Metschnikowia bicuspidata

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Metschnikowia bicuspidata | Eriocheir sinensis | horizontal transmission

1. Introduction

Crustacean culture is often accompanied by a variety of diseases, among which viral and bacterial diseases are widespread. However, studies have shown that yeast, including *Metschnikowia*, *Cryptococcus*, *Candida*, *Pichia*, *Debaryomyces*, *Torulopsis*, *Fonsecaea*, and *Exophiala*, are pathogenic to crustaceans [1][2][3][4], causing substantial economic losses to the aquaculture industry. For example, the yeast species *Candida sake*, *Pichia anomala*, *Endomyces fibuliger*, *C. famata*, and *Torulopsis mogii* are pathogenic to the freshwater prawn, *Macrobrachium rosenbergii* [5][6]. *Exophiala cancerae* and *Fonsecaea brasiliensis* are pathogenic to the mangrove land crab, *Ucides cordatus* [7], and *Metschnikowia bicuspidata* is pathogenic to the Chinese swimming crab, *Portunus trituberculatus* [8].

The Chinese mitten crab, *Eriocheir sinensis*, is an important farmed crustacean species in China, with 778, 682 tons produced in 2019 [9]. An emerging disease, which is commonly known as “milky disease”, occurred in *E. sinensis* farms in Panjin city, northeast China, in the winter of 2018 [10]. The symptoms of diseased crabs were characterized by opaque whitish muscles and milky hemolymph, inactive and anorexic behavior, and staying at the shallow end of the pond. The disease had a mortality rate of over 20% [11], and the mortality of infected crabs is rising. Thus, the disease has caused serious production and economic losses in the crab farming industry. The main pathological feature of the disease is the presence of severe myopathy, discrete necrotic lesions, and severe colonization of the pathogen in the muscles, heart, gills, and other organs. Subsequently, milky disease is of great concern to aquaculturists and researchers. Using molecular methods [11][12], the pathogen responsible for milky disease was isolated and identified as the yeast *Metschnikowia bicuspidata*.

Metschnikowia bicuspidata is an opportunistic pathogenic yeast that is distributed in marine and freshwater environments worldwide. Many species have been reported to host *M. bicuspidata*, including the bait organisms, *Daphnia* and *Artemia* [13][14], and more notably, aquatic animals of high economic value, such as salmon, *Oncorhynchus tshawytscha* [15], freshwater cultured shrimp, *M. rosenbergii* [5][6][16], and marine cultured crab, *P. trituberculatus* [4]. An outbreak of *M. bicuspidata* in Taiwan from May 2001 to December 2003 resulted in the cumulative mortality of 20%–95% in *M. rosenbergii* and, in California, *M. bicuspidata* led to the cumulative mortality of 34.5% in larval *O. tshawytscha* [15]. In addition, *M. bicuspidata* occurs in mixed bacterial infections. For example, *M. bicuspidata* was co-infected with *Vibrio alginolyticus* in *M. rosenbergii* [17]. Therefore, this yeast pathogen is easily and widely spread.

Clarifying the transmission route of pathogens is important for the prevention and control of aquaculture disease. *Daphnia magna* infected with *M. bicuspidata* release a large number of ascospores into the environment after death [18]. Healthy *D. magna* can then become infected after ingesting the ascospores [18]. The mature ascospores of *M. bicuspidata* are needle-shaped, allowing them to penetrate their host more easily [19]. In addition, *M. bicuspidata* can spread among different species via the food chain. For example, *M. bicuspidata* infects the brine shrimp *Artemia*, which can act as a vector for transmission to larval salmon [15]. *M. bicuspidata* is a fungal pathogen, and there is no effective drug for treating the infection. Although some antifungal drugs and biological remedies have shown effective anti-pathogenic effects in vitro [8][20], utilization is difficult in aquaculture settings and, more importantly, they are not preferred as the crabs will ultimately be used for human consumption. Therefore, preventing the transmission of *M. bicuspidata* is the best method of control, and research should focus on this aspect. However, it is unclear how healthy crabs are infected.

2. Metschnikowia bicuspidata

Metschnikowiabicuspidata is a pathogenic yeast that was first identified in *Daphnia* [21]. This pathogen can infect various economically important aquatic animals, such as *P. trituberculatus*, *M. rosenbergii*, and *E. sinensis*, which results in substantial economic losses to the aquaculture industry [8][16][22]. Therefore, it is important to identify the route of transmission to effectively control mycoses in these cultured species. Previous research has shown that salmon can also be infected with *M. bicuspidata*, via *Artemia* as the vector, but it cannot be infected directly from the water or through intramuscular injection [15]. Unlike vertebrates, crustaceans have an open tube cycle. Hemolymph not only flows through the heart and blood vessels but also into the cell space. Therefore, *M. bicuspidata* can be rapidly infected by injection. This has been confirmed in *P. trituberculatus*, *M. rosenbergii*, and *E. sinensis* [4][11][12][16]. However, to the best of knowledge, there have been no studies that discuss intra-species or inter-species transmission.

In general, aquatic animal pathogens are transmitted via horizontal transmission, vertical transmission, or both [23][24][25]. The route of transmission is related to the type of pathogen. Generally, pathogens that are spread through vertical transmission have low virulence, which keeps the host alive, while those that are spread through horizontal transmission are often highly virulent, which accelerates the diffusion rate following the death of the host [26][27][28]. Fungi are mainly spread through horizontal transmission.

Here, it was found that *M. bicuspidata* did not colonize germ cells and was not detected in fertilized eggs and hatched larvae (zoeae 1–5 and megalopae), which indicated that *M. bicuspidata* was not transmitted vertically. Similarly, the prevalence of *M. bicuspidata* in *M. rosenbergii* was highest in adult prawns (73%), followed by juveniles (25%) and post-larvae (2%), but no yeast infections were detected in larvae [5]. Owing to the metamorphosis and rapid development of the larval stage, an infection experiment of the larval stage was not included in this investigation, and further studies are required to determine whether infection with *M. bicuspidata* is possible during the metamorphosis stage. A previous investigation demonstrated that the infection rate in young crabs was low, but the infection rate in the adult stage was high [10], which is consistent with the observations in *M. rosenbergii* [16], although the reason for the high rate of infections in adults remains unclear. Chen et al. [16] postulated that despite adults having a more mature immune defense system, they are more likely than young shrimp to encounter yeast in pond water or sediment because they have a longer life span and tend to consume dead or dying shrimp that have been infected with the yeast.

Similar to other fungi, *M. bicuspidata* is mainly transmitted through horizontal transmission. In the present study, healthy crabs were infected with the yeast following the ingestion of infected tissues, indicating that cannibalism is a mode of infection. The *Eriocheir sinensis* is aggressive and often cannibalizes other crabs, especially when the shell has not hardened after molting [29][30]. Pathogen transmission through mutual encroachment is also common in crustaceans, such as with the white spot syndrome virus (WSSV) in *P. vannamei* [31]. In high-density breeding conditions, it is difficult to avoid cannibalism, and disease-carrying organisms are easy to consume given their weakened state, which further facilitates the transmission of pathogens. Co-cultured carnivorous fish can significantly reduce the incidence of WSSV in *P. vannamei* [32] because carnivorous fish rapidly consume moribund shrimp, which prevents transmission of the pathogen to healthy shrimp. However, it is difficult to use this method in crab cultures, as it is difficult for common fish to consume the hard, thickened shell of crabs, and paddy field environments are also not conducive to the polyculture of fish.

Soto et al. [33] compared the transmission rate of WSSV in *P. vannamei* through feeding and by cohabitation. Their results showed that the transmission rate of WSSV through cohabitation was 0.01, while that from feeding was 0.46, which indicates that cohabitation is not a major route of transmission for WSSV. It is demonstrated that healthy crabs can still be infected when they are not in contact with diseased crabs and during mixed breeding, healthy crabs can become infected by the ingestion of feces or water containing the pathogen. In addition, this investigation revealed that healthy crabs can become infected when exposed to water contaminated with *M. bicuspidata*. Owing to the high concentration of *M. bicuspidata* (10^7 cells/mL) used in the experiment, the infection rate from immersion was also high, but such a concentration is unusual during the culture process. Therefore, the minimal concentration of yeast in the culture environment requires further investigation. Previous research has shown that yeast mainly infects aquatic organisms via wounds caused by aggression or mechanical injury [34][35][36], although it is demonstrated that healthy crabs without external lesions can be infected by yeast, which is consistent with the findings of Stentiford et al. [37]. Lu et al. [5] also suggested that *M. rosenbergii* infection occurs mainly through mouth and gill contact with the yeast in water and sediments.

Through comparing the three modes of infection, the infection rate of the group that was fed infected tissue was higher than the bath immersion and cohabitation groups, suggesting that cannibalism is more likely to result in infection. The survey of juvenile crabs and berried crabs in the overwintering ponds in the Panjin area also revealed that the infection rate was high [10]. This may be related to the high-density culture under the ice during the overwintering period, without access to shelter, which results in the cannibalism of infected crabs. It also revealed that feeding, bath immersion, and cohabitation did not reach a 100% infection rate, further suggesting that there were individuals with a strong resistance to *M. bicuspidata* in the population, which highlights a promising avenue for the future breeding of disease-resistant *Eriocheir sinensis* and other species.

Many studies have been carried out with respect to the treatment of fungal diseases. Ma et al. [12][38] found that common antifungal drugs (polyenes, triazoles, and fluorocytosine) markedly inhibited *M. bicuspidata* in vitro. Zhang et al. [20], found that massoia lactone, derived from liamocins produced by *Aureobasidium melanogenum*, performed well against *M. bicuspidata*. However, the absorption, distribution, metabolic kinetics, and associated by-products of these antifungal treatments have not been well investigated. Considering factors such as food safety and production cost, it is unclear whether these treatments can be used safely on a large scale. Some researchers have tried to control the disease with biological approaches, including using marine killer yeast against *M. bicuspidata* [8][39][40][41]. Although these killer yeasts can inhibit *M. bicuspidata* in a laboratory environment, it is unclear how they may safely be used in complex aquacultur environments and achieve a significant effect. It is expected that safe and effective drugs against yeast infections will be developed in the near future. Until that time, prevention is the best strategy. Furthermore, since transmission of the pathogen can occur via contaminated water and infected tissues, as demonstrated in the present study, additional measures to reduce infection include disinfecting the water and bait organisms, reducing the culture density, and removing dead individuals.

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