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Counting and identification of molds and yeasts in dry salted shrimp commercialized in Rio Branco, Acre, Brazil

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Abstract

Dry salted shrimp is a popular food in Acre cuisine, especially as a main ingredient in “tacacá” and “rabada no tucupi”. However, different intrinsic and extrinsic factors can result in accelerated deterioration and microbial proliferation, causing food poisoning to the consumer. The objective was to perform the counting and identification of molds and yeasts in dry salted shrimp in establishments that sell typical foods in the municipality of Rio Branco, state of Acre. Shrimp samples from six establishments were collected in sterile bottles, identified, refrigerated and transported to the Laboratory of Infectious Diseases of Animals at the Federal University of Acre, for the manual counting of colony-forming units and identification of fungal genera. As a result, the number of molds and yeasts in the dried salted shrimp samples varied between $1.0x10^2$ and $1.02x10^6$ CFU/g. Nine fungal genera were identified: *Aspergillus*, *Penicillium*, *Cladosporium*, *Malassezia*, *Fusarium*, *Exophiala*, *Candida*, *Curvularia* and *Trichosporon*. The most frequent were *Aspergillus*, *Penicillium* and *Cladosporium*, these being potential producers of mycotoxins. The presence of molds and yeasts in the samples of dried salted shrimp shows the need for greater hygienic-sanitary rigidity of this fish at different stages of manipulation and processing as preventative measures for public health.

Keywords: *Aspergillus*; *Cladosporium*; *Penicillium*; fungus; *Litopenaeus vannamei*.

Practical Application: Detection of biological contaminants in animal products and the risk for One Helth.

1 Introduction

Shrimp farming is one of the major areas of aquaculture, being a major activity in the fishing industry in Brazil (Negreiros & Santos, 2015). Currently, *Litopenaeus vannamei*, also known as Pacific white shrimp, is the main crustacean grown and commercialized in the nation. Several characteristics, such as rusticity, adaptation to salinity and low nutritional requirements, have favored extensive breeding over the years, representing about 99.8% of crustacean production, despite being an exotic species (Ballester et al., 2013).

The *L. vannamei* is widely consumed in several states (Negreiros & Santos, 2015), with a fundamental participation in Acre gastronomy, mainly because it is the main ingredient of “tacacá” and “rabada no tucupi”, typical dishes of the region. The sale of this crustacean occurs mostly in open markets, given the more affordable value, although there is generally greater exposure to inadequate handling and packaging hygienic and sanitary conditions, which impairs the quality of the product (Lima & Santos, 2014; Silva et al., 2017a; Martins & Ferreira, 2018).

In this way, shrimp are susceptible to contamination from different sources. In addition, they are a product with high perishability and physicochemical characteristics that can favor accelerated microbial deterioration and proliferation (Chen et al., 2020).

Previous studies have highlighted fungi as microorganisms responsible for food contamination and deterioration, including shrimp (Barreto et al., 2016; Chen et al., 2019; Oliveira et al., 2019), with negative impacts for the economic and health sector. In addition, fungi produce mycotoxins that are deposited in food and pose risks to public health. The main agents involved belong to the genres *Fusarium*, *Penicillium* and *Aspergillus* (Sun et al., 2014; Kim et al., 2020).

The World Organization for Animal Health establishes diagnostic methods and recommends sampling for health surveillance purposes regarding aquatic animals taken to market, to reduce the risk of contamination. Adherence to these procedures guarantees the periodic evaluation of the product’s microbiological quality, as well as safety to the consumer’s health. (Laurin et al., 2019). However, the search for fungi and potential mycotoxins in shrimp is scarce in the literature.

In this sense, the objective was to perform the counting and identification of molds and yeasts in dried salted shrimp from the main establishments that sell typical foods in the municipality of Rio Branco, state of Acre.

2 Materials and methods

For the study, six fixed establishments for the sale of typical foods (“tacacá” and “rabada no tucupi”) were selected in the city

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of Rio Branco (9°58’26” S; 67°48’27” W), state of Acre, Brazilian Western Amazon. The separate portion of dry salted shrimp, which would be necessary for the production of each individual typical dish, was collected from each establishment. The samples were stored in sterile bottles, identified and transported in an insulated box to the Laboratory of Infectious Diseases of Animals of the Teaching and Research Unit in Veterinary Medicine of the Federal University of Acre.

Initially, 25g of shrimp in 225 mL of 0.1% peptone water were added to produce the initial dilution in the concentration 10^{-1}. The homogenization of the mixture was carried out on an orbital shaker for 10 minutes. Then, serial dilutions were produced 10^{-2} and 10^{-3}.

Aliquots containing 0.1 mL of each dilution were transferred, in duplicate, to Potato Dextrose Agar 4% medium (PDA) (Acumedia®, Indaiatuba, SP), supplemented with 0.2% chloramphenicol, using the surface plating method. The plates were incubated at 25 °C for up to five days. The result was expressed in Colony Forming Unit per gram (CFU/g) and the calculation was performed based on the dilution 10^{-3} (Silva et al., 2017b).

The growth of fungal colonies was observed, with manual counting, followed by purification in PDA and analysis of phenotypic characteristics. The macromorphological aspects analyzed were color, texture, surface, verse and reverse pigmentation (Murray et al., 2017; Brasil, 2013). Regarding microscopic characterization, the evaluation for filamentous fungi was based on specialized literature, whereas for yeast fungi, the identification key recommended by the National Health Surveillance Agency was used, including germ tube test, microculture on blade, urease test and capsule research (Brasil, 2004). The images of the isolates were obtained by photographic capture under an optical microscope, with 100x magnification, in immersion oil and lactophenol blue staining. The results obtained were analyzed using descriptive statistics (Rouquayrol & Gurgel, 2017).

3 Results

All samples of dry salted shrimp analyzed, from the six establishments that sell typical foods in Rio Branco, state of Acre, resulted in the growth of molds and/or yeasts in culture medium.

The mold and yeast count varied between $1.0x10^2$ and $1.02x10^6$ UFC/g. The samples from most establishments (83.3% - 5/6) had a count equal to or greater than $1.0x10^4$ CFU/g (Table 1).

Nine fungal genera were identified, according to the macro and microscopic aspects of the colonies, in addition to the complementary tests (Table 2). *Aspergillus* sp. (33.33%), *Penicillium* sp. (33.33%) and *Cladosporium* sp. (33.33%) were detected more frequently (Figure 1).

4 Discussion

The establishments selected for the study purchase shrimp from the Central Market of Rio Branco, Acre, an open market environment, which has great participation in the regional market, because it sells the raw material with a price significantly lower than the industrialized products. However, in these environments, the guarantee regarding the origin, packaging and handling in accordance with the recommended hygienic conditions, must be carefully analyzed (Martins & Ferreira, 2018).

Table 1. Counting of molds and yeasts detected in dried salted shrimp sold in the municipality of Rio Branco, Acre.

Establishments	Counting of molds and yeasts (*CFU/g)
1	$1.0x10^4$
2	$1.0x10^4$
3	$1.0x10^2$
4	$1.0x10^4$
5	$1.02x10^6$
6	$2.2x10^5$

*CFU: Colony Forming Unit.

Table 2. Frequency of molds and yeasts identified in samples of dried salted shrimp sold in the municipality of Rio Branco, Acre.

Gerena fungal	Frequency
Aspergillus sp.	33.33%
Candida sp.	16.67%
Cladosporium sp.	33.33%
Curvularia sp.	16.67%
Exophiala sp.	16.67%
Fusarium sp.	16.67%
Malassezia sp.	16.67%
Penicillium sp.	33.33%
Trichosporon sp.	16.67%

In addition to the manipulation of raw materials by vendors and the unfavorable factors inherent to open markets, the way the crustacean is prepared in the establishment that sells typical dishes also reflects on the quality of the food and the possible risks to the consumer’s health (Lima & Santos, 2014; Barreto et al., 2016; Silva et al., 2017a).

In addition, factors intrinsic to fish, such as high water activity, pH close to neutrality, potential for redox and chemical composition, contribute to its more accelerated deterioration (Freire et al., 2016). When these factors are combined with the high temperatures of the Amazon Region, they culminate in rapid decomposition of the product, because they are an adequate environment for the growth of different microorganisms (Martins & Ferreira, 2018).

In Brazil, the current law that regulates the standards necessary for a product to be considered suitable for human consumption does not establish a permitted value for the presence of fungi in foods from the salting process. However, the amount allowed under the Technical Regulation on Good Practices for Food Services, Resolution of the Collegiate Board - RCB No. 12 (Brasil, 2001), is $1.0x10^4$ CFU/g for other foods that have a level of moisture and water activity, similar to shrimp (Oliveira et al., 2019).

The indicative values of fungal contamination were above the limit established by the RCB No. 12 (Brasil, 2001) in 33.3% (2/6) of the analyzed samples. Similarly, a study conducted with samples

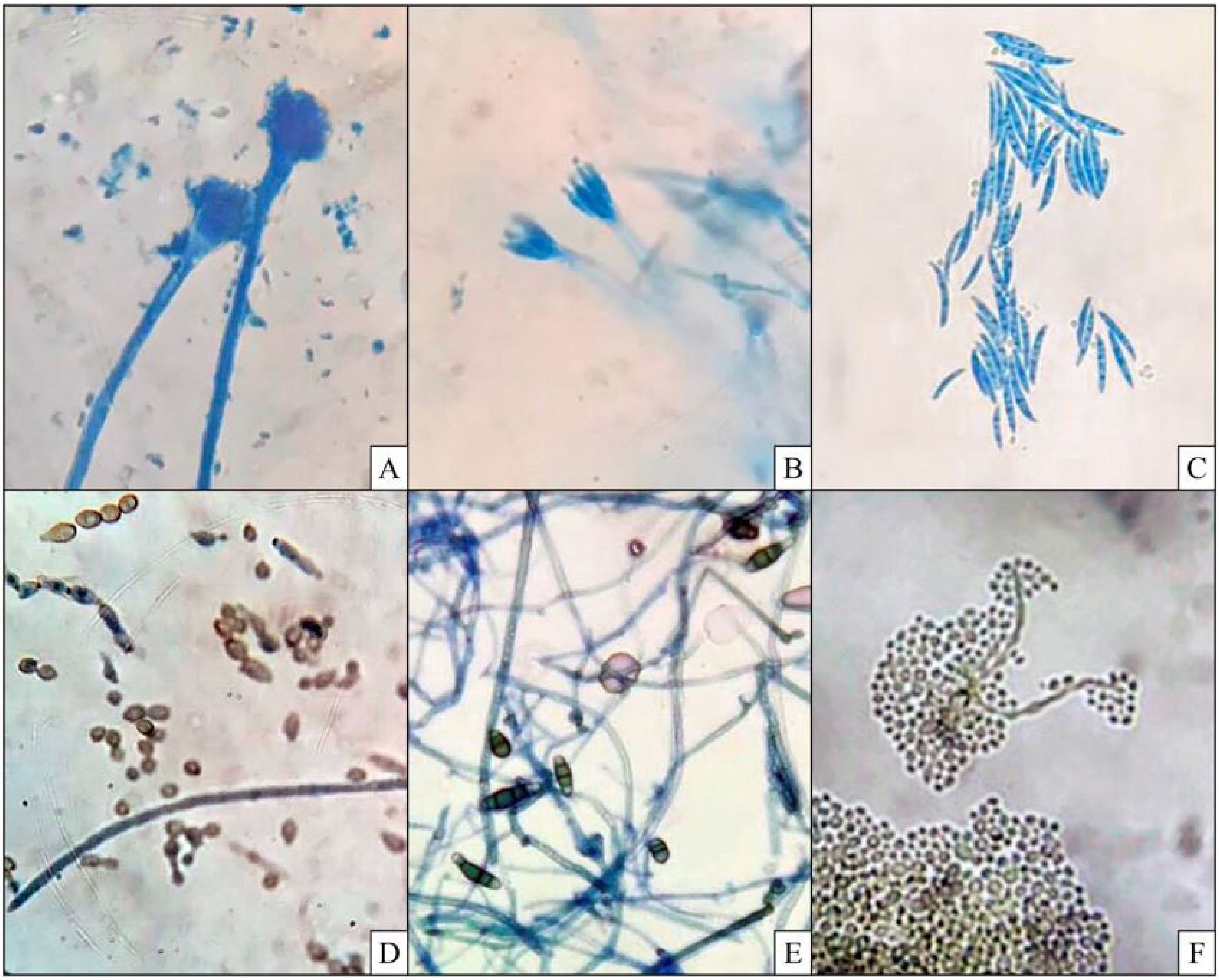

Figure 1. Micromorphology of fungal isolates identified in samples of dried salted shrimp sold in the municipality of Rio Branco, Acre. (A) *Aspergillus*; (B) *Penicillium*; (C) *Fusarium*; (D) *Cladosporium*; (E) *Curvularia*; (F) *Exophiala*. Coloring with lactophenol blue, obj. 100x.

of salted shrimp sold on the beaches of Maranhão, identified the presence of molds and yeasts, showing that 36.36% of the counts were between $1.8x10^4$ and $9.0x10^4$ CFU/g, and 9.09% presented counts of $1.5x10^5$ CFU/g (Ferreira et al., 2012), while in the state of Bahia, values ranging from <1.0x10 to $5.0x10^2$ CFU/g for filamentous fungi (Barreto et al., 2016).

In the study by Nunes et al. (2013), in turn, it was observed that 100% (21/21) of the shrimp samples analyzed in the state of Pará presented fungal contamination, with values between $1.0x10^2$ to $1.21x10^7$ CFU/g, this interval is similar to that found in the present work.

According to the results obtained, it is believed that fungal contamination of salted shrimp occurs frequently. However, these results may suffer significant variations according to the methodology used, including the geographical location of the study, origin and procedures for handling shrimp (Ferreira et al., 2012; Nunes et al., 2013; Barreto et al., 2016).

It is also verified that the processing of dry salting is not a unique and sufficient method to make the food free of microorganisms (Empresa Brasileira de Pesquisa Agropecuária, 2018). Fungal species called halophilic tolerate high concentrations of salinity, allowing their maintenance in the product even after the salting process (Silva et al., 2015), as recorded in this study. For example, Zhou et al. (2018), Yang et al. (2018) and Das et al. (2019) described *Trichothecium roseum*, *Aspergillus unguis* and *Aspergillus terreus*, respectively, as halophilic fungi of marine origin, detected in shrimp samples.

In general, the mycological investigations in shrimp samples only do the CFU count, without the precise identification of the fungal genera, compromising epidemiological studies (Barreto et al., 2016; Freire et al., 2016). Oliveira et al. (2019), when analyzing shrimp samples of *Macrobrachium amazonicum*, also isolated *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium* and *Curvularia* in Sabouraud dextrose agar, coinciding with our results demonstrated in PDA.

Describing this fungal variability related to the contamination of crustaceans is essential to better understand the dynamics of pathogens and the characteristics of harmful bioactive and mycotoxins to the consumer population of products of marine origin, establishing measures for the prevention and control of possible risks to public health, because certain fungal molecules are responsible for poisoning (Calvet et al., 2012; Barreto et al., 2016; Kim et al., 2020).

Among microorganisms identified in this study, it is considered that *Aspergillus*, *Penicillium* and *Fusarium* are potential producers of mycotoxins, which can cause serious neurogenic, teratogenic, carcinogenic and immunosuppressive problems (Calvet et al., 2012; Sun et al., 2014; Kim et al., 2020). The *Cladosporium*, in turn, is generally considered a food contaminant, but some species are pathogenic to humans, related to skin infections, rhinosinusitis, brain abscess and septicemia (Menezes et al., 2017; Garcia et al., 2018; Ahn et al., 2019; Batra et al., 2019).

As for the genus *Exophiala*, a previous study recorded the presence of this demea fungus in crabs (Guerra, 2010), however, there is no report in the literature about its identification in shrimp, this being the first description in *L. vannamei*. In humans, it has high pathogenicity, being associated with necrotizing, allergic and pulmonary skin disorders, and meningoencephalitis, especially in immunosuppressed patients (Góralska et al., 2018; Hagiya et al., 2019; Klasinc et al., 2019; Sato et al., 2019).

There are no reports in the literature on contamination of *L. vannamei* by fungi of the genus *Candida* (Boone, 1931). A previous study recorded the presence of some yeast species in shrimp *M. amazonicum*, concluding that the genus may be part of the microbiota of crustaceans (Brilhante et al., 2014). Infection by *Candida* in humans can result in mucocutaneous and systemic diseases in immunosuppressed hosts when the infectious load is high, despite being considered a commensal of the skin and gastrointestinal tract (Maranhão et al., 2019; Farahyar et al., 2020).

Similarly, *Trichosporon* and *Malassezia* were associated with the fungal microbiota of the hepatopancreas and intestine of *L. vannamei* (Boone, 1931). In humans, *Trichosporon* is the etiological agent of white piedra, benign superficial lesions, but which can spread (Huang et al., 2019). It can also be associated with urinary disorders and esophagitis (Francisco et al., 2019; Méndez-Tovar et al., 2019).

Malassezia is reported as a commensal for the skin of humans and animals, but can result in pityriasis versicolor, with the development of seborrheic and atopic dermatitis (Choi et al., 2020). Both fungal genera are considered opportunistic and are more detrimental in immunocompromised hosts, which can even result in septicemia (Challapilla et al., 2019; Sah et al., 2019).

Regarding the genus *Curvularia*, there is no description in the literature of the contamination of this fungus in shrimp samples. However, in a previous study, the presence of *Curvularia* in fish was reported (Ding et al., 2019). In humans, the microorganism is occasionally identified in cases of keratitis and rhinosinusitis in association with allergic disorders (Lakhundi et al., 2017; Edelmayer et al., 2019). Skin and neurological infections have also been mentioned (Ter Beest et al., 2020).

Most fungi isolated from dried salted shrimp samples can be considered pathogenic to humans, especially in situations of the immunocompromised, along with the fact that the implications for the gastrointestinal tract through the consumption of contaminated food is still little explored (Chen et al., 2019).

Thus, the results obtained in this study indicate the relevance of different fungal genera as food contaminants that are based on the use of *L.* vannamei. The action of inspection bodies in the marketing of shrimp is essential, as well as health education for traders of products of animal origin, in order to provide a quality product and low microbiological risk to the consumer market.

5 Conclusion

The presence of molds and yeasts in the samples of dried salted shrimp, originating from the main establishments that sell typical foods in Rio Branco, Acre, highlights the need for hygienic-sanitary control in the different stages of handling and processing of food produced with *L. vannamei*.

Several fungal genera have been described as shrimp contaminants, including potential mycotoxin producers, such as *Aspergillus*, *Penicillium* and *Fusarium*, indicating the importance of studies in the area to support measures to prevent and control possible intoxications for consumers.

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