Isolation and identification of lactic acid bacteria and yeasts with probiotic ability from the intestine of gilthead seabream

(Sparus aurata)

Mehdi Kamali Kolochani¹, Seyed Mohammad Mousavi², Isaac Zamani^{3*}, Vahid Yavari² and Mohammad Zakeri²

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Abstract

Lactic acid bacteria are the most common bacteria used as probiotics in aquaculture. This study aimed to isolate and identify lactic acid bacteria and yeasts with probiotic potential from the intestine of gilthead seabream. Five fish were randomly selected (mean weight: 279.88±17.67 gr) from Nixa Design and Development Farm located in Charak port and 25 fish fries (mean weight: 39.43±9.67 gr) from Tiab Pran Qeshm farm in Qeshm Island. The selected fish had a healthy appearance and were also chosen randomly. Lactic acid bacteria and yeasts were isolated and purified from the intestines of the specimens and identified based on morphological characteristics and molecular sequences. Then these isolates were evaluated based on fundamental probiotic indicators, including acid resistance, bile salts, antagonistic properties, and Haemolytic activity for fish. 12 isolates were purified based on color, shape, and colony size. Then, two yeasts and five bacteria with different morphology were identified using gram staining and microscopic examinations. All lactobacillus isolates had antagonistic properties against the pathogenic bacterium Vibrio harveyi. Two strains of yeast; Rhodotorula mucilaginosa CBS 316 and Wickerhamiella infanticola CBS 7922, were isolated. The lactic acid bacterium isolated from the intestine of the gilthead seabream included two genera of Enterococcus and Bacillus respectively. The results of probiotic potency tests showed that isolates 1 (bacteria), 3, and 6 (yeast) had the best performance. From the obtained data, it was concluded that the use of a combination of bacteria and yeasts as probiotics in aquaculture has higher efficiency.

Keywords: Aquaculture, Probiotic, Bacteria, 16S rDNA, ITS

^{*} Corresponding Author: Isaac Zamani, Assistant Professor, Department of Marine Biology, Faculty of Marine Science and Oceanography, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran E-mail: zamani@kmsu.ac.ir



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¹ PhD Graduated in fisheries, Faculty of Marine Natural Resources, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran

² Professor, Department of Fisheries, Faculty of Marine Natural Resources, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran

³ Assistant Professor, Department of Marine Biology, Faculty of Marine Science and Oceanography, Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran

Refrences

- Arihara, K., Ota, H., Itoh, M., Kondo, Y., Sameshima, T., Yamanaka, H., Akimoto, M., Kanai, S., and Miki, T. (1998). Lactobacillus acidophilus group lactic acid bacteria applied to meat fermentation. Journal of Food Science 63, 544-547.
- Axelsson, L. (1998). Lactic acid bacteria: Classification and physiology. In, S. Salminen and A. Von Wright (eds). Lactic Acid Bacteria: Microbiology and Functional Aspects. Inc, New York.
- Bagni, M., Romano, N., Finoia, M., Abelli, L., Scapigliati, G., Tiscar, P. G., Sarti, M., and Marino, G. (2005). Short-and long-term effects of a dietary yeast β-glucan (Macrogard) and alginic acid (Ergosan) preparation on immune response in sea bass (Dicentrarchus labrax). *Fish & Shellfish Immunology* 18, 311-325.
- Bakke, I., Coward, E., Andersen, T., and Vadstein, O. (2015). Selection in the host structures the microbiota associated with developing cod larvae (G adus morhua). *Environmental microbiology* 17, 3914-3924.
- Bourouni, O. C., El Bour, M., Calo-Mata, P., and Barros-Velàzquez, J. (2012). "Antimicrobial resistance and potential probiotic application of Enterococcus spp. in sea bass and sea bream aquaculture," Citeseer.
- Bucio, A., Hartemink, R., Schrama, J. W., Verreth, J., and Rombouts, F. M. (2006). Presence of lactobacilli in the intestinal content of freshwater fish from a river and from a farm with a recirculation system. Food microbiology 23, 476-482.
- Buntin, N., Chanthachum, S., and Hongpattarakere, T. (2008). Screening of lactic acid bacteria from gastrointestinal tracts of marine fish for their potential use as probiotics. Songklanakarin Journal of Science & Technology 30.
- Cao, L., Wang, L., Yang, L., Tao, S., Xia, R., and Fan, W. (2015). Long-term effect of early-life supplementation with probiotics on preventing atopic dermatitis: A meta-analysis. *Journal of Dermatological Treatment* 26, 537-540.
- Carbone, D., and Faggio, C. (2016). Importance of prebiotics in aquaculture as immunostimulants. Effects on immune system of Sparus aurata and Dicentrarchus labrax. Fish & Shellfish Immunology 54, 172-178.
- Chabrillón, M., Rico, R., Arijo, S., Diaz-Rosales, P., Balebona, M., and Moriñigo, M. (2005). Interactions of microorganisms isolated from gilthead sea bream, Sparus aurata L., on Vibrio harveyi, a pathogen of farmed Senegalese sole, Solea senegalensis (Kaup). *Journal of fish diseases* 28, 531-537.
- Chauhan, A., and Singh, R. (2019). Probiotics in aquaculture: a promising emerging alternative approach. *Symbiosis* 77, 99-113.
- Duarte, C. M., Holmer, M., Olsen, Y., Soto, D., Marbà, N., Guiu, J., Black, K., and Karakassis, I. (2009). Will the oceans help feed humanity? BioScience 59, 967-976.
- El-Saadony, M. T., Alagawany, M., Patra, A. K., Kar, I., Tiwari, R., Dawood, M. A., Dhama, K., and Abdel-Latif, H. M. (2021). The functionality of probiotics in aquaculture: An overview. *Fish & Shellfish Immunology* 117, 36-52.
- FAO Food and Agricultural Organization, (2016). The State of World Fisheries and Aquaculture 2016. FAO, Rome.
- Forberg, T., Sjulstad, E. B., Bakke, I., Olsen, Y., Hagiwara, A., Sakakura, Y., and Vadstein, O. (2016). Correlation between microbiota and growth in Mangrove Killifish (Kryptolebias marmoratus) and Atlantic cod (Gadus morhua). *Scientific reports* 6, 1-9.
- Gatesoupe, F. (2007). Live yeasts in the gut: natural occurrence, dietary introduction, and their effects on fish health and development. *Aquaculture* 267, 20-30.
- Gatesoupe, F. J. (1999). The use of probiotics in aquaculture. *Aquaculture* 180, 147-165.
- Gatesoupe, F.-J. (2008). Updating the importance of lactic acid bacteria in fish farming: natural occurrence and probiotic treatments. *Journal of molecular microbiology and biotechnology* 14, 107-114.
- Ghosh, S., Sinha, A., and Sahu, C. (2007). Effect of probiotic on reproductive performance in female livebearing ornamental fish. Aquaculture Research 38, 518-526.
- Giatsis, C., Sipkema, D., Smidt, H., Heilig, H., Benvenuti, G., Verreth, J., and Verdegem, M. (2015). The impact of rearing environment on the development of gut microbiota in tilapia larvae. *Scientific reports* 5, 1-15.
- Gomez-Gil, B., Roque, A., and Turnbull, J. F. (2000). The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. *Aquaculture* 191, 259-270.

- Grześkowiak, Ł., Isolauri, E., Salminen, S., and Gueimonde, M. (2011). Manufacturing process influences properties of probiotic bacteria. *British Journal of Nutrition* 105, 887-894.
- Hall, T., Biosciences, I., & Carlsbad, C. (2011). BioEdit: an important software for molecular biology. GERF Bull Biosci, 2(1), 60-61.
- Hoseinifar, S. H., Sun, Y.-Z., Zhou, Z., Van Doan, H., Davies, S. J., and Harikrishnan, R. (2020). Boosting immune function and disease bio-control through environment-friendly and sustainable approaches in finfish aquaculture: herbal therapy scenarios. *Reviews in Fisheries Science & Aquaculture* 28, 303-321.
- Igarashi, T., Aritake, S., and Yasumoto, T. (1999). Mechanisms underlying the hemolytic and ichthyotoxic activities of maitotoxin. *Natural toxins* 7, 71-79.
- Izadpanah, E., Saffari, S., Torfi Mozanzadeh, M., Mousavi, S.M., Pasha-Zanoosi, H. 2022. Nano-selenium supplementation in plant protein-based diets changed thyroid hormones status and hepatic enzymes activity in *Acanthopagrus arabicus* female broodfish and their offspring. Aquaculture Report, 24, https://doi.org/10.1016/j.aqrep.2022.101134
- Kavitha, M., Raja, M., and Perumal, P. (2018). Evaluation of probiotic potential of Bacillus spp. isolated from the digestive tract of freshwater fish Labeo calbasu (Hamilton, 1822). *Aquaculture Reports* 11, 59-69.
- Keyshams, P., Mousavi, S.M., Zakeri, M., Kochanian, P., Souri, M. 2021. Effects of different levels of commercial food supplement, Biotronic® Top3 on biochemical and immune parameters of *Litopenaeus vannamei*, Iranian Veterinary Journal, 17(1): 33-45. doi: 10.22055/ivj.2021.263466.2334
- Khademzade, O., Zakeri, M., Haghi, M., Mousavi, S.M. 2020. The effects of water additive *Bacillus cereus* and *Pediococcus acidilactici* on water quality, growth performances, economic benefits, immunohematology, and bacterial flora of white leg shrimp (*Penaeus vannamei* Boone, 1931) reared in earthen ponds. Aquaculture Research, 51: 1759-1770. doi: https://doi.org/10.1111/are.14525
- Kim, J. Y., Hahn, H. J., Choe, Y. B., Lee, Y. W., Ahn, K. J., and Moon, K. C. (2013). Molecular biological identification of Malassezia yeasts using pyrosequencing. *Annals of dermatology* 25, 73-79.
- Koščová, J., Nemcová, R., Gancarčíková, S., Jonecová, Z., Bomba, A., and Buleca, V. (2006). Effect of two plant extracts and Lactobacillus fermentum on colonization of gastrointestinal tract by Salmonella enterica var. Düsseldorf in chicks. *Biologia* 61, 775-778.
- Lauzon, H., Gudmundsdottir, S., Pedersen, M., Budde, B., and Gudmundsdottir, B. (2008). Isolation of putative probionts from cod rearing environment. *Veterinary microbiology* 132, 328-339.
- Lavelle, E., and Harris, J. (1997). The processing of an orally administered protein antigen in the digestive tract of rainbow trout, Oncorhynchus mykiss. Comparative Biochemistry and Physiology Part A: Physiology 117, 263-275.
- Mahdhi, A., Kamoun, F., Messina, C., and Bakhrouf, A. (2012). Probiotic properties of Brevibacillus brevis and its influence on sea bass (Dicentrarchus labrax) larval rearing. *African Journal of Microbiology Research* 6, 6487-6495.
- Makridis, P., Martins, S., Vercauteren, T., Van Driessche, K., Decamp, O., and Dinis, M. T. (2005). Evaluation of candidate probiotic strains for gilthead sea bream larvae (Sparus aurata) using an in vivo approach. *Letters in applied microbiology* 40, 274-277.
- Mao, Q., Sun, X., Sun, J., Zhang, F., Lv, A., Hu, X., and Guo, Y. (2020). A candidate probiotic strain of Enterococcus faecium from the intestine of the crucian carp Carassius auratus. *AMB Express* 10, 1-9.
- Meidong, R., Khotchanalekha, K., Doolgindachbaporn, S., Nagasawa, T., Nakao, M., Sakai, K., and Tongpim, S. (2018). Evaluation of probiotic Bacillus aerius B81e isolated from healthy hybrid catfish on growth, disease resistance, and innate immunity of Pla-mong Pangasius bocourti. *Fish & shellfish immunology* 73, 1-10.
- Merrifield, D. L., and Ringo, E. (2014). "Aquaculture nutrition: gut health, probiotics, and prebiotics," John Wiley & Sons.
- Mohammadian, T., Tabandeh, M., and Gharibi, D. (2014). Isolation and biochemical identification of potentially probiotic bacteria from Barbus grypus intestine. *Iranian Veterinary Journal* 10, 88-97.
- Moroni, F., Naya-Català, F., Piazzon, M. C., Rimoldi, S., Calduch-Giner, J., Giardini, A., Martínez, I., Brambilla, F., Pérez-Sánchez, J., and Terova, G. (2021). The Effects of Nisin-Producing Lactococcus lactis Strain Used as Probiotic on Gilthead Sea Bream (Sparus aurata) Growth, Gut Microbiota, and Transcriptional Response. *Frontiers in Marine Science* 8, 364.

- Mukherjee, A., and Ghosh, K. (2016). Antagonism against fish pathogens by cellular components and verification of probiotic properties in autochthonous bacteria isolated from the gut of an I ndian major carp, C atla catla (H amilton). *Aquaculture Research* 47, 2243-2255.
- Navarrete, P., and Tovar-Ramírez, D. (2014). Use of yeasts as probiotics in fish aquaculture. *Sustainable aquaculture techniques* 1, 135-72.
- Nayak, S. K. (2010). Role of gastrointestinal microbiota in fish. Aquaculture Research 41, 1553-1573.
- Nikoskelainen, S., Salminen, S., Bylund, G., and Ouwehand, A. C. (2001). Characterization of the properties of human-and dairy-derived probiotics for prevention of infectious diseases in fish. Applied and Environmental Microbiology 67, 2430-2435.
- Olafsen, J. A. (2001). Interactions between fish larvae and bacteria in marine aquaculture. *Aquaculture* 200, 223-247.
- Pérez-Sánchez, T., Balcázar, J., García, Y., Halaihel, N., Vendrell, D., De Blas, I., Merrifield, D., and Ruiz-Zarzuela, I. (2011). Identification and characterization of lactic acid bacteria isolated from rainbow trout, Oncorhynchus mykiss (Walbaum), with inhibitory activity against Lactococcus garvieae. Journal of fish diseases 34, 499-507.
- Ramesh, D., Vinothkanna, A., Rai, A. K., and Vignesh, V. S. (2015). Isolation of potential probiotic Bacillus spp. and assessment of their subcellular components to induce immune responses in Labeo rohita against Aeromonas hydrophila. *Fish & shellfish immunology* 45, 268-276.
- Ringø, E., Zhou, Z., Vecino, J. G., Wadsworth, S., Romero, J., Krogdahl, Å., Olsen, R., Dimitroglou, A., Foey, A., and Davies, S. (2016). Effect of dietary components on the gut microbiota of aquatic animals. A neverending story? *Aquaculture Nutrition* 22, 219-282.
- Román, L., Padilla, D., Acosta, F., Sorroza, L., Fátima, E., Déniz, S., Grasso, V., Bravo, J., and Real, F. (2015). The effect of probiotic Enterococcus gallinarum L-1 on the innate immune parameters of outstanding species to marine aquaculture. *Journal of Applied Animal Research* 43, 177-183.
- Roosta, Z., and Hoseinifar, S. H. (2016). The effects of crowding stress on some epidermal mucus immune parameters, growth performance, and survival rate of tiger barb (Pentius tetrazona). *Aquaculture Research* 47, 1682-1686.
- Sahu, M. K., Swarnakumar, N., Sivakumar, K., Thangaradjou, T., and Kannan, L. (2008). Probiotics in aquaculture: importance and future perspectives. *Indian journal of microbiology* 48, 299-308.
- Sarra, M., Taoufik, G., Benjamin, B., Yannick, F., and Khaled, H. (2013). Isolation and characterization of enterococci bacteriocinic strains from Tunisian fish viscera.
- Schleifer, K. H., and Kilpper-Bälz, R. (1984). Transfer of Streptococcus faecalis and Streptococcus faecium to the genus Enterococcus nom. rev. as Enterococcus faecalis comb. nov. and Enterococcus faecium comb. nov. *International Journal of Systematic and Evolutionary Microbiology* 34, 31-34.
- Schulze, A. D., Alabi, A. O., Tattersall-Sheldrake, A. R., and Miller, K. M. (2006). Bacterial diversity in a marine hatchery: balance between pathogenic and potentially probiotic bacterial strains. *Aquaculture* 256, 50-73.
- Selvaraj, V., Sampath, K., and Sekar, V. (2006). Adjuvant and immunostimulatory effects of β-glucan administration in combination with lipopolysaccharide enhances survival and some immune parameters in carp challenged with Aeromonas hydrophila. *Veterinary immunology and immunopathology* 114, 15-24.
- Sica, M. G., Brugnoni, L. I., Marucci, P. L., and Cubitto, M. A. (2012). Characterization of probiotic properties of lactic acid bacteria isolated from an estuarine environment for application in rainbow trout (Oncorhynchus mykiss, Walbaum) farming. Antonie Van Leeuwenhoek 101, 869-879.
- Stephens, W. Z., Burns, A. R., Stagaman, K., Wong, S., Rawls, J. F., Guillemin, K., and Bohannan, B. J. (2016). The composition of the zebrafish intestinal microbial community varies across development. *The ISME journal* 10, 644-654.
- Sugita, H., Hirose, Y., Matsuo, N., and Deguchi, Y. (1998). Production of the antibacterial substance by Bacillus sp. strain NM 12, an intestinal bacterium of Japanese coastal fish. Aquaculture 165, 269-280.

- Sugiura, S. H., Roy, P. K., and Ferraris, R. P. (2006). Dietary acidification enhances phosphorus digestibility but decreases H+/K+-ATPase expression in rainbow trout. Journal of Experimental Biology 209, 3719-3728.
- Suzer, C., Çoban, D., Kamaci, H. O., Saka, Ş., Firat, K., Otgucuoğlu, Ö., and Küçüksari, H. (2008). Lactobacillus spp. bacteria as probiotics in gilthead sea bream (Sparus aurata, L.) larvae: effects on growth performance and digestive enzyme activities. *Aquaculture* 280, 140-145.
- Tallapragada P, Rayavarapu B, Rao PP, Ranganath N, Veerabhadrappa P (2018) Screening of potential probiotic lactic acid bacteria and production of amylase and its partial purification. J Genetic Eng Biotechnol 16:357–362
- Thankappan, B., Ramesh, D., Ramkumar, S., Natarajaseenivasan, K., and Anbarasu, K. (2015). Characterization of Bacillus spp. from the gastrointestinal tract of Labeo rohita—towards to identify novel probiotics against fish pathogens. *Applied biochemistry and biotechnology* 175, 340-353.
- Thilsted, S. H., Thorne-Lyman, A., Webb, P., Bogard, J. R., Subasinghe, R., Phillips, M. J., and Allison, E. H. (2016). Sustaining healthy diets: The role of capture fisheries and aquaculture for improving nutrition in the post-2015 era. Food Policy 61, 126-131.
- Trinh, L. T., Bakke, I., and Vadstein, O. (2017). Correlations of age and growth rate with microbiota composition in Atlantic cod (Gadus morhua) larvae. *Scientific reports* 7, 1-9.
- Vadstein, O. (1997). The use of immunostimulation in marine larviculture: possibilities and challenges. *Aquaculture* 155, 401-417.
- Vadstein, O., Attramadal, K. J., Bakke, I., and Olsen, Y. (2018). K-selection as microbial community management strategy: a method for improved viability of larvae in aquaculture. *Frontiers in Microbiology* 9, 2730.
- Van Doan, H., Hoseinifar, S. H., Dawood, M. A., Chitmanat, C., and Tayyamath, K. (2017). Effects of Cordyceps militaris spent mushroom substrate and Lactobacillus plantarum on mucosal, serum immunology and growth performance of Nile tilapia (Oreochromis niloticus). *Fish & shellfish immunology* 70, 87-94.
- Varela, J., Ruiz-Jarabo, I., Vargas-Chacoff, L., Arijo, S., León-Rubio, J., García-Millán, I., Del Río, M. M., Moriñigo, M., and Mancera, J. (2010). Dietary administration of probiotic Pdp11 promotes growth and improves stress tolerance to high stocking density in gilthead seabream Sparus auratus. *Aquaculture* 309, 265-271.
- Vatsos, I., Kotzamanis, Y., Henry, M., Angelidis, P., and Alexis, M. (2010). Monitoring stress in fish by applying image analysis to their skin mucous cells. *European journal of histochemistry: EJH* 54.
- 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.
- 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.
- Vijayabaskar, P., and Somasundaram, S. (2008). Isolation of bacteriocin producing lactic acid bacteria from fish gut and probiotic activity against common fresh water fish pathogen Aeromonas hydrophila. *Biotechnology* 7, 124-128.
- Vine, N. G., Leukes, W., Kaiser, H., Daya, S., Baxter, J., and Hecht, T. (2004). Competition for attachment of aquaculture candidate probiotic and pathogenic bacteria on fish intestinal mucus. *Journal of fish diseases* 27, 319-326.
- Wang, A., Ran, C., Wang, Y., Zhang, Z., Ding, Q., Yang, Y., Olsen, R. E., Ringø, E., Bindelle, J., and Zhou, Z. (2019). Use of probiotics in aquaculture of China—a review of the past decade. *Fish & shellfish immunology* 86, 734-755.
- Wang, J., Ji, H., Wang, S., Liu, H., Zhang, W., Zhang, D., and Wang, Y. (2018). Probiotic Lactobacillus plantarum promotes intestinal barrier function by strengthening the epithelium and modulating gut microbiota. *Frontiers* in microbiology 9, 1953.
- Wanka, K. M., Damerau, T., Costas, B., Krueger, A., Schulz, C., and Wuertz, S. (2018). Isolation and characterization of native probiotics for fish farming. *Bmc Microbiology* 18, 1-13.
- Zhang, W., Liu, M., and Dai, X. (2013). Biological characteristics and probiotic effect of Leuconostoc lactis strain isolated from the intestine of black porgy fish. Brazilian journal of microbiology 44, 685-691.