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Colonization and Probiotic Effect of *Metschnikowia* sp. C14 in the Intestine of Juvenile Sea Cucumber, *Apostichopus japonicus*

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Abstract Viable cell count was used to determine whether *Metschnikowia* sp. C14 can colonize the intestine of juvenile sea cucumber *Apostichopus japonicus*. Sea cucumber individuals were divided into two groups, which were fed the control diet for 38 days or the C14-supplemented diet at 10^5 cells g^{-1} diet for 28 days, then the control diet from day 29 to day 38. The number of C14 cells in the intestine of sea cucumber fed the C14-supplemented diet significantly increased from day 7 to day 28, and decreased from day 29 to day 38. Sea cucumber fed with the diet containing C14 showed a significant increase in trypsin activity and lipase activity from day 21 to day 33 compared with the control. Feeding C14 significantly improved the phagocytic activity and respiratory burst in coelomocytes from day 21 to day 35 and from day 14 to day 38, respectively. In addition, there was an obvious enhancement in lysozyme activity (from day 21 to day 38 or day 33), phenoloxidase activity (from day 21 to day 28) and total nitric oxide synthase activity (from day 14 to day 38) in coelomic fluid supernatant and/or coelomocyte cell lysate supernatant compared with the control. There were significant positive correlations between the number of C14 cells colonizing the intestine and trypsin activity of the intestine, lysozyme activity of the coelomic fluid supernatant and coelomocyte lysate supernatant from sea cucumber. These data suggested that the number of C14 cells should be maintained at 10^5 cfu (colony-forming units) g^{-1} intestine material for the maximum benefit.

Key words *Apostichopus japonicas*; feeding duration; colonization; *Metschnikowia* sp. C14; digestive enzyme activity; immune parameter

1 Introduction

Probiotics are live microorganisms that can benefit the health of the host (FAO/WHO, 2001). For the aquatic animals, the beneficial effect may be reached by various mechanisms including, for example, nutritional complement, improvement of the digestibility of feed, enhancement of innate immunity, antibacterial activity, stimulation of biological processes, improvement of the quality of the water among others (Verschuere *et al*., 2000; Prado *et al*., 2010; Newaj-Fyzul *et al*., 2014). In recent years, bacterial and yeast probiotics such as *Bacillus subtilis* (Zhao *et al.*, 2012), *Bacillus* sp. BC26 (Liu *et al*., 2013), *B. baekryungensis* (Yan *et al.*, 2014a), *B. cereus* (Yang *et al*., 2015; Zhao *et al*., 2016), the mixture of *B. subtilis* and *B. cereus* (Li *et al*., 2015), *Hanseniaspora opuntiae* (Ma *et al.*, 2013, 2014), lactic acid bacteria (Li *et al*., 2018), *Metschnikowia* sp. (Liu *et al*., 2012; Yang *et al.*, 2014), *Paracoccus marcusii* (Yan *et al.*, 2014b), *Pseudoalteromonas elyakovii* (Chi *et al*., 2014), *Rhodotorula benthica* (Wang *et al*., 2015), *R. mucilaginosa* (Zhang *et al*., 2017), *Rhodotorula* sp. (Yang *et al*., 2015a, 2015b), *Shewanella japonica* (Chi *et al.*, 2014), *Vibrio* sp. (Liu *et al*., 2017) and *Vibrio tasmaniensis* (Chi *et al*., 2014) have been demonstrated to improve growth, digestive enzyme activity and immune response of sea cucumber *Apostichopus japonicas*, and enhance their resistance to pathogen attack. In addition, dietary administration of probiotics do not affect significantly the intestinal microbiota of sea cucumber (Ma *et al*., 2018, 2019). Analysis of gut microbiota revealed a significant difference in the relative abundance of *Lactococcus garviaeae* between diseased and healthy sea cucumbers (Zhang *et al.*, 2018). The intestinal microbiota homeostasis of sea cucumber can be improved by probiotics (Yang *et al*., 2017).

Several studies have applied different feeding durations varying between 7 and 45 days to improve the immune response in sea cucumber (Liu *et al*., 2012; Ma *et al.*, 2013; Chi *et al*., 2014) though the reason for choosing different durations is not clear. Three strains of marine yeast have been confirmed to be capable of colonizing in sea cucumber intestine for at least 31 days after cessation of feeding

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following 15 days of administration (Ma *et al.*, 2014; Yang *et al.*, 2014; Yang *et al*., 2015a). In the South African abalone *Haliotis midae*, there is a positive correlation between yeast/bacterium quantity and enzyme activity (Macey and Coyne, 2006). The yeast *Metschnikowia* sp. C14 was originally isolated from the intestine of healthy adult sea cucumber (Liu *et al*., 2012). It can effectively colonize the juvenile sea cucumber intestine *via* dietary supplementation and improve their specific growth rate and digestive enzyme activity (Yang *et al*., 2014) and immune response and resistance to pathogen infection (Liu *et al*., 2012). Nevertheless, no information is available for the correlation between the number of probiotic cells in sea cucumber intestine and the probiotic effect. Therefore, the aim of this study was to determine the effect of C14 with different feeding durations on digestive enzyme activity and immune response of sea cucumber.

2 Materials and Methods

2.1 Yeast and Diet Preparation

The yeast C14 was cultured overnight in yeast-peptone-dextrose (YPD) broth at 25℃ with constant shaking. The cellular suspension was centrifuged at $1000\times g$ and 4℃ for 10 min with the pellet washed and re-suspended in 0.9% NaCl. The concentration of the yeast suspension was adjusted to about 10^6 cells mL^{-1} using a haemocytometer slide. The formulation and proximate composition of the control diet were prepared following the method described by Liu *et al*. (2012). Suspension was added to the control diet and mixed thoroughly to achieve 1×10^5 cells g^{-1}, which was prepared each day to guarantee the vitality of C14. Selection of the C14 dose in the diet was based on the data documented previously (Liu *et al*., 2012).

2.2 Feeding Trail

Sea cucumber individuals purchased from a commercial farm were acclimated to the rearing condition for two weeks. Then selected sea cucumber individuals with similar size (0.92 ± 0.01 g) were randomly distributed into six plastic tanks (100 L), 100 each. Sea cucumber in three tanks was fed the C14-supplemented diet for 28 days, then the control diet from day 29 to day 38 while the animal in other three tanks was fed the control diet for 38 days. Sea cucumber was fed once a day at 16:00. Water temperature ranged from 17℃ to 22℃, salinity from 33 to 34 and acidity from pH 7.8 to 8.2. Dissolve oxygen was maintained at or near saturation by aeration *via* air stones. Fifty liters of water each tank was replaced with fresh seawater every day.

2.3 Sample Collection

After the start of feeding, ten sea cucumber individuals each tank were randomly sampled for enumeration of live yeast, digestive enzyme activity and immune parameter assays on days 7, 14, 21, 28, 29, 31, 33, 35 and 38. Before sampling, sea cucumber was starved for 16 h (Liu *et al*., 2013). Fifty microliters of coelomic fluid from each of ten sea cucumber individuals was pooled and mixed with an equal volume of isotonic aqueous anticoagulant solution (Xing *et al.*, 1998). Four hundred microliters of the coelomic fluid was taken for phagocytic activity and respiratory burst tests while the remaining was centrifuged at $800\times g$ and 4℃ for 10 min with the supernatant collected and used directly for lysozyme (LSZ), phenoloxidase (PO) and total nitric oxide synthase (T-NOS) activities assays. The coelomocyte lysate supernatant (CLS) was prepared following the method described by Ma *et al*. (2018) and used for LSZ, PO and T-NOS activities assays.

The intestines from ten sea cucumber individuals were pooled and homogenized in nine volumes of sterile 0.9% NaCl. A portion of the intestinal homogenate was taken for enumeration of live yeasts, and the remaining was centrifuged with the supernatant used for digestive enzyme activity analysis.

2.4 Protein Content Assay

The soluble protein content of the coelomic fluid, CLS and the supernatant of intestine homogenates were measured following the method described by Bradford (1976).

2.5 Determination of the Number of C14 in Intestine

Intestinal homogenates were serially diluted using 0.9% NaCl, and 0.1 mL volume was spread onto the surface of duplicate plates of yeast-peptone-dextrose agar and incubated at about 25℃ for 7 days. The yeast cell number is recorded as colony-forming units (cfu) per gram of fresh intestine material (cfu g^{-1}).

2.6 Digestive Enzyme Activity Measurement

Trypsin, amylase and lipase activities were measured according to the methods described by Ma *et al*. (2018).

2.7 Immune Parameter Assay

Phagocytic activity of coelomocytes was measured using the uptake of neutral red stained zymosan particles based on the method of Ma *et al*. (2013). The respiratory burst or superoxide anion generation of coelomocytes was measured spectrophotmetrically using a nitroblue tetrazolium (NBT) assay (Song and Hsieh, 1994) except that the assaying temperature was changed from 37℃ to room temperature (about 20℃). The absorbance of the dissolved cytoplasmic formazan was read at 630 nm and expressed as NBT activity per 100 μL coelomic fluid (Sajeevan *et al.*, 2006). LSZ activity in coelomic fluid supernatant (CF) and CLS was estimated following the method of Ma *et al*. (2018). PO activity was determined spectrophotometrically using L-3,4-dihydroxyphenylalanine as a substrate and trypsin as an elicitor as previously described by Ma *et al.* (2013). T-NOS activity was determined by colorimetric analysis using a commercial test kit (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions except that the temperature for the reaction was changed to room temperature (about 20℃).

2.8 Statistical Analysis

Statistical analysis was conducted using the SPSS 19.0 for windows. Data of the digestive enzyme activity and immune parameter between two groups were analyzed using an independent samples *t*-test. The number of live yeast was analyzed using one-way analysis of variance. If significance was detected, Tukey's multiple range test was used to compare the means between sampling dates. Pearson's product moment correlation coefficient was used to determine the strengths of the association between digestive enzyme activity/innate immune parameter and yeast cell numbers. Prior to statistical analysis, the yeast cell number was logarithm-transformed to alleviate heteroscedasticity. Differences were considered significant if $P<0.05$.

3 Results

3.1 Colonization and Persistence of C14 in the Intestine of Sea Cucumber

C14 was only isolated from the intestine of sea cucumber fed the probiotic-supplemented diet. Moreover, the number of C14 cells was significantly increased from day 7 to day 28 ($P<0.05$) with the highest 4.43×10^5 cfu g^{-1} on day 28. However, the C14 cell number in the intestines decreased significantly within 10 days following cessation of feeding with the C14-supplemented diet ($P<0.05$) (Fig.1). There was no significant difference in C14 cell number between day 21 and day 33.

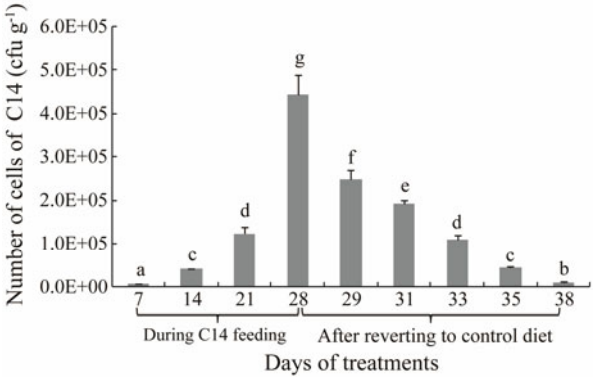

Fig.1 The number of C14 cells in the intestines of sea cucumber during 28 days of feeding with C14-supplemented diet and after reverting to control diet for another 10 days. Means without a same letter differed significantly ($P<0.05$) between the feeding regimes.

3.2 Digestive Enzyme Activity

The intestinal trypsin and lipase activities in sea cucumber fed with C14-supplemented diet for 21 and 28 days were significantly higher than those in the animal fed the control diet ($P<0.05$) (Figs.2–3). Furthermore, there was significant difference in trypsin and lipase activities between two groups from day 29 to day 33 after the cessation of feeding ($P<0.05$). However, no significant difference in amylase activity was observed between the control and the C14 feeding groups.

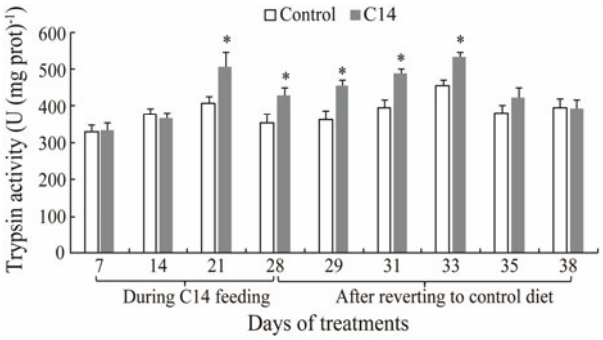

Fig.2 Intestinal trypsin activity of sea cucumber cross 38 days of feeding; Data represent means±SD ($n=3$). * Significant difference ($P<0.05$) from the control group at the same sampling date.

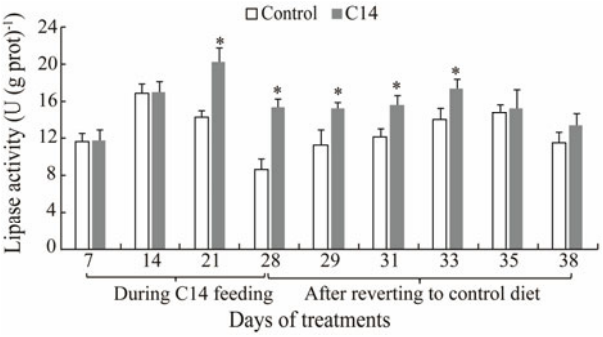

Fig.3 Intestinal lipase activity of sea cucumber cross 38 days of feeding; Data represent means±SD ($n=3$). * Significantly difference ($P<0.05$) from the control group at the same sampling date.

A correlation analysis was conducted to determine the strength of the association between digestive enzyme activity and the number of C14 cells in intestine in sampling period. The Pearson's product moment correlation between trypsin activity and the C14 cell number was positive ($R=0.68$; $P<0.05$). Nevertheless, there was no correlation between lipase or amylase activity and the C14 cell number (Table 1).

Table 1 Pearson's correlations between parameters and logarithm-transformed numbers of C14 cells

Parameter	Correlation
Trypsin activity	0.68*
Lipase activity	0.55
Amylase activity	0.24
Phagocytic activity	0.58
Respiratory burst	0.54
LSZ (CF)	0.82**
LSZ (CLS)	0.83**
PO (CF)	0.61
PO (CLS)	0.48
T-NOS (CF)	0.63
T-NOS (CLS)	0.55

Notes: * Significantly different at $P<0.05$; ** Significantly different at $P<0.01$ ($n=9$).

3.3 Immune Parameter

The phagocytic activity of sea cucumber fed C14-supple-

mented diet showed a significant increase compared to the control after 21 and 28 days of feeding ($P<0.05$) (Fig.4). Moreover, there was significant difference in phagocytic activity between two groups from day 29 to day 35 after the cessation of feeding ($P<0.05$) (Fig.4).

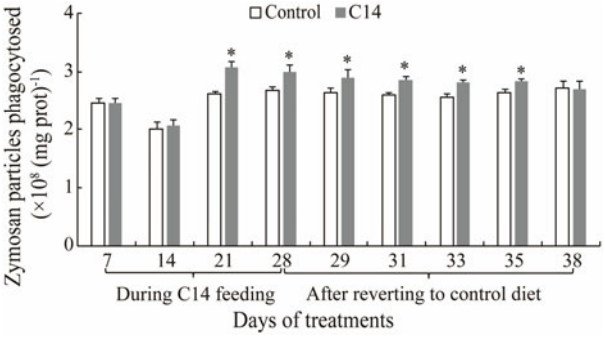

Fig.4 Phagocytic activity in coelomocytes of sea cucumber over 38 days of feeding. Data represent means±SD ($n=3$). * Significantly difference ($P<0.05$) from the control group at the same sampling date.

Sea cucumber fed with C14-supplemented diet for 14, 21 and 28 days had a significant higher respiratory burst than those fed the control diet ($P<0.05$). When the animal was shifted to the control diet, significant differences were still observed between two groups from day 29 to day 38 ($P<0.05$) (Fig.5).

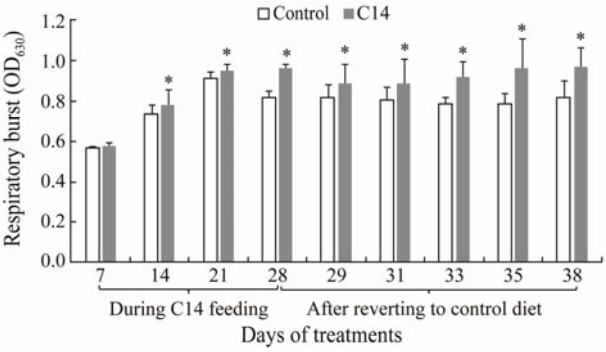

Fig.5 Respiratory burst in coelomocytes of sea cucumber over 38 days of feeding. Data represent means±SD ($n=3$). * Significantly difference ($P<0.05$) from the control group at the same sampling date.

There was a significant increase in the LSZ activity of CF and CLS of sea cucumber fed C14-supplemented diet on days 21 and 28 compared with the level observed in the control ($P<0.05$) (Figs.6–7). Furthermore, the LSZ activity of CF and CLS from the experimental sea cucumber was statistically higher than those of control animal from day 29 to day 38, and from day 29 to day 33, respectively, after the cessation of feeding ($P<0.05$).

Sea cucumbers fed with C14-supplemented diet had significantly higher PO activity in the CF on days 21 and 28, and in the CLS on day 14 compared with those fed the control diet ($P<0.05$) (Figs.8–9). However, no significant difference in PO activity was observed between two groups on the other days.

The T-NOS activity in the CF and CLS of sea cucumber fed C14-supplemented diet was statistically different from that of control from day 14 to day 38 ($P<0.05$) (Figs.10–11).

Different digestive enzyme activities and immune parameters were observed among the 38 days from the control group. A correlation analysis was conducted to determine the strength of the association between innate immune parameters and the number of C14 cells in the intestines over the 38-day sampling period. The Pearson's product moment correlation between LSZ activity in CF/CLS and the C14 cell numbers revealed a positive correlation ($R=0.82/0.83$; $P<0.01$). Conversely, no correlation was found between the other innate immune parameters and C14 cell numbers (Table 1).

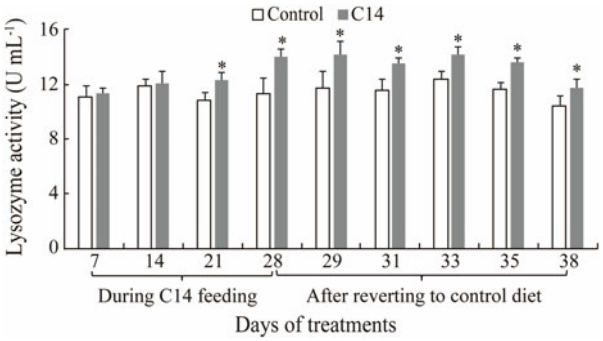

Fig.6 Lysozyme activity in coelomic fluid supernatant of sea cucumber over 38 days of feeding. Data represent means ±SD ($n=3$). * Significantly difference ($P<0.05$) from the control group at the same sampling date.

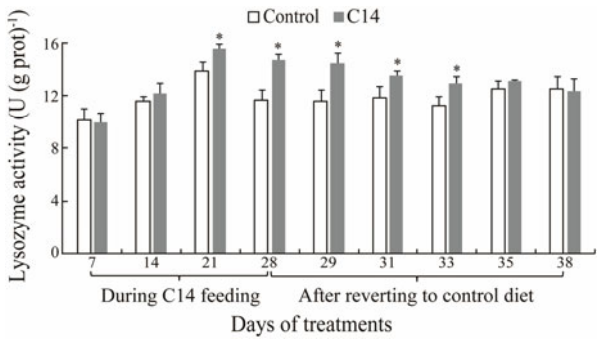

Fig.7 Lysozyme activity in coelomocyte lysate supernatant of sea cucumber over 38 days of feeding. Data represent means±SD ($n=3$). * Significantly difference ($P<0.05$) from the control group at same sampling date.

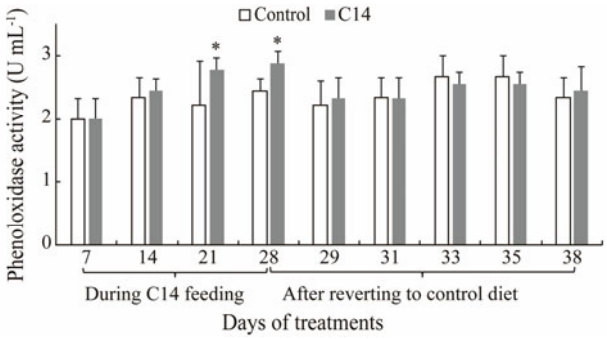

Fig.8 Phenoloxidase activity in coelomic fluid supernatant of sea cucumber over 38 days of feeding. Data represent means±SD ($n=3$). * Significantly difference ($P<0.05$) from the control group at the same sampling date.

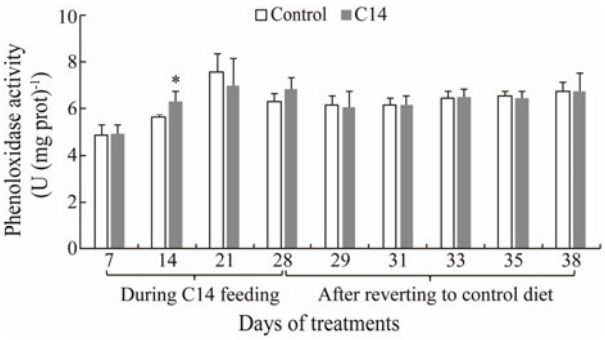

Fig.9 Phenoloxidase activity in coelomocyte lysate supernatant of sea cucumber over 38 days of feeding. Data represent means ± SD (n=3). * Significantly difference (P< 0.05) from the control group at the same sampling date.

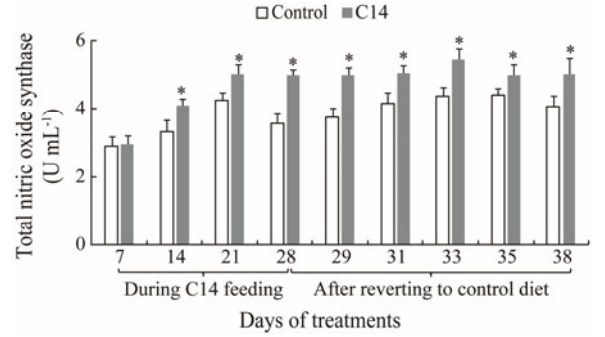

Fig.10 Total nitric oxide synthase activity in coelomic fluid supernatant of sea cucumber over 38 days of feeding. Data represent means ± SD (n=3). * Significantly difference (P <0.05) from the control group at the same sampling date.

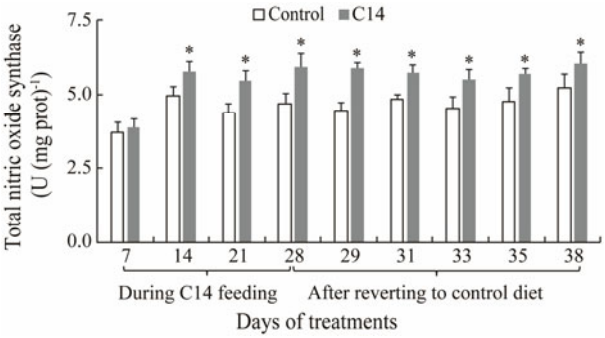

Fig.11 Total nitric oxide synthase activity in coelomocyte lysate supernatant of sea cucumber over 38 days of feeding. Data represent means ± SD (n=3). * Significantly difference (P<0.05) from the control group at the same sampling date.

4 Discussion

In the present study, C14 was only recovered from the intestine of sea cucumber receiving the yeast diet. This suggests that C14 can survive and withstand the condition of the sea cucumber intestine. The number of C14 cells reached about 10^5 cfu g^{-1} after sea cucumber fed with C14-supplemented diet at 10^5 cells g^{-1} for 21 to 28 days. However, the number of C14 cells dropped one order of magnitude 7 days after sea cucumber was shifted to the control diet. There was significant difference in trypsin activity between two groups from day 21 to day 33. We observed a positive correlation between the intestinal trypsin activity and the number of C14 cells while no correlation was observed between the C14 cell number and lipase/ amylase activity. Although the yeast is able to colonize the intestine of *A. japonicas* (Yang *et al.*, 2014), cells must be at a concentration of approximately 10^5 cfu g^{-1} to increase the trypsin activity. Similar results have also been observed in different abalone species. Feeding South African abalone with a diet containing each of the three probionts (*Vibrio midae* SY9.8, *Cryptococcus* sp. SS1, and *Debaryomyces hansenii* AY1) at a concentration of approximately 10^7 cells g^{-1} for 21 days resulted in cultivatable probionts at 10^6 to 10^7 cfu g^{-1} gut material (Macey and Coyne, 2006). Once the probiotic-supplemented feeding was stopped, the number of probiont cells declined. There was a positive correlation between the number of *Cryptococcus* sp. SS1 cells and amylase activity and between the number of *Vibrio midae* SY9.8 cells and protease activity in abalone intestine while no correlation was found between the number of *Debaryomyces hansenii* AY1 cells and protease and/or amylase activities (Macey and Coyne, 2006). A positive correlation was also observed between amylase or protease activity and the number of *Enterococcus* sp. s6 cells in the gut of Japanese abalone *Haliotis gigantea* whereas there was no correlation between amylase or protease activity and the number of *Lactobacillus* sp. a3 cells (Iehata *et al.*, 2009).

Marine yeasts have been used in sea cucumber aquaculture and were verified to have effects on non-specific immune response parameters such as phagocytic activity and LSZ, PO and T-NOS activities (Liu *et al.*, 2012; Ma *et al*., 2013; Wang *et al.*, 2015; Yang *et al.*, 2015b; Zhang *et al.*, 2017). Although different feeding durations, such as 30, 45 or 56 days, were chosen in the different reports about effects of dietary yeasts on the immune parameters of sea cucumber (Liu *et al*., 2012; Ma *et al*., 2013; Wang *et al*., 2015; Yang *et al*., 2015b; Zhang *et al.*, 2017), the basis for choosing these periods was unclear. In the present study, sea cucumber fed a diet containing live cells of C14 showed significantly higher phagocytic activity/ respiratory burst of coelomocytes from day 21/14 to day 35/38, higher LSZ activity of CF/ CLS from day 21 to day 38/33, and higher T-NOS activity of CF/ CLS from day 14 to day 38 than the control. A correlation analysis suggested a positive association between colonization of C14 in the intestine and LSZ activity of CF and CLS, indicating that cultivatable C14 cells should be maintained at 10^5 cfu g^{-1} to make a significant contribution to the cellular and humoral immune responses of sea cucumbers.

In addition, the varied enzyme activities/immune parameters observed in the control group over the 38 days may be related to environmental factors, such as temperature, and metabolism and growth of sea cucumbers, as well as other factors.

5 Conclusions

Yeast C14 can successfully colonize sea cucumber intestine when supplemented with control diet. There is a

positive correlation between the number of probiotic cells colonizing intestine and the trypsin activity/LSZ activity.

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References

Bradford, M. M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, **72**: 248-254.

Chi, C., Liu, J. Y., Fei, S. Z., Zhang, C., Chang, Y. Q., Liu, X. L., and Wang, G. X., 2014. Effect of intestinal autochthonous probiotics isolated from the gut of sea cucumber (*Apostichopus japonicus*) on immune response and growth of *A. japonicus*. *Fish and Shellfish Immunology*, **38**: 367-373.

FAO/WHO, 2001. *Health and Nutritional Properties of Probiotics in Food Including Powder Milk with Live Lactic Acid Bacteria*. Food and Agriculture Organization and World Health Organization Joint report, 34pp.

Iehata, S., Inagaki, T., Okunishi, S., Nakano, M., Tanaka, R., and Maeda, H., 2009. Colonization and probiotic effects of lactic acid bacteria in the gut of the abalone *Haliotis gigantea*. *Fisheries Science*, **75**: 1285-1293.

Li, C., Ren, Y. C., Jiang, S. H., Zhou, S., Zhao, J. S., Wang, R. J., and Li, Y. M., 2018. Effects of dietary supplementation of four strains of lactic acid bacteria on growth, immune-related response and genes expression of the juvenile sea cucumber *Apostichopus japonicus* Selenka. *Fish and Shellfish Immunology*, **74**: 69-75.

Li, J. G., Xu, Y. P., Jin, L. J., and Li, X. Y., 2015. Effects of a probiotic mixture (*Bacillus subtilis* YB-1 and *Bacillus cereus* YB-2) on disease resistance and non-specific immunity of sea cucumber, *Apostichopus japonicus* (Selenka). *Aquaculture Research*, **46**: 3008-3019.

Liu, J., Han, H., Sun, F. X., Zhang, C. Y., Cao, S. Q., Zhao, N. X., and Ma, Y. X., 2013. Effects of dietary live *Bacillus* sp. BC26 on digestive enzyme activity, immune response and disease resistance against *Vibrio splendidus* infection in juvenile sea cucumber *Apostichopus japonicus*. *Journal of Dalian Ocean University*, **28**: 568-572 (in Chinese with English abstract).

Liu, N. N., Zhang, S. S., Zhang, W. W., and Li, C. H., 2017. *Vibrio* sp. 33 a potential bacterial antagonist of *Vibrio splendidus*, pathogenic to sea cucumber (*Apostichopus japonicus*). *Aquaculture*, **470**: 68-73.

Liu, Z. M., Ma, Y. X., Yang, Z. P., Li, M., Liu, J., and Bao, P. Y., 2012. Immune responses and disease resistance of the juvenile sea cucumber *Apostichopus japonicus* induced by *Metschnikowia* sp. C14. *Aquaculture*, **368-369**: 10-18.

Ma, Y. X., Li, L. Y., Bao, P. Y., Li, M., Chen, W., and Chang, Y. Q., 2018. Effects of combined dietary administration of *Rhodotorula* sp. H26 and *Bacillus* sp. BC26 on growth, immunity and intestinal microbiota in juvenile sea cucumber, *Apostichopus japonicus*. *Aquaculture Research*, **49**: 3792-3803.

Ma, Y. X., Li, L. Y., Li, M., Chen, W., Bao, P. Y., Yu, Z. C., and Chang, Y. Q., 2019. Effects of dietary probiotic yeast on growth parameters in juvenile sea cucumber, *Apostichopus japonicus*. *Aquaculture*, **499**: 203-211.

Ma, Y. X., Liu, Z. M., Yang, Z. P., Bao, P. Y., Zhang, C. Y., and Ding, J. F., 2014. Effects of *Hanseniaspora opuntiae* C21 on the growth and digestive enzyme activity of juvenile sea cucumber *Apostichopus japonicas*. *Chinese Journal of Oceanology and Limnology*, **32**: 743-748.

Ma, Y. X., Liu, Z. M., Yang, Z. P., Li, M., Liu, J., and Song, J., 2013. Effects of dietary live yeast *Hanseniaspora opuntiae* C21 on the immune and disease resistance against *Vibrio splendidus* infection in juvenile sea cucumber *Apostichopus japonicus*. *Fish and Shellfish Immunology*, **34**: 66-73.

Macey, B. M., and Coyne, V. E., 2006. Colonization of the gastrointestinal tract of the farmed South African abalone *Haliotis midae* by the probionts *Vibrio midae* SY9, *Cryptococcus* sp. SS1, and *Debaryomyces hansenii* AY1. *Marine Biotechnology*, **8**: 246-259.

Newaj-Fyzul, A., Al-Harbi, A. H., and Austin, B., 2014. Review: Developments in the use of probiotics for disease control in aquaculture. *Aquaculture*, **431**: 1-11.

Prado, S., Romalde, J. L., and Barja, J. L., 2010. Review of probiotics for use in bivalve hatcheries. *Veterinary Microbiology*, **145**: 187-197.

Sajeevan, T. P., Philip, R., and Singh, I. S. B., 2006. Immunostimulatory effect of a marine yeast *Candida sake* S165 in *Fenneropenaeus indicus*. *Aquaculture*, **257**: 150-155.

Song, H. L., and Hsieh, Y. T., 1994. Immunostimulation of tiger shrimp (*Penaeus monodon*) hemocytes for generation of microbicidal substances: Analysis of reactive oxygen species. *Developmental Comparative Immunology*, **18**: 201-209.

Verschuere, L., Rombaut, G., Sorgeloos, P., and Verstraete, W., 2000. Probiotic bacteria as biological control agents in aquaculture. *Microbiology and Molecular Biology Reviews*, **64**: 655-671.

Wang, J. H., Zhao, L. Q., Liu, F., Wang, H., and Xiao, S., 2015. Effect of potential probiotic *Rhodotorula benthica* D30 on the growth performance, digestive enzyme activity and immunity in juvenile sea cucumber *Apostichopus japonicus*. *Fish and Shellfish Immunology*, **43**: 330-336.

Xing, J., Leung, M. F., and Chia, F. S., 1998. Quantitative analysis of phagocytosis by amebocytes of a sea cucumber, *Holothuria leucospilota*. *Invertebrate Biology*, **117**: 67-74.

Yan, F. J., Tian, X. L., and Dong, S. L., 2014a. Effect of *Bacillus baekryungensis* YD13 supplemented in diets on growth performance and immune response of sea cucumber (*Apostichopus japonicus*). *Journal of Ocean University of China*, **13**: 805-810.

Yan, F. J., Tian, X. L., Dong, S. L., Fang, Z. H., and Yang, G., 2014b. Growth performance, immune response, and disease resistance against *Vibrio splendidus* infection in juvenile sea cucumber *Apostichopus japonicus* fed a supplementary diet of the potential probiotic *Paracoccus marcusii* DB11. *Aquaculture*, **420-421**: 105-111.

Yang, G., Peng, M., Tian, X. L., and Dong, S. L., 2017. Molecular ecological network analysis reveals the effects of probiotics and florfenicol on intestinal microbiota homeostasis: An example of sea cucumber. *Scientific Report*, **7**: 4778.

Yang, G., Tian, X. L., Dong, S. L., Peng, M., and Wang, D. D., 2015. Effects of dietary *Bacillus cereus* G19, *B. cereus* BC-01, and *Paracoccus marcusii* DB11 supplementation on the growth, immune response, and expression of immune-related genes in coelomocytes and intestine of the sea cucumber (*Apostichopus japonicus* Selenka). *Fish and Shellfish Immunology*, **45**: 800-807.

Yang, Z. P., Sun, J. M., and Xu, Z., 2015a. Beneficial effects of *Rhodotorula* sp. C11 on growth and disease resistance of juvenile Japanese spiky sea cucumber *Apostichopus japonicus*.

Journal of Aquatic Animal Health, **27**: 71-76.

Yang, Z. P., Sun, J. M., Xu, Z., Zhang, C. C., and Zhou, Q., 2014. Beneficial effects of *Metschnikowia* sp. C14 on growth and intestinal digestive enzymes of juvenile sea cucumber *Apostichopus japonicus*. *Animal Feed Science and Technology*, **197**: 142-147.

Yang, Z. P., Xu, Z., Zhou, Q., Zhang, C. C., and Sun, J. M., 2015b. Effects of dietary supplementation of marine yeast *Rhodotorula* sp. C11 on digestive enzyme activity and immune response in juvenile sea cucumber *Apostichopus japonicas. Progress in Fishery Science*, **36**: 107-112 (in Chinese with English abstract).

Zhang, K., Liu, J. F., Dong, Q., Zhang, C. X., Xiao, S., Wang, H., and Wang, J. H., 2017. Effects of dietary addition of *Rhodotorula mucilaginosa* on performance of *Apostichopus japonicaus. Journal of Dalian polytechnic University*, **36**: 6-9 (in Chinese with English abstract).

Zhang, Z., Xing, R. L., Lv, Z. M., Shao, Y. N., Zhang, W. W., Zhao, X. L., and Li, C. H., 2018. Analysis of gut microbiota revealed *Lactococcus garviaeae* could be an indicative of skin ulceration syndrome in farmed sea cucumber *Apostichopus japonicus. Fish and Shellfish Immunology*, **80**: 148-154.

Zhao, Y. C., Yuan, L., Wan, J. L., Sun, Z. X., Wang, Y. Y., and Sun, H. S., 2016. Effects of potential probiotic *Bacillus cereus* EN25 on growth, immunity and disease resistance of juvenile sea cucumber *Apostichopus japonicus*. *Fish and Shellfish Immunology*, **49**: 237-242.

Zhao, Y. C., Zhang, W. B., Xu, W., Mai, K. S., Zhang, Y. J., and Liufu, Z. G., 2012. Effects of potential probiotic *Bacillus subtilis* T13 on growth, immunity and disease resistance against *Vibrio splendidus* infection in juvenile sea cucumber *Apostichopus japonicus*. *Fish and Shellfish Immunology*, **32**: 750-755.

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