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STUDY ON FISH GUT MICROBIOTA SEASONAL AND DIURNAL FLUCTUATION AND SERUM MELATONIN

Dr. Dhankesh Meena

Assistant professor, scrs government college

Sawai Madhopur (Rajasthan)

ABSTRACT

The rhythmicity of the gut microbiota is characterized by a more complicated multilayer network comprising all taxonomic levels of microbial taxa and their metabolites, in contrast to the rigidly hierarchical organization that is present in the circadian clock system. One thing that should be taken into consideration, however, is that the functionality of the rhythmicity of the gut microbiota is strongly dependent on the circadian clock of the host as well as the physiological status of the host. During this discussion, we talked about the daily rhythmicity of the gut microbiota, its significant involvement in the physiology, health, and metabolism of the host, and the crosstalk that occurs between the rhythmicity of the gut microbiota and the circadian rhythm of the host. Through the acquisition of this knowledge, the groundwork is laid for the development of chronotherapies that target the microbiota of the gut. On the other hand, the mechanism of production, the beneficial effects of gut microbial rhythmicity on the host, and the dynamic microbial–host interplay are not yet fully understood and require additional research.

Keywords: *gut microbiota, serum melatonin.*

INTRODUCTION

Early research on the fish gut microbiome relied on culture-based approaches, which revealed that the makeup of the microbiome may change depending on the time of year, the phases of the life cycle, and the different settings that the fish were exposed to. The results of these tests also led to the discovery of a number of probiotic bacteria, including different species of Bacillus and Lactobacillus. Due to the fact that culture-based studies only show a very small fraction of the microorganisms that are associated with fish guts, a great number of uncommon and unique taxa that are difficult to cultivate are not taken into consideration. Furthermore, the culture-dependent method is a strategy that is utilized when the emphasis is placed on the "variety" of the taxonomic groups that make up the fish gut microbiome rather than the "abundance" of these groups. In order to uncover the true diversity of the fish gut microbiome, it is necessary to conduct culture-independent evaluation or metagenomics in order to do so. Using next-generation sequencing (NGS) platforms and directly mining the DNA of the community as part of the metagenomics technique allows researchers to circumvent the difficulties associated with culture-based investigations in order to identify microorganisms that cannot be cultivated and taxonomic groups that are in low abundance. It is anticipated that the developments in next-generation sequencing systems and the quickly developing bioinformatics tools would make it possible to discern the whole taxonomic profiles of many fish species, along with forecasts of the key functional taxa.

There are several limits associated with both the culture-dependent and metagenomics methodologies, just like there are with any other technology. The particular purpose of an investigation is a significant factor that should guide the selection of a methodology. Metagenomics, on the other hand, is able to measure the full depth of bacterial diversity in fish guts, so bypassing the limitations of culture-based approaches. Culture-based approaches, on the other hand, are utilized with the intention of obtaining novel commensals in the form of pure isolates wherever possible. The taxonomic classification of these organisms is also the foundation upon which bacteriology is built. The scientific study of the classification of bacteria into groups or taxa on the basis of their evolutionary relatedness and the similarities they share with one another is known as bacterial taxonomy. The polyphasic approach, which integrates morphological, genotypic, physiological, and chemotaxonomic classification of bacteria, has been the approach to bacterial taxonomy that has gained the most widespread adoption over the course of the past two decades. In spite of the fact that it is still commonly employed in the present day, the polyphasic technique is currently on the verge of being modified as a result of the dramatic advances that have occurred in sequencing technology in this'OMICS' era. The employment of genomic data in the process of characterizing the novel isolate for appropriate taxonomic placement is made possible through the utilization of whole genome sequencing (WGS), which provides substantial information on the novel isolate's evolutionary relationships. The use of conventional methods, such as those for %G+C content measurement and DNA-DNA Hybridization (DDH) techniques, has been successfully replaced by the use of tools for calculating Average Nucleotide Identity (ANI), Average Amino Acid Identity (AAI), and digital DDH (dDDH). This is due to the advancements that have been made in genome sequencing and bioinformatics tools. Furthermore, in this day and age of taxogenomics, classical taxonomy is connected to the examination of the genome sequence in order to achieve a higher level of taxonomic precision.

OBJECTIVES

1. to study on fish gut microbiota seasona.
2. to study diurnal fluctuation.

The Gut Microbiota in Fish

The eggs, the water around them, and the first meal are the three elements that are responsible for the microbial colonization of fish larvae. Cytophaga, Flavobacterium, and Pseudomonas were shown to be the most prevalent species at this point in time, according to the findings of some preliminary research that investigated bacteria that were connected with fish eggs. A number of recent research have produced outcomes that are correlated with one another, while others have produced wholly different findings. There were even some early studies that acknowledged the existence of inter-species variance. As an illustration, the bacterial colonization of cod, Gadus morhua L., and halibut, Hippoglossus hippoglossus, eggs was found to be distinct from one another. At this point, it is generally acknowledged that the bacteria that initially colonize an egg are species-specific, with variances being controlled by variations in the binding glycoproteins that are present on the egg surface. Furthermore, the microbiota of the water immediately around the eggs determines the types of bacteria that come into contact with them and, as a result, have the opportunity to colonize. When sterile larvae hatch, they take in the bacteria that are connected with the chorion. These bacteria are the first to colonize the growing gastrointestinal tract (GIT). Once the fish larvae start drinking water to regulate their osmoregulation, subsequent resident bacteria are acquired. The

microbiota then becomes further varied by feeding, which further contributes to the diversity of the microbiota. To begin, the gastrointestinal tract (GIT) of freshly hatched larvae typically contains a small number of microorganisms. Numerous studies have demonstrated that nutrition has a significant role in the formation of the microbial community in the gut, and that significant diversification takes place from the very first meal. As fish develop, it appears that the diversity of bacteria expands, much as it does in people. This is an interesting phenomenon. The authors of the review of the intestinal microflora of fish larvae and fry summarized the findings of twenty-four research that revealed the bacterial genera that were present in the intestinal tracts of freshwater and marine fish when they were in the larval and fry phases. Vibrio, Pseudomonas, Cytophaga, Flavobacterium, and the family Enterobacteriaceae were the bacteria that were reported the most frequently among the 11 marine species. Vibrio was reported fifteen times, Pseudomonas nine times, Cytophaga eight times! According to the investigations, there were between three and four genera and families on average (Table 1). In a study that compared the gut microbiota of 12 adult bony fish, researchers discovered bacteria belonging to 17 different phyla. The majority of the species had between 7 and 15 different phyla, which is a significantly higher average than the microbiota revealed in the evaluation of egg and larval. There is a rather stable gut microbiota that is established within the first fifty days of life for many animals, despite the fact that the microbial population shifts depending on the life stage and surrounding environment. This was proved by a definitive study that was conducted with Danio rerio, a kind of zebrafish. The study reported that a core microbial community is supported by selective pressures produced by the host system, regardless of the environmental circumstances.

Melatonin on Fish in Aquaculture

Taking into account the multipotent physiological actions of MEL, recent studies, particularly those conducted on various commercially significant fish groups, have opened up a new possibility for the application of the most convenient and reliable measures for the manipulation of endogenous MEL levels. This is done in order to elicit the desired physiological response, which may be of considerable interest in aquaculture. It should come as no surprise that the manipulation of light conditions in the surroundings is considered to be a traditional method for modifying the circulating MEL milieu in the fish that are of interest. Nevertheless, the utilization of such approaches contains a number of restrictions when it comes to field research. As a result, the hunt for a substitute has become an absolute necessity for aquaculture purposes. As a consequence of this discovery, numerous attempts have been made to increase the concentrations of endogenous MEL in the gut and, consequently, in the circulation by the utilization of various dietary supplements.

Research Methodology

Material and methods

In order to recognize strain CT19T as a unique species belonging to the genus Salinicoccus, a full analysis of the strain was carried out using the polyphasic approach to bacterial taxonomy. This technique incorporates genotypic, phenotypic, physiological, and chemotaxonomic characterisation. In addition, the genome of the strain was sequenced in order to make a comparison with the phylogenetic neighbor that was the closest to it as well as all of the other Salinicoccus species genomes that were accessible. This was done in order to establish the strain CT19T as a new species. Figure 1 provides a summary of the procedures that were utilized in order to finish the taxo-genomic characterisation of the organism.

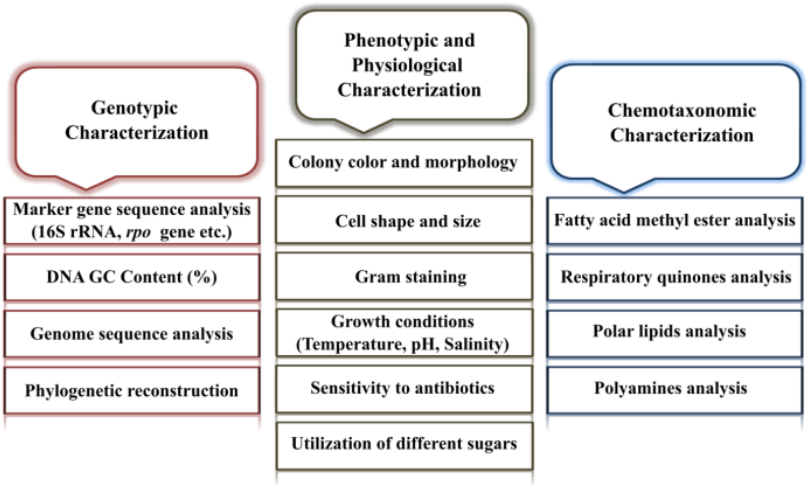

Figure 1: Schematic representation of the methods employed under polyphasic approach for characterization of bacteria.

The genome of strain CT19T was compared with other genomes of the genus Salinicoccus that were accessible using the taxo genomics methodology. This comparison was carried out in addition to the procedures that are illustrated in Figure 1. Additionally, the functionalities that were encoded within the genome of the CT19T strain were explored and analyzed. In the next sections, each strategy will be described in further detail.

Genotypic and genomic characterization

Marker gene sequence analysis and identification of closest neighbors

In accordance with the instructions provided in goal I, the 16S rRNA marker gene was amplified and sequenced. Using EzTaxon-e-Server, we were able to determine which phylogenetic neighbors were the most closely related.

Phylogenetic tree construction

Sequences from all other recognized species were obtained from GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and aligned with CLUSTAL W using a gap opening penalty of 15 and a gap extension penalty of 6.66 for both pairwise and multiple alignments. This was done in order to perform phylogenetic reconstruction based on the 16S rRNA gene. Within the MEGA 7.0 software, the neighbor-joining, maximum-likelihood, and maximum-parsimony (ML and MP; Fitch, 1971) algorithms were utilized in order to read the alignments. Through the utilization of the Jukes Cantor model with total deletion, the NJ tree was inferred. The Jukes-Cantor model and the Nearest-Neighbor-Interchange (NNI) heuristic search technique were utilized across all sites in order to generate the machine learning phylogeny. The search method known as Subtree-Pruning-Regrafting (SPR) was utilized for MP. For the purpose of determining the tree topologies that were produced, a bootstrap analysis was carried out with one thousand resamplings.

Genome sequencing and assembly

The genome of strain CT19T was sequenced at Beijing Genomics Institute (BGI), Hong Kong, China, using an Illumina HiSeq 4000 platform. The genome of S. hispanicus J-82T was sequenced using an Illumina HiSeq X platform at AgriGenome Labs Pvt Ltd, Hyderabad, Telangana, India. Both of these genomes were sequenced utilizing genomic sequencing technology. A paired end library was created for strain CT19T and S. hispanicus J-82T, with insert sizes of 270 bp and 300 bp, respectively. The library preparation was carried out with the assistance of the NEB Ultra Kit. SOAPnuke was incorporated with the purpose of including quality control and pre-processing of the raw data. Reads were filtered using the following parameters: a low quality threshold of -l 20; a low quality rate of -q 0.4; a N rate threshold of -n 0.1; and a maximum number of mismatches with the adapter of -n 0.1. Through the use of the -d flag, PCR duplications and -M 3 were eliminated. A multi-kilometer technique was utilized in order to carry out the assembly process in SPAdes version 3.1.0 (99, 103, 107, 111, 115, 119, 121, 125, 127). The final assemblies that had contigs that were longer than 500 base pairs were validated with the help of QUAST version 4.5. RNAmmer v1.2 and ARAGORN were utilized in order to ascertain the rRNA and tRNA genes that are contained within the genome.

Determination of GC content

With the help of QUAST version 4.5, the GC content (in percentages of moles) of the final assembled genomes was evaluated.

Results and Discussion

After being taken from the foregut of mirror carp, the contents were diluted in a series of steps and then spread out on marine agar (MA; 2216, Difco). After three days of incubation at 30 degrees Celsius on an MA plate, the strain CT19T was observed to have a colony that ranged in color from orange to pink (Figure 2). In order to conduct regular testing, the strain was kept on MA slants at a temperature of 4 degrees Celsius. Additionally, it was stored for an extended period of time as 20% (v/v) glycerol suspensions at a temperature of -80 degrees Celsius.

Figure 2: Strain CT19T grown on Marine Agar (MA; 2216, Difco) after incubation at 30 °C for 48 h.

Genotypic and genomic characterization

Marker gene sequence analysis

The genomic DNA was extracted and observed as a crisp, intact band of high intensity upon 0•8% agarose gel electrophoresis (Figure 3A). This was done in order to identify the strain CT19T.

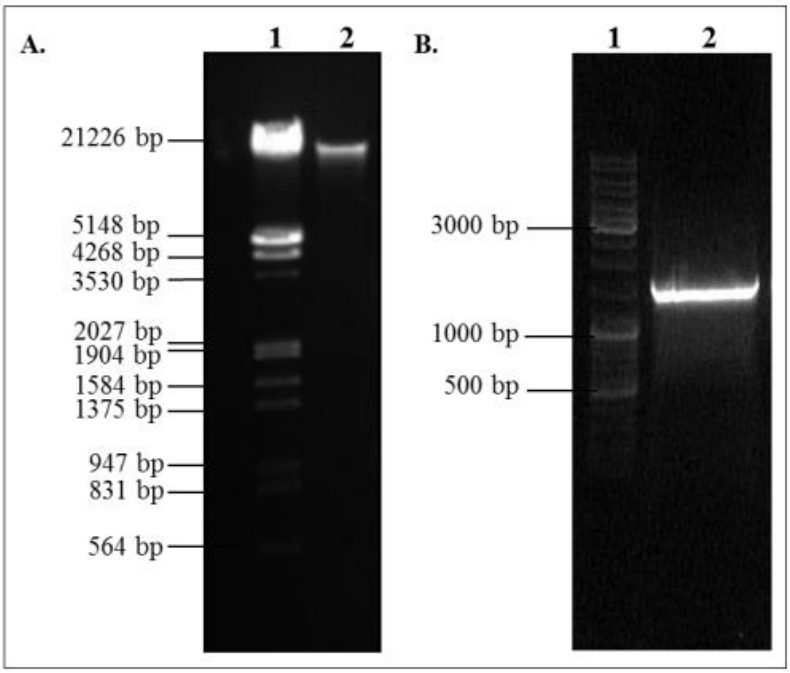

Figure 3.: Electrophoretogram of 0·8% agarose gel electrophoresis showing

Based on the results obtained from the NanoDrop ND-1000 spectrophotometer (owned by Thermo Scientific), the concentration of the genomic DNA was determined to be 753 ng µl-1. Additionally, the A260/A280 ratio was calculated to be 1.83, indicating that the DNA was devoid of any RNA contamination. In Figure 3.B, the 16S rRNA gene was visualized as a thick band by 0•8% agarose gel electrophoresis. This was accomplished through the use of PCR to amplify the gene. The size of the band was approximately 1500 base pairs, which is approximately the same as the size of the 16S rRNA gene found in bacteria (Figure 3. B). Following the sequencing of the gene, it was combined into a virtually full sequence that was 1437 base pairs in length.

The closest phylogenetic neighbors of strain CT19T utilizing were discovered to be Salinicoccus hispanicus J-82T (=DSM 5352T ; 97.4% 16S rRNA gene sequence identity) followed by S. sesuvii CC-SPL15-2 T (=DSM 23267T ; 96.4%), S. amylolyticus JC304T (=KCTC 33661T ; 95.6%) and S. roseus DSM 5351T (95.4%). There was a lower than 94.5% likelihood of identifying with any of the other members of the genus Salinicoccus. Under the accession number MK249750, the 16S rRNA gene sequence of the bacterium CT19T was uploaded to GenBank with the National Center for Biotechnology Information.

Because the strain CT19T shared a 16S rRNA gene sequence similarity of more than 97% with only one neighbor, which is the standard species demarcation limit, it was chosen for taxo-genomic characterization in order to determine whether or not it represented a new species.

Phylogenetic tree construction

In order to determine the phylogenetic relationships between the strain CT19T sequence variation, the 16S rRNA gene was utilized as a marker gene. Despite the fact that the gene has remained substantially conserved throughout the course of evolution, the hypervariable areas that are contained within its sequence offer sufficient diversity to be taken into consideration for the phylogenetic delineation of various taxonomic groups. A consistent tree topology was produced as a consequence of the three approaches that were utilized for the reconstruction of phylogeny. These methods included neighbor-joining, maximum-likelihood, and maximum-parsimony algorithms. Figure 4 shows that strain CT19T formed a clade with S. hispanicus J-82T (=DSM 5352T) and S. sesuvii CC-SPL15-2 T (=DSM 23267T). Additional support for the clade was provided by strong bootstrap values (92%) on the data.

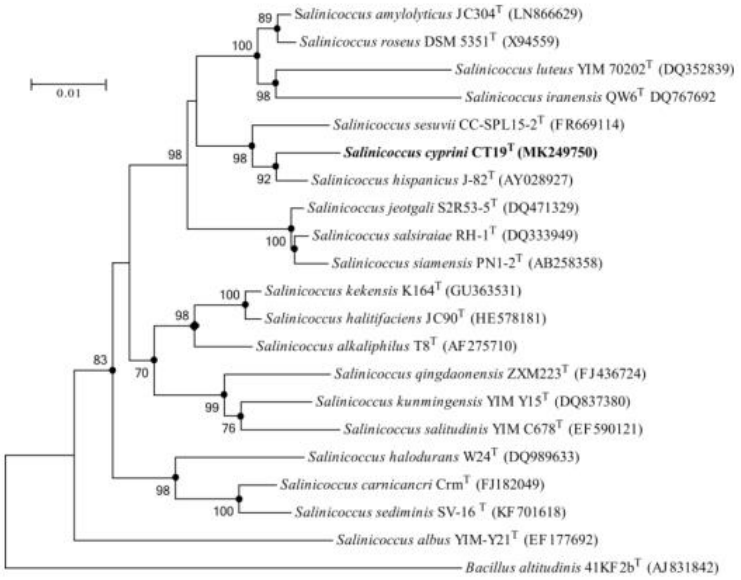

Figure 4: Phylogenetic relationships of strain CT19T with other representative members of genus Salinicoccus based on nearly complete 16S rRNA gene sequence data.

After that, the bacterial strains S. hispanicus J-82T (=DSM 5352T) and S. sesuvii CCSPL15-2 T (=DSM 23267T) were obtained from the DSMZ in Germany for the purpose of conducting comparison testing with the strain CT19T.

Conclusion

Based on the evidence that has been obtained up to this point, it appears that the composition of food is one of the most important environmental cues that govern the production of MEL in the gastrointestinal tract region. The function of MEL as a fish food additive on numerous physiological parameters related with the improvement of growth and reproduction to act as a critical synchronizer in aquaculture is therefore the primary focus of research that is currently being conducted all over the world. It has been demonstrated in a number of studies that the consumption of MEL or its precursor L-Trp through the diet leads to an increase in the production of MEL in the gut of rainbow trout and European sea bass. Because the increased level of MEL within the gut, which can be the result of either the administration of MEL or the addition of L-Trp to the food, is transported to all of the central and peripheral organs, the possibility of its actions in an endocrine manner in regulating various body functions is something that should be taken into consideration. Oral administration of MEL has been shown to promote the equilibrium of microorganisms in the stomach of

zebra fish, according to reports from recent investigations. One of the most significant challenges in aquaculture is the presence of pathogenic bacterial infections connected to disease. It is well established that endogenous MEL in freshwater carp protects the gut against these infections. In fish, the most important effect of exogenous MEL therapy is to boost reproductive performance. This is accomplished by increasing the fecundity rates, the percentage of fertilization, and the quality of both male and female seeds. Consequently, the introduction of MEL or L-Trp into fish food as an additive is becoming an increasingly popular technique with the purpose of eliciting a MEL-induced reaction in fish. As a conclusion, the current state of knowledge clearly provides reasonable indications that taking into consideration the multipotent functions of MEL, the utilization of this hormone or its precursor as an additive in fish food may be an effective tool for achieving better fish growth and a higher rate of reproduction in an aquaculture system that is sustainable.

References

1. Backström, T., and Winberg, S. (2017). Serotonin coordinates responses to social stress-what we can learn from fish. Front. Neurosci. 11:595. doi: 10.3389/fnins.2017.00595

2. Baekelandt, S., Milla, S., Cornet, V., Flamion, E., Ledor,é, Y., Redivo, B., et al. (2020). Seasonal simulated photoperiods influence melatonin release and immune markers of pike perch Sander lucioperca. Sci. Rep. 10, 1–10. doi: 10.1038/s41598-020-59568-1

3. Boyd, C. E., D'Abramo, L. R., Glencross, B. D., Huyben, D. C., Juarez, L. M., Lockwood, G. S., et al. (2020). Achieving sustainable aquaculture: historical and current perspectives and future needs and challenges. J. World Aquac. Soc. 51, 578–633. doi: 10.1111/jwas.12714

4. Cardinali, D. P. (2019). Melatonin: clinical perspectives in neurodegeneration. Front. Endocrinol. 10:480. doi: 10.3389/fendo.2019.00480

5. Choi, J. Y., Kim, N. N., Choi, Y. J., Park, M. S., and Choi, C. Y. (2016). Differential daily rhythms of melatonin in the pineal gland and gut of goldfish Carassius auratus in response to light. Biol. Rhythm Res. 47, 145–161. doi: 10.1080/09291016.2015.1094964

6. Costello, C., Cao, L., Gelcich, S., Cisneros-Mata, M., Free, C. M., Froehlich, H. E., et al. (2020). The future of food from the sea. Nature 588, 95–100. doi: 10.1038/s41586-020-2616-y

7. Devi, H. S., Rajiv, C., Mondal, G., Khan, Z. A., Dharmajyoti Devi, S., Yumnamcha, T., et al. (2016). Melatonin bio-synthesizing enzyme genes (Tph1, Aanat1, Aanat2, and Hiomt) and their temporal pattern of expression in brain and gut of a tropical carp in natural environmental conditions. Cogent Biol. 2:1230337. doi: 10.1080/23312025.2016.1230337

8. Devi, S. D., Mondal, G., Khan, Z. A., Sarma, H. K., and Chattoraj, A. (2021). Differential gene expression and immunohistochemical localization of the key melatonin biosynthesizing enzymes in the testis of zebrafish (Danio rerio). Biol. Rhythm Res. 2021, 1–17. doi: 10.1080/09291016.2021.1926078

9. Food Agriculture Organization (2019). Fishery and Aquaculture Statistics. Global Aquaculture Production 1950–2017 (FishstatJ). Rome: FAO Fisheries and Aquaculture Department.

10. Fortes-Silva, R., Oliveira, I. E., Vieira, V. P., Winkaler, E. U., Guerra-Santos, B., and Cerqueira, R. B. (2016). Daily rhythms of locomotor activity and the influence of a light and dark cycle on gut microbiota species in tambaqui (Colossoma macropomum). Biol. Rhythm Res. 47, 183–190. doi: 10.1080/09291016.2015.1094972

11. Gao, Y., Yang, C., Huang, C., Zhang, G., Ji, H., and Dong, W. (2019). The effects of melatonin supplement on paddlefish (Polyodon spathula) sperm quality and ATP content in sperm during in vitro storage. Aquaculture 503, 475–482. doi: 10.1016/j.aquaculture.2019.01.010

12. Gesto, M., Álvarez-Otero, R., Conde-Sieira, M., Otero-Rodiño, C., Usandizaga, S., Soengas, J. L., et al. (2016). A simple melatonin treatment protocol attenuates the response to acute stress in the sole Solea senegalensis. Aquaculture 452, 272–282. doi: 10.1016/j.aquaculture.2015.11.006

13. Hasan, K. N., and Banerjee, G. (2020). Recent studies on probiotics as beneficial mediator in aquaculture: a review. J. Basic Applied Zool. 81:53. doi: 10.1186/s41936-020-00190-y

14. Hasan, K. N., Pal, P. K., and Maitra, S. K. (2016). Diurnal and seasonal profiles of melatonin in the liver and their correlates of ovarian functions under natural photo-thermal conditions in adult carp Catla catla. Biol. Rhythm Res. 47, 947–965. doi: 10.1080/09291016.2016.1212535

15. Hasan, K. N., Pal, P. K., and Maitra, S. K. (2020). Temporal relationship between the levels of melatonin and different antioxidants in the liver of a surface feeding carp Catla catla. Biol. Rhythm Res. 51, 373–391. doi: 10.1080/09291016.2018.1533728

16. He, C., Wang, J., Zhang, Z., Yang, M., Li, Y., Tian, X., et al. (2016). Mitochondria synthesize melatonin to ameliorate its function and improve mice oocyte's quality under in vitro conditions. Int. J. Mol. Sci. 17:939. doi: 10.3390/ijms17060939

17. Khan, Z. A., Yumnamcha, T., Rajiv, C., Sanjita Devi, H., Mondal, G., Devi, S. D., et al. (2016). Melatonin biosynthesizing enzyme genes and clock genes in ovary and whole brain of zebrafish (Danio rerio): differential expression and a possible interplay. Gen. Comp. Endocrinol. 233, 16–31. doi: 10.1016/j.ygcen.2016.05.014

18. Kulczykowska, E., Kalamarz-Kubiak, H., Gozdowska, M., and Sokołowska, E. (2018). Cortisol and melatonin in the cutaneous stress response system of fish. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 218, 1–7. doi: 10.1016/j.cbpa.2018.01.003

19. Kulczykowska, E., Kleszczyńska, A., Gozdowska, M., and Sokołowska, E. (2017). The time enzyme in melatonin biosynthesis in fish: day/night expressions of three aralkylamine N-acetyltransferase genes in three-spined stickleback. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 208, 46–53. doi: 10.1016/j.cbpa.2017.03.005

20. Lutfi, E., Basili, D., Falcinelli, S., Morillas, L., Carnevali, O., Capilla, E., et al. (2021). The probiotic Lactobacillus rhamnosus mimics the dark-driven regulation of appetite markers and melatonin receptors' expression in zebrafish (Danio rerio) larvae: understanding the role of the gut microbiome. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 2021:110634. doi: 10.1016/j.cbpb.2021.110634