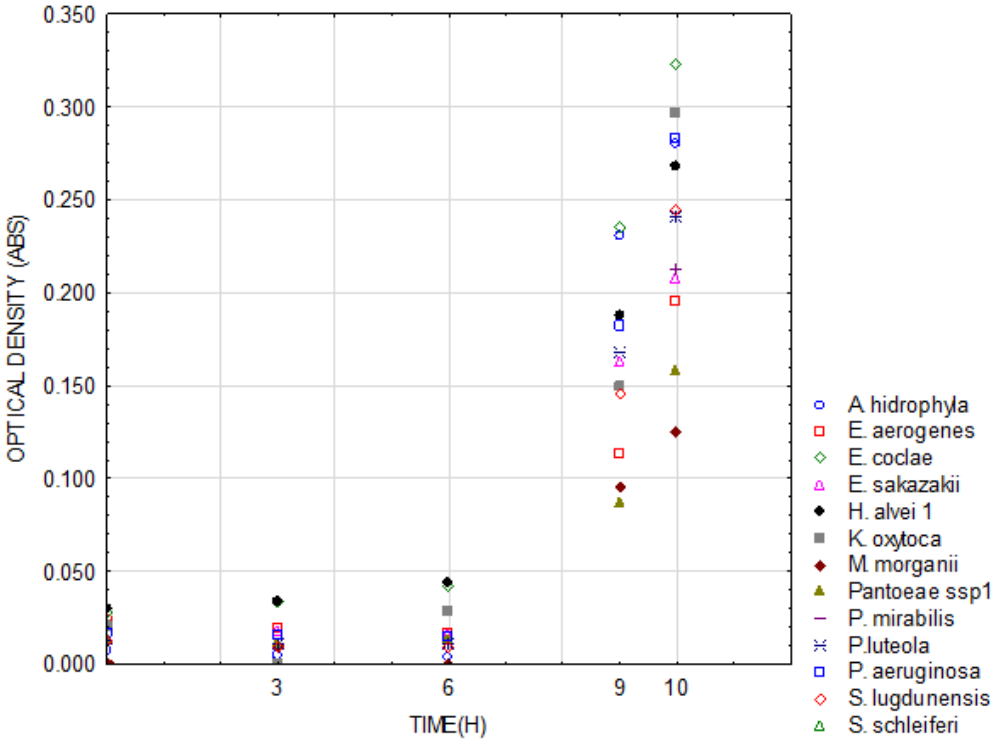

Figure 6. Lag phase measured through spectrophotometry at 10°C.

Table 2. ANOVA for optical density measured through spectrophotometry (620 nm) at 37°C.

Bacterial strains/temperature	F	p
Aeromonas hidrophyla	351.12	0.00
Enterobacter aerogenes	6492.10	0.00
Enterobacter clocae	1495.46	0.00
Enterobacter sakazakii	1229.58	0.00
Hafnia alvei 1	4217.81	0.00
Klebsiella oxytoca	1841.33	0.00
Morganella morganii	3786.19	0.00
Pantoea spp. 1	3858.13	0.00
Proteus mirabilis	815.01	0.00
Pseudomonas luteola	1589.93	0.00
Pseudomonas aeroginosa	896.03	0.00
Staphylococcus lugdunensis	414.56	0.00
Staphylococcus schleiferi	1274.05	0.00

Table 3. ANOVA for optical density measured through spectrophotometry (620 nm) at 15°C.

Bacterial strains/temperature	F	p
Aeromonas hidrophyla	10.78	0.00
Enterobacter aerogenes	72.31	0.00
Enterobacter clocae	26.68	0.00
Enterobacter sakazakii	117.71	0.00
Hafnia alvei 1	1080.73	0.00
Klebsiella oxytoca	312.63	0.00
Morganella morganii	1232.05	0.00
Pantoea spp. 1	62.23	0.00
Proteus mirabilis	696.90	0.00
Pseudomonas luteola	8324.10	0.00
Pseudomonas aeroginosa	9304.56	0.00
Staphylococcus lugdunensis	3722.63	0.00
Staphylococcus schleiferi	2287.08	0.00

The lag phase of the majority of the bacteria studied (except E. *sakazakii*) at 37°C was approximately 2 h (Figure 4). At 15°C, the lag phase was also at least 2 h and at 4 h, *E. clocae,* E. *sakazakii* and *K. oxytoca* were already in log phase. When they were exposed to temperatures of 10°C, the lag phase was at least 6 h.

Table 4. ANOVA for optical density measured through spectrophotometry (620 nm) at 10°C.

Bacterial strains/temperature	F	p
Aeromonas hidrophyla	505.01	0.00
Enterobacter aerogenes	164.45	0.00
Enterobacter clocae	645.99	0.00
Enterobacter sakazakii	88.74	0.00
Hafnia alvei 1	614.83	0.00
Klebsiella oxytoca	32.16	0.00
Morganella morganii	446.89	0.00
Pantoea spp. 1	421.68	0.00
Proteus mirabilis	632.45	0.00
Pseudomonas luteola	1271.25	0.00
Pseudomonas aeroginosa	1184.08	0.00
Staphylococcus lugdunensis	417.88	0.00
Staphylococcus schleiferi	283.53	0.00

This means that a safe condition of refrigeration temperature/time to prevent the multiplication of these microorganisms is about 10°C/6 h. Damasceno et al. (2015) noted that 16 strains isolated from Piramutaba (*Brachyplatystoma vailantii*) and Butterfly peacock bass (*Cichla ocellaris*) did not achieve growth for 6 h at 10°C.

This result is consistent with the FDA (2011), which suggests that raw fish should be kept at 10°C throughout processing to inhibit the growth and toxin production of pathogenic bacteria. Thus, the adoption of correct measures in the fish industry, such as appropriate conservation through cold treatment and maintaining hygienic practices, tends to reduce the risk of transmitting the disease-causing agents and is able to produce a quality product at the end of the production chain (Lopes et al., 2012).

Conclusion

The microbiological assessment of filhote and dourada suggests these Amazon fish species sold in the Ver-o-Peso market have mesophilic and psychrotrophic bacteria counts within the limits set by the Brazilian legislation. The microorganisms selected after isolation underwent a significant variation ($p<0.05$) when they were subjected to different refrigeration temperatures for up to 10 h, which yields the optimal refrigeration temperature/time condition (10°C/6 h) to prevent the multiplication of these microorganisms.

Conflict of interests

The authors have not declared any conflict of interest.

REFERENCES

Agnese AP, Oliveira VM, Silva PPO, Silva PPO, Oliveira GA (2001). Count of heterotrophic aerobic mesophilic bacteria and enumeration of total and fecal coliforms in fresh fish marketed in the city of Seropédica – RJ. Hig. Aliment. 15(88):67-70.

Alonso JC (2002). Space-time standard of population structure and current state of the fishing Golden exploration of dourad (*Brachyplatystoma flavicans*, Castelnau, 1855) in Estuary-Amazonas-Solimoes. Doctoral dissertation, National Institute for Research in the Amazon — INPA, Manaus (AM), Brazil.

Amaral GV, Freitas DGC (2013). Quality index method to determine the freshness of fish. Cienc. Rural 43(11):2093-2100.

Angelini R, Fabrè NN, Silva-Jr UL (2006). Trophic analysis and fishing simulation of the biggest Amazonian catfish. Afr. J. Agric. Res. 1(5):151-158.

Anzai Y, Kudo Y, Oyaizu H (1997). The phylogeny of the genera *Chryseomonas, Flavimonas,* and *Pseudomonas* supports synonymy of these three genera. Int. J. Syst. Bacteriol. 47:249-251.

Apun K, Asiah MY, Jugang K (1999). Distribution of bacteria in tropical freshwater fish and ponds. Int. J. Environ. Health Res. 9: 285-292.

Araújo EC, Moura LL, Martins AGLA, Carvalho ASS (2012). Evaluation of microbiological quality and physicochemical of Tambaqui (*Colossoma macropomum*) sold in the municipal market in Açailândia –Ma. VII CONEPPI, Tocantins. P 1:6.

Austin B (2002). The bacterial microflora of fish. Sci. World J. 2:558-572.

Bal'a MFA, Podolak R, Marshall DL (2000). Microbial and color quality of fillets obtained from steam-pasteurized deheaded and eviscerated whole catfish. Food Microbiol. 17:625-631.

Barthem R, Goulding M (1997). The catfish connection. Columbia University Press, New York. 144p.

Bellamy R, Barkham T (2002). *Staphylococcus lugdunensis* infection sites: predominance of abscesses in the pelvic girdle region. Clin. Infect. Dis. 35:32-34.

Brazil. Ministry of Agriculture, Livestock and Food Supply (1997). Decree no. 185 of May 13th, 1997. Approves technical regulation for regulation and quality of fresh fish. Official Gazette of the Federative Republic of Brazil. Brasilia – DF.

Brazil Ministry of Agriculture, Livestock and Food Supply (2003). Instruction no. 62 of August 26th, 2003 - Formalizes the analytical methods of microbiological analyzes for control of animal products and water. Official Gazette of the Federative Republic of Brazil. Brasilia – DF.

Brazil Ministry of Health. (2001). Resolution RDC no. 12 of January 2nd, 2001. Approves the Technical Regulation on Microbiological Standards for Food. Official Gazette of the Federative Republic of Brazil. Brasilia – DF.

Carvajal-Vallejos FM, Duponchelle F, Desmarais E, Cerqueira F, Querouil S, Nunez J, Garcia C, Renno JC (2014). Genetic structure in the Amazonian catfish *Brachyplatystoma rousseauxii*: Influence of life history strategies. Genetic 142(4):323-336.

Cercenado E (2009). *Staphylococcus lugdunensis*: um coagulase negative staphylococci different from others. Enferm. Infec.. Microbiol. Clín.. 27(3):139-42.

Chajecka-Wierzchowska W, Zadernowska A, Nalepa B, Sierpinska M, Łaniewska-Trokenheim L (2015). Coagulase-negative staphylococci (CoNS) isolated from ready-to-eat food of animal origin e Phenotypic and genotypic antibiotic resistance. Food Microbiol. 46:222-226.

Coelho MIS, Mendes ES, Cruz MCS, Bezerra SS, Silva RPP (2010). Evaluation of microbiological quality of mineral water consumed in the metropolitan area in Recife – Pernambuco. Acta Scientiarum Health Sci. 32(1):1-8.

Connor BJ, Kopecky RT, Frymoyer PA, Forbes BA (1987). Recurrent Pseudomonas (CDC Group Ve-1) peritonitis in a patient undergoing continuous ambulatory peritoneal dialysis. J. Clin. Microbiol. 25:

1113-1114.
Córser PI, Ferrari G, Martinez YB, Salas EM, Cagnasso MA (2000). Proximate analysis, fatty acid profile, essential amino acids and mineral content in twelve species of fish of commercial importance in Venezuela. Arch Latinoam Nutr. 50 (2):187-94.
Crandall C, Abbott SL, Zhao YO, Probert W, Janda JM (2006). Isolation of toxigenic *Hafnia alvei* from a probable case of hemolytic uremic syndrome. Infection 34:227-229.
Cruz-Romero M, Kelly AL, Kerry JP (2008). Influence of packaging strategy on microbiological and biochemical changes in high-pressure-treated oysters (*Crassostrea gigas*). J. Sci. Food. Agric. 88(15):2713-2723.
Damasceno EIT, Pantoja LNG, Figueiredo HM, Meller LH, Rodrigues AMC (2015). Microbiota of two species of commercially importante fish in the Amazon region (Belém-Pa-Brazil): Butterfly peacock bass (*Cichla ocellaris*) and Piramutaba (*Brachyplatystoma vailantii*). Afr. J. Microbiol. Res. 9(9):572-580.
Doe PE, Heruwati ES (1988). A model for the prediction of the microbial spoilage of sun-dried tropical fish. J. Food Eng. 8:47-72.
DSMZ (German Collection of Microorganisms and Cell Cultures) (2008). Bacterial Nomenclature up-to-date: Approved lists, validation lists.
Ercolini D, Russo F, Nasi A, Ferranti P, Villani F (2009). Mesophilic and psychrotrophic bacteria from meat and their spoilage potential *in vitro* and in beef. Appl. Environ. Microbiol. 75:1990-2001.
FDA (Food and Drug Administration) (2011). Fish and Fishery Products Hazards and Controls Guidance, 4th edition.
Franco BDGM, Landgraf M (2005). *Microbiology of foods*. São Paulo: Atheneu. 196p. [in portuguese]
Franco, BDGM, Landagraf M (2006). *Microbiology of foods.* São Paulo: Atheneu, 182p.
Franco, BDGM, Landgraf M, Destro MT (1996). Food Microbiology. Atheneu São Paulo, Brazil. 183p.
Frank KL, Patel R. *Staphylococcus lugdunensis*: Not the average coagulase-negative *Staphylococcus species*. Clin. Microbiol. Newsl. 30(8):55-62.
Freney J, Hansen W, Etienne J, Vandenesch F, Fleurette J (1988). Postoperative infant septicemia caused by *Pseudomonas luteola* (CDC group Ve-1) and *Pseudomonas oryzihabitans* (CDC group Ve-2). J. Clin. Microbiol. 26:1241-1243.
Gonzalez-Rodriguez, MN, Sanz JJ, Santos JA, Otero A, Garcia-Lopez, ML (2002). Foodbourne pathogenic bacteria in prepackage fresh retail portion of farmed rainbow trout and salmon stored at three degrees C. Int. J. Food Microbiol. 76 (1-2):135-141.
Gorczyca E, Len PP (1985). Mesophilic spoilage of bay trout (*Arripis trurta*), bream *(Acanthropagrus butchri)* and mullet (Aldrichettaforsteri). In: A. Reilly (editor), Spoilage of Tropical Fish and Product Development, FAO Fish. Rep. 317 Suppl. FAO, Rome, Italy. pp. 123-132.
Gram L (1992). Spoilage of three Senegalese fish species stored in ice at ambient temperature. Fishing News Books, Blackwell, Oxford, pp. 225-233.
Gram L (1995). Bacteriological changes. In H. H. Huss (Ed.), Quality and quality changes in fresh fish (pp.51–64). Rome: FAO Fisheries Technical Papers.
Gram L, Huss HH (1996). Microbiological spoilage of fish and fish products. Int J Food Microbiol. 33:121-137.
Gram L, Wedell-Neergaard C, Huss HH (1990). The bacteriology of fresh and spoiling Lake Victorian Nile perch (*Lutes niloficus).* Int. J. Food Microbial. 10:303-316.
Guizani N, Al-Busaydi MA, Al-Belushi IM, Mothershaw A, Rahman MS (2005). The effect of storage temperature on histamine production and the freshness of yellowfin tuna (*Thunnus albacares*). Food Res. Int. 38:215-222.
Hammes WP, Hertel C (1998). New developments in meat starter cultures. Meat Sci. 49:S125-S138.
Harrigan WF (1998). Laboratory methods in food microbiology. 3rd ed. California: Academic. 519p.
Hawkins RE, Moriarty RA, Lewis DE, Oldfield EC (1991). Serious infections involving the CDC group Ve bacteria *Chryseomonas luteola* and *Flavimonas oryzihabitans*. Rev. Infect. Dis. 13:257-260.
Hernandez-Milan B, Nenendez-Rivas V (1998). Hafnia in acute infantile gastroenteritis. An Esp Pediatr. 48:331.
Holt JG, Krieg NR, Sneath PHA (1994). Bergey´s Manual of Determinative Bacteriology . 9.ed. Maryland: Williams & Wilkins. 1106p.
ICMSF (2005). Microorganisms in Foods 6: Microbial Ecology of Food Commodities, 2nd edition. New York: Kluwer Academic/Plenum Publishers. 174:235.
ICMSF (International Commission on Microbiological Specifications for Foods) (1986). Microorganisms in Foods. Sampling for microbiological analysis: Principles and specific applications. 2. ed. Londres: Blackwell Scientific Publications. 181:193.
Janda JM, Abbott SL (2006). The genus Hafnia: from soups to nuts. Clin. Microbiol. Ver. 19:12-28.
Kiska DL, Gilligan PH (1999). *Pseudomonas*. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH. Manual of Clinical Microbiology, 7th Ed. American Society for Microbiology Press, Washington DC. pp. 517-525.
Kloos WE, Schleifer KH (1986). Genus IV. Staphylococcus Rosenbach 1884. Bergey's manual of systematic bacteriology. 2:965-1595.
Koffi-Nevri R, Ouina TST, Koussemon M, Brou K (2011). Chemical composition and lactic microflora of Adjuevan, A Traditional Ivorian Fermented Fish Condiment. Pak. J. Nutr. 10(4):332-337.
Laguna E, Gil M, Fontanals D, Brullet E (1992). Colits and Hafnia alvei. Med. Clin. (Barcelona). 98:556.
Lazarin EM, Almeida Filho ES, Ritter DO, Mello CA, Correa GSS, Ignácio CMS (2011). Occurrence of *Aeromonas* spp. and psychotropic microorganisms and estimate of the period of validity of commercial painted fillet (coruscans Pseudoplatystoma) kept under refrigeration. Arq. Bras. Med. Vet. Zootec. 63(6):1541-1546.
Lindberg AM, Ljungh A, Ahrne S, Lofdahl S, Molin G (1998). Enterobacteriaceae found in high numbers in fish, minced meat and pasteurised milk or cream and the presence of toxin encoding genes. Int. J. Food Microbiol. 39:11-17.
Liston J (1980). Advances in Fish Science and Technology. Connell, J. J.; Ed.; Fishing News Books Ltd.: Farnham, Surrey. pp. 138-157.
Liu CH, Lin WL, Wang CC, Lee KL, Tsai MC. J (2007). Young-infant sepsis combined with urinary tract infection due to *Hafnia alvei*. Formos. Med. Assoc. 106:S39-S43.
Lopes IS, Ferreira EM, Pereira DM, Pereira LS, Cunha MCS, Costa FN (2012). Yellow fish (*acoupa Cynoscion*) landed: microbiological characteristics and quality of ice used in its conservation. Inst Adolfo Lutz J. 71(4):677-84.
Maia AA, Cantisani ML, Esposto EM, Silva WCP, Rodrigues ECP, Rodrigues DP, Lazaro NS (2009). Antimicrobial resistance of *Pseudomonas aeruginosa* isolated from fish and cuts/chicken giblets. Ciênc. Tecnol. Aliment. 29(1):114-119.
Martuscelli M, Crudele MA, Gardini F, Suzzi G (2000). Biogenic amine formation and oxidation by *Staphylococcus xylosus* strains from artisanal fermented sausages. Lett. Appl. Microbiol. 31:228-232.
Massaguer PR (2006). *Microbiology of foods processes*. São Paulo: Varela. 258p [in spainsh].
Morton RD (2001). Aerobic Plate Count. Am. J. Public Health 4th ed. Washington – DC. pp. 63-67.
Oku I, Amakoromo ER (2013). Microflora of fresh and smoke-dried fish in Yenagoa metropolis, Nigeria. Afr. J. Microbiol. Res. 7:4451-4456.
Oliveira NMS, Oliveira WRM, Nascimento LC, Silva JMSF, Vicente E, Fiorini JE, Bressan MC (2008). Physical-chemical evualution of tilapia fillets (Oreochromis niloticus) submitted to sanitization. Ciênc. Tecnol. Aliment. 8(1):83-89.
Orden B, Franco A (1994). Isolation of Hafnia in acute gastroenterits. Med. Clin. 103:436-437.

Otto MP, Foucher B, Dardare E, Géromê P (2013). Severe catheter related bacteremia due to *Pseudomonas luteola*. Med Maladies Infect. 43:170-173.

Paludan-Muller C, Dalgaard P, Huss HH, Gram L (1998). Evaluation of the role of *Carnobacterium piscicola* in spoilage of vacuum and modified atmosphere-packed-smoked salmon stored at 5°C. Int. J. Food Microbiol. 39:155-166.

Petrere Junior M, Barthem RB, Córdoba EA, Gomez, BC (2004). Review of the large catfish fisheries in the upper Amazon and the stock depletion of piraı´ba (Brachyplatystoma filamentosum Lichtenstein). Rev. Fish Biol. Fisher 14:403-414.

Poutanen SM, Baron EJ. (2001). *Staphylococcus lugdunensis*: a notably distinct coagulase-negative *Staphylococcus*. Clin. Microbiol. Newsl. 23(19): 147-50.

Reina J, Borrell N (1991). Clinical significance of Hafnia isolation in the feces of children with acute gastroenteritis. Enferm. Infect. Microbiol Clin. 9:658-659.

Reina J, Hervas J, Borrell N (1993). Acute gastroenteritis caused by *Hafnia alvei* in children. Clin. Infect. Dis. 16:443.

Rodrigues TP, Freitas MQ, Marsico ET (2008). Assessment quality of Nile tilapia (*Oreochromis niloticus*) cultivated, eviscerated and stored on ice. Rev. Bras. Cienc. Vet. 15: 67-71.

Rohav G, Simhon A, Mattan Y, Moses AE, Sacks T (1995). Infections with *Chryseomonas luteola* (CDC group Ve-1) and *Flavimonas oryzihabitans* (CDC group Ve-2). Medicine 74:83-88.

Santos TM, Martins RT, Santos WLM, Martins NE (2008). Visual inspection and bacteriological, physico-chemical evaluations of piramutaba fillet (*Brachyplatistoma vaillanti*) frozen. Arq. Bras. Med. Vet. Zootec. 60(6):1538-1545.

Seral C, Castillo FJ, Lorente MT, Varea M, Clavel A, Rubio MC, Gomez-Lus R (2001). The *eaeA* gene is not found in *Hafnia alvei* from patients with diarrhea in Aragon, Spain. Int. Microbiol. 4:81-82.

Shewan J (1977). The bacteriology of fresh and spoiling fish and the biochemical changes induced by bacterial action. Proceedings of the conference on handling, processing and marketing of tropical fish. London: Tropical Products Institute. 5:51-66.

Silver MR, Felegie TP, Sorkin MI (1985). Unusual bacterium, group Ve-2, causing peritonitis in a patient on continuous ambulatory peritoneal dialysis. J. Clin. Microbiol. 21:838-839.

Thong Thi AN, Noseda B, Samapundo S, Nguyen BL, Rasschaert G, Heyndrickx M (2013). Microbial ecology of Vietnamese Trash (*Pangasius hypophthalmus*) fillets during processing. Int. J. Food Microbiol. 167:144-152.

Van der Mee-Marquet N, Achard A, Mereghetti L, Danton A, Minier M, Quentin R (2003). *Staphylococcus lugdunensis* infections: high frequency of inguinal area carriage. J. Clin. Microbiol. 41:1404-1409.

Vandenesch F, Eykyn SJ, Etienne J, Lemozy J (1995). Skin and post-surgical wound infections due to *Staphylococcus lugdunensis*. Clin. Microbiol. Infect. 1:73-74.

Vilhelmsson O, Hafsteinsson H, Kristjansson JK (1997). Extremely halotolerant bacteria characteristic of fully cured and dried cod. Int. J. Food Microbiol. 36:163-170.

Vishwanath W, Lilabati H, Bijen M (1998). Biochemical, nutritional and microbiological quality of fresh and smoked mud eel fish *Monopterus alhus* - a comparative study. Food Chem. 61(1/2):153-156.

Vivas J, Padilla D, Real F, Bravo J, Grasso V, Acosta F (2008). Influence of environmental condition on biofilm formation by *Hafnia alvei* strains. Vet. Microbiol. 129:150-155.

Yagoub SO (2009). Isolation of Enterobacteriaceae and Pseudomonas spp. from raw fish sold in fish market in Khartoum state. J. Bacteriol. 1(7):085-088.