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Short communication

Viability of the etiologic agent of the Lethargic Crab Disease, *Exophiala cancerae*, during cooking of the mangrove-land crab: Does this traditional dish represent a risk to humans?

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ABSTRACT

One of the most typical seafood dishes in the Brazilian coastal areas is the cooked mangrove-land crab, *Ucides cordatus* (L.). Mortality events of *U. cordatus* were reported from a large extension for over 15 years, especially in the Northeast region. These mortalities are known to be triggered by an epizooty known as Lethargic Crab Disease, for which the putative etiological agent is a new species of black yeast, *Exophiala cancerae* De Hoog, Vicente, Najafzadeh, Harrak, Seyedmousavi, & Boeger. Although there is no compelling evidence that this fungus may represent a zoonosis, there is great public concern regarding consumption of crabs from affected regions. Thus, this study evaluates the efficiency of cooking procedures on the inactivation of the etiologic agent. The variation of the internal temperature of crabs and tests of the activity of *E. cancerae* to temperature under simulated cooking condition were determined and the results were analyzed combined. The results indicate that crab's core body attains the boiling water temperature about an average of 14 min after exposition. Furthermore, short intervals of exposure (30 s) to such boiling temperatures were sufficient to warrant inactivation of *E. cancerae*. Thus, the traditional mode of preparation of the mangrove-land crab is sufficient to inactivate the pathologic agent and the consumption of sick or carrier animals should not represent a potential public health risk.

1. Introduction

Every week, a large number of mangrove-land crabs, *Ucides cordatus* (Linnaeus 1763) (Brachyura: Ocypodidae), is consumed in coastal regions of the northeast Brazil. It represents one of the most traditional dishes, with specialized restaurants and special festivities throughout the year in several localities in the region.

The species has an important position as a fishery resource in the northern and northeastern coast of Brazil, especially to impoverished communities as it does not require specialized equipment for its capture. Only in the city of Bragança (state of Pará), where the artisanal activity of crabs harvest may reach an annual income of 7 tons per km^2 of mangrove, it has produced financial returns sufficient not only to the sustain crab fishermen families but also to stimulate the local commerce (Araújo, 2006, 176 p.).

Traditionally, mangrove-land crab is consumed after cooking in water and salt (with spices, occasionally) for about 25 min, depending on the desired texture of the crab meat (Pedrosa & Cozzolino, 2001). The species is never consumed raw. However, after capture, crabs are transported alive in trucks without any hygienic control, packed in fabric bags, or simply tied to each other, always along with the mud residues they are taken. Afterwards, crabs are exposed for marketing without any preventive hygienic treatment, except for the washing performed by the consumer (Vieira et al., 2004).

For this reason, the number of investigations directed to the isolation of pathogens associated to food-borne diseases in mangrove-land crab meat is increasing. Among these studies, Theophilo and Vieira (1994) isolated several strains of *Vibrio parahaemoliticus* in samples of living *U. cordatus* marketed in restaurants of Praia do Futuro (city of Fortaleza, State of Ceará); this facultative anaerobic bacteria is recognized as an important pathogen of humans and aquaculture animals (Wong, Chen, Liu, & Liu, 1999). Further, Vieira et al. (2004) also isolated serovars of *Vibrio* spp., *Salmonella senftenberg*, *Salmonella poona*, representative species of

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Enterobacteriaceae and of Pseudomonadaceae from raw samples of *U. cordatus*. These last four taxa of microorganisms inhabit the intestinal tract of humans, which indicates probable contamination by sewage (direct or indirect) in the capture sites of these crabs.

However, according to the resolution of the National Sanitary Surveillance Agency (ANVISA, Brazil) – Resolution DRC n°12 of 02 January 2001, which is applied to food destined for human consumption, just analysis of only one restricted group of bacteria is required to fish and fisheries products (coagulase-positive *Staphylococcus*, *Salmonella* sp. and thermotolerant coliforms—at 45 °C) and no reference values are determined for other microorganisms.

Besides these well-known human pathogens, there is an increased awareness in the region concerning the consequences of ingestion of the black yeast *Exophiala cancerae* De Hoog, Vicente, Najafzadeh, Harrak, Seyedmousavi, & Boeger (De Hoog, Vicente, Najafzadeh, Harrak, & Seyedmousavi, 1999), which is considered the causative agent of the Lethargic Crab Disease (LCD) (Orélis-Ribeiro, Boeger, Vicente, & Ostrensky, 2011). The LCD is an epidemic infirmity responsible for numerous mortality events of *U. cordatus* since 1997, periodically affecting several mangroves along Brazilian coast (Boeger, Pie, Ostrensky, & Patella, 2005; Boeger et al., 2007). This same species has also been reported from a toad with signs of systemic mycosis, drink water, fruit drink, and a human skin infection (De Hoog et al., 1999).

Studies of species of black yeast phylogenetically close to the LCD etiologic agent, such as *Exophiala dermatitidis* and *Exophiala spinifera*, postulate the ingestion as a possible route for systemic infections in humans by these microorganisms (De Hoog, Poonwan, Gerrits, & Van Den Ende, 1999; Hiruma et al., 1993). Thus, considering the cooking technique of *U. cordatus*, it has become of utmost importance to assess whether this process represents risk of infection by *E. cancerae* for the consumers. Therefore, the goal of this study is to elucidate this important public health concern by determining the time of inactivation of the fungal elements under simulated cooking conditions. Further, as LCD is a systemic phaeohyphomycosis of the mangrove-land crab, we evaluated the time required for the internal portions of the crab to reach the temperature of the boiling water while cooking.

2. Materials and methods

2.1. Evaluating the time to boiling temperature reaches crab innermost tissues

Healthy specimens of *U. cordatus* were collected in Ilha das Peças (Bay of Antonina, State of Paraná, southern coast of Brazil), displaying a commercial size that range between 6.1 and 7.3 cm. Crabs were euthanized by pitting. A total of 8 crabs were introduced individually in an aluminum-based pot with boiling water at a temperature of 98 °C. The internal temperature of each crab was measured at each 15-s interval, with the support of a digital food thermometer (Digital Thermo; Thermopress, France) (inserted in the carapace, immediately ventral to the heart) and a stopwatch (Traceable®).

2.2. Testing resistance of Exophiala cancerae to the temperature of cooking

Prior to the *in vitro* tests used for evaluate the resistance of *E. cancerae* (CBS strain 120420) to the temperature of cooking, mycelia fragments stored in Sabouraud Dextrose Agar (Himedia) were plated in Mycosel Agar (Pronadisa) and incubated for two weeks (at 25 °C). This strain was isolated and purified from moribund crab tissues during an LCD's mortality outbreak in the state of Sergipe, northeast Brazil. The yeast cells and elements of hyphae obtained from the recovered colonies were suspended in saline solution (2.5%, the same physiological salinity of *U. cordatus* hemolymph—Harris & Santos, 1993), resulting in a stock solution of approximately 2×10^7 fungal elements per mL. Manipulations were performed in a horizontal laminar air-flow workstation (LabCon Co., Purifier Class II). Aliquots of 50 μL of this solution were transferred to 4 microtubes (0.2 mL, Axygen®). Each microtube was exposed to the temperature of 98 °C in a thermocycler (Mastercycler personal, Eppendorf) for different time-intervals (0, 30, 120, and 300 s). Afterwards, the contents of these tubes were individually plated in Mycosel Agar and incubated for 14 days at 25 °C. Subsequently, digital photographs (with a Sony MVC-CD500) for the resulting 4 Petri dishes (90 × 15 mm) were analyzed with the support of the SigmaScan Pro 5.0® program tools (Systat Software Inc.). All images were converted to scales of gray and measurements were limited to elements with strict intensity threshold (over 60 pixels), i.e. areas occupied by CFUs (Colony-forming units) of *E. cancerae*. This experiment was repeated 5 times.

3. Results and discussion

Except for a single mangrove-land crab that only reached its maximum internal temperature after about 20 min of cooking (specimen 06), the other animals (87.5%) required a maximum of 14 min to reach 98 °C (Fig. 1). Furthermore, in the resistance analysis to cooking temperature, the colony growth of *E. cancerae* was observed only in the control group (0 s), in which colonies occupied an average of 1549 mm^2 of the Petri dishes (Fig. 2).

Thus, our results strongly indicate that the causative agent of the LCD is inactivated or dead within 15 min of the coction process of the crabs. This cooking time is almost half of the time typically used in the preparation of the traditional dish of this crab species and, thus, *E. cancerae* should not represent a potential public health problem under this scenario.

Sterflinger (1998), measuring the lethal temperature of rock-inhabiting black yeasts, noted that dehydration is an important prerequisite for these microorganisms withstand high temperatures. In their study, the dried mycelia stand temperature up to 120 °C for at least 30 min, retaining full-growth activity after transfer to fresh medium. However, for physiologically active and fully hydrated thalli, the lethal temperature is between 35 °C and 75 °C. Hence, these results corroborate our study.

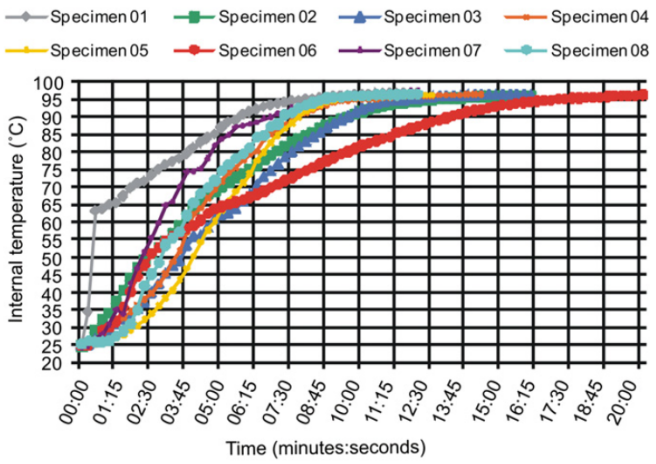

Fig. 1. Dynamic of the increasing in *U. cordatus* core body temperature along the exposition to coction process.

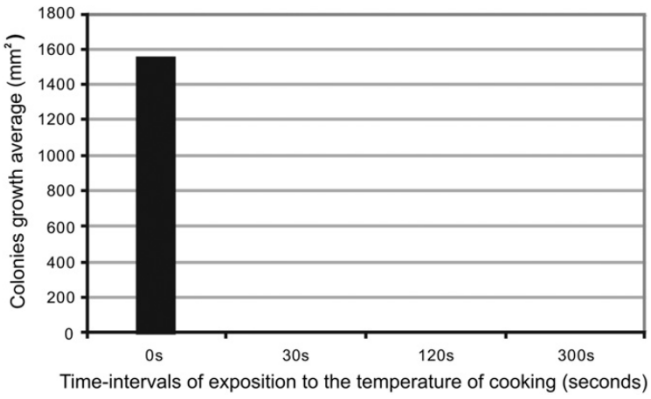

Fig. 2. Average CFU growth of *Exophiala cancerae* (CBS strain 120420) after exposition to the cooking (boiling water) for different time-intervals.

Although LCD does not seem to pose a threat as food items, they may still affect crab grabbers and other people who handle and transport these animals to their final destinations via other infection pathways. Indeed, De Hoog et al. (1999) indicate that the "despite its maximum growth temperature of 33 °C, the species possesses intrinsic virulence factors. These may be expressed particularly in external tissues of the extremities patients with reduced blood circulation, e.g. to underlying diabetes."

Therefore, future evaluation of the pathogenicity of the LCD-causative agent to mammalian hosts, through mice intraperithoneal and superficial inoculations, will provide a better understanding of the outcomes of a possible cross infection of these microorganisms.

Despite the characteristic ubiquity of black yeasts, there are few reports of contamination of food by these microorganisms. Kazanas (1986) isolated a strain of *E. dermatitidis*(Kano)de Hoog & Hermanides-Nijhof, 1977 from edible desiccated mushrooms, a dermatropic as well as neurotropic agent that causes cutaneous and disseminated phaeohyphomycosis (Ajello, 1975; Ajello, Georg, Steigbigel, & Wang, 1974). By intraperithoneal inoculations and intragastric intubations in mice, the authors confirmed the potential for systemic infection of these black yeasts by both routes.

Thus, responsible public policies depend strongly on additional evaluation of the infection potential of the black yeast species, as highlighted by their recognized harmful effects. Some of the clinical signs of black yeast infections are discreet and might remain concealed for a long time, particularly given that crab grabbers often live in remote areas with poor health assistance. This is an area that deserves special attention by health officials in the regions affected by LCD.

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References

Ajello, L. (1975). Phaeohyphomycosis: definition and etiology. In *Mycoses* (pp. 126–133). Washington, D.C: Pan American Health Organization, Scientific publ. no. 304.

Ajello, L., Georg, L. K., Steigbigel, R. T., & Wang, C. J. K. (1974). A case of phaeohyphomycosis caused by a new species of Phialophora. *Mycologia, 66*, 490–498.

Araújo, A. R. (2006). *Fishery statistics and commercialization of the mangrove crab, Ucides cordatus (L.), in Bragança – Pará – Brazil*. Unpublished Ph.D. thesis, University of Bremen.

Boeger, W. A., Pie, M. R., Ostrensky, A., & Patella, L. (2005). Lethargic Crab Disease: multidisciplinary evidence supports a mycotic etiology. *Memórias do Instituto Oswaldo Cruz, 100*(2), 161–167.

Boeger, W. A., Pie, M. R., Vicente, V. A., Ostrensky, A., Hungria, D., & Castilho, G. G. (2007). Histopathology of the mangrove land crab *Ucides cordatus* (Ocypodidae) affected by Lethargic Crab Disease. *Disease of Aquatic Organisms, 78*, 73–81.

De Hoog, G. S., Poonwan, N., Gerrits, & Van Den Ende, A. H. G. (1999). Taxonomy of *Exophiala spinifera* and its relationship to *E. jeanselmei*. *Studies in Mycology, 43*, 33–42.

Harris, R. R., & Santos, M. C. F. (1993). Sodium uptake and transport (Na+K+) ATPase changes following Na+ depletion and low salinity acclimation in the mangrove crab *Ucides cordatus* (L.). *Comparative Biochemistry Physiology, 105*, 35–42.

de Hoog, G. S., & Hermanides-Nijhof, E. J. (1977). *The black yeasts and allied hyphomycetes. Studies in Mycology no. 15*. Baarn, The Netherlands: Centraalbureau voor Schimmelcultures.

Hiruma, M., Kawada, A., Ohata, H., Ohnishi, Y., Takahashi, H., Yamazaki, M., et al. (1993). Systemic phaeohyphomycosis caused by *Exophiala dermatitidis*. *Mycoses, 36*, 1–7.

Kazanas, N. (1986). Pathogenicity of a fungus resembling *Wangiella dermatitidis* isolated from edible mushrooms. *Applied and Environmental Microbiology, 51*, 261–267.

Orélis-Ribeiro, R., Boeger, W. A., Vicente, V. A., Chammas, M., & Ostrensky, A. (2011). Fulfilling Koch's postulates confirms the mycotic origin of Lethargic Crab Disease. *Antonie Leeuwenhoek, 99*(3), 601–608. doi:10.1007/s10482-010-9531-4.

Pedrosa, L. F. C., & Cozzolino, S. M. F. (2001). Composição centesimal e de minerais de mariscos crus e cozidos da cidade de Natal/RN. *Ciência e Tecnologia de Alimentos, 21*(2), 154–157.

Sterflinger, K. (1998). Temperature and NaCl-tolerance of rock-inhabiting meristematic fungi. *Antonie Van Leeuwenhoek, 74*, 271–281.

Theophilo, G. N. D., & Vieira, R. H. S. F. (1994). Study of *Vibrio parahaemolyticus* in raw and cooked crabs used for marketing in Fortaleza, Ceará State. *Bol SBCTA, 28*(2), 134–142, São Paulo.

Vieira, R. H. S. F., Lima, E. A., Sousa, D. B. R., Reis, E. F., Costa, R. G., & Rodrigues, D. P. (2004). *Vibrio spp.* and *Salmonella spp.*, presence and susceptibility in crab *Ucides cordatus*. *Revista do Instituto de Medicina Tropical de São Paulo, 46*(4), 179–182.

Wong, H. C., Chen, M. C., Liu, S. H., & Liu, D. P. (1999). Incidence of highly genetically diversified *Vibrio parahaemolyticus* in seafood imported from Asian countries. *International Journal of Food Microbiology, 52*, 181–188.