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Impacts of probiotics on feeding technology and its application in aquaculture

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Review

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ABSTRACT

Probiotic is a useful microorganism that directly or indirectly used to protect the host animal against pathogens. The bacterial pathogens are becoming more and more resistant to antimicrobial drugs, pesticides, and disinfectants that are used in aquatic disease control and high growth of production. For this reason, the probiotics study in aquaculture is a rising demand to ensure eco-friendly sustainable aquaculture as an alternative to antibiotics. The advantages of such probiotics enhance feed value, enzymatic stimulation in the digestive system, and reverse habitat for pathogens, influence anti-mutagenic activity, and improved immune response. The research of probiotics in gut microbiota of aquatic animals has not characterized adequately, and their impact on the environment is not widely deliberated. The impact on enzyme activity related to the fish metabolism system should be well-identified by further study. The application and the bio-security of probiotics in fish should be properly evaluated. The farmer should have a brief knowledge about the probiotics organism and should be careful during applying to the culture field.

Keywords: Application, aquaculture, bio-security, micro-organism, probiotics

1. Introduction

Aquaculture is an important and emerging foodproducing sector in the world. The fishery yearbook 2017 has stated that 153 million tones, likely 89 % of total fishery production, was used for human consumption and among them 45 % live and fresh fish was used directly as human consumption [1]. The production is always hindered by the diseases and environmental adverse situation that result in severe financial losses for the farmers. The use of antibiotics has been increased in the past decades in fishery sectors and poultry farming [2] to enhance the efficiency of feeding and disease control [3]. Due to using antibiotics in aquaculture the growth of antimicrobialresistant pathogens has also increased [4]. There is a great risk that resistant bacteria can transfer from aquaculture species to human. That's why now we need to develop new feeding techniques to solve this issue. In this case, eco-friendly treatments like probiotics can play an effective role in feeding technology in aquaculture.

The word "probiotics" was first used by Parker, 1974. According to his definition, probiotics are such types of organisms and substances that contribute to gut microbial balance [5]. Probiotics can be emphasized as a nutritious food source and as an eco-friendly biological control mediator [6]. In Fuller's (1989) definition, the live microbial feed element seems to be beneficial for the host by improving microbial balance in the intestine [7]. Moriarty named probiotics shortly as "water additives" in 1998. Therefore, several terms are generally used to illustrate probiotics such as "beneficial", "eco-friendly", or "healthy" bacteria [8].

Aquatic host and microorganism are correlated with each other in their life cycle. This correlation can be treated in a useful way. Bacteria in the aquatic condition influence the activity of gut microbiota as well as vice versa [9]. The microbes that are used in probiotics may help in detoxification of host as well as food digestion in the gut [10, 11]. For this purpose different health and growth regulatory elements such as probiotics, prebiotics, symbiotic and other functional supplements can be applied [12]. Where antibiotics can't work efficiently, microbial involvement can possibly play a functional role to ensure sustainable and eco-friendly alternatives in aquaculture production [13, 14].

A huge number of researches and application has been going on the aquaculture system. The Vibrio alginolyticus isolated probiotics in shrimp hatcheries have been used successfully from earlier [15]. It has resulted that, probiotics treatments can be effective in growth performance, disease control, spawning, gut activity, and hematological parameters in different fishes such as sea bass [16], rainbow trout [17], snook [18, 19], sturgeon [12], and shrimp [20]. This is very important to have brief knowledge about the selection of probionts, use of probiotics, mode of action, safety guideline as well as their characterization [21]. According to the above studies, all these probiotics applications are probably connected with gut or gut microbial function. So, it is cleared that the scientific approach should need to know the details about genomic function with another organ and activity with different enzymes related to the metabolism.

2. Selection and source of probiotics

The selection of probiotics bacteria is very important because the evidence from researchers is very a few that are practically used. It may create an adverse situation for the host due to select inappropriate microbiota. The selection steps should be specific; the adaptation ability of the organism must be correlated with the different host and environmental factors [22]. The mechanisms of probiotics function are very crucial to understand and should have good knowledge about the characterization of potential probiotics which will be used in the farm [23]. Probiotics are generally selected by bio-security considerations of the following methods such as (a) Methods of processing, (b) Method of management of the probiotics and (c) Host body location to apply microorganisms [24].



Fig 1: General criteria for selecting probiotics

As a probiotics LAB (lactic acid bacteria) such as Lactobacilli and Bifidobacteria have been broadly used and researched in terrestrial animals and humans [25]. So, it's so far becoming popular as healthpromoting functional foods as well as prophylactic, therapeutic and growth enhancements for the production of aquatic animals and human health. The gut microbiota of human and aquatic animals is dominated by gram-positive or facultative anaerobic bacteria. Bifidobacterium, Streptococcus, Lactobacillu and Streptomyces are the potential probionts among this microbiota [26-29]. LABs are normally found in the fish intestine. This type of bacteria can survive in acidic and bile environment of the intestine helps to convert lactose into lactic acid and thus reduce pH in the gastrointestinal tract [30]. At present, the sporeforming *Bacillus sp* and Yeast have been used widely because of its adhesion abilities, produce antimicrobial peptides and immune stimulation. The source of the LAB is fermented products like pickled vegetables, buttermilk, kimchi, pancake, soy sauce, etc. Applications of gram-negative facultative anaerobic bacteria in the digestive organ for some crustacean and herbivorous fish have come on succeeding [20]. For marine fish, Pseudomonas and Vibrio are commonly applied in the posterior intestine, and Aeromonas, Plesiomonas, and Enterobacteriaceae are fairly used in freshwater fish. Bacillus sp. as probiotics is popular because of spore-forming and long-lasting shelf life. It's noticed that some other types of commercial

probiotics feeds also found in the market which does not emphasize too much on the different publishers.

3. How do probiotics work?

The application of probiotics in aquafarming is modern technology. This actually begun by applying in finfish juveniles but recently it is applied to shrimp larvae. The bacteria taken from the surrounding environment are commonly ingested with the feed or mixed with drinking water in the filter feeder case [32]. There are various mechanisms performed in the probiotics process in the pond. One research showed that probiotics application reduce hatching time seven days/months and 21 days/year [33]. The properties of bacteria in ambient water are very crucial in this technology. Bacillus PC465 secluded from the intestine of Fenneropenaeus chinensis that works against WSSV (white spot syndrome virus) and improves the health condition and resistance in Litopenaeus vannamei [34]. Probiotic creates a biosecure environment and acts as transport stress resistance [35]. It creates an adverse habitat to the pathogens of the host animal. Useful bacteria take the place of harmful bacteria, furthermore de-colonized the pathogens and eventually destroy those [36]. Mechanisms action of different probiotics is potentially different such as (a) Making a bio-security shell on the gut, young larvae, and egg surface, (b) Increase metabolism of cells like enzyme or vitamins, (c) Formation of the colony and peptide bonding and (d) Stimulation of the immune system



Fig 2: Probiotics mechanism (Tan LT-H et al., 2016) [31].

3.1. Competitive exclusion of harmful bacteria

Competitive exclusion (CE) includes the addition of a harmless bacteria culture of single or various strains to the gut intestinal territory of animals in order to reduce the pathogenic bacteria colony. Good bacteria always compete with bad bacteria for nutrients that are hosted by cell surfaces. This is actually discouraging to the harmful bacteria. The useful bacteria can change pH in the gut that reduces the growth of pathogens. So the lower number of pathogens indicates the lower cause of diseases. Competitive exclusion is now a phenomenon to prevent or reduces the competing bacterial colony and creates a challenging environment in the same position on the gut by a recognized microorganism. A study on Nile tilapia treated with 1×10^6 and 1 \times 10⁴ CFU g⁻¹ of *B. amyloliquefaciens* for 30 days resulted in higher resistance against harmful Y. ruckeri or C. perfringens [37]. Obtaining a sustainable, pleasant and controlled microorganism is the goal of probiotics feeds that is designed in competitive exclusion. Prevention of replication of pathogenic microbiota and decolonization by making competition during taking nutrients, produce adverse habitat on the mucosa [8].





For making a bond with microorganism, various types of tactics such as electrostatic interactions, lipoteichoic acids, hydrophobic, passive forces, static forces and structure of adhesion are applied [38]. Adhesion and colonization in the gut help to protect against pathogens through competition for concerning tissue and nutrients.

3.2. Creation of adverse habitat of pathogenic bacteria

Probiotics feeds can make an adverse habitat for the pathogens in the system. It is known that different species of bacteria can't be a friend to each other; they always act as antagonism [39]. That's why probiotics microbiota can play a major role to destroy potential pathogens. The chemical antagonistic compounds created by probionents are toxic or destructive to the other pathogens habitat. The presence of pro-bacteria creates an antibacterial environment in the host's intestine as well as in culture water. This prevents the proliferation of active pathogenic microbes and even eliminates these. The structure and antibacterial compound activity should be more clear and up to date on the present situation. Moreover, researchers should demonstrate that the antimicrobial compound whatever produced in live conditions. If the production of probiotics is only for the situation demand but no specific future planning, the pathogen eventually developed their resistance against the probionts. Then probiotics would be ineffective like antibiotic treatment [40]. So the risk assessment and sustainability against pathogenic organism is very important to assure.

3.3. Stimulation of immunity against pathogenic microorganisms

The stimulation of immune response is done for host bio-security applying various biological functions by probionents to kill pathogens. In a word, it can be said that "protection against pathogens". It is found similarity in fish and higher vertebrate's immune system and both have two essential components (a) The innate, natural or nonspecific security system that is formed by a series of a cellular and humoral mechanism and improve immunity and resistance, e.g. common carp [41], angelfish [42], olive flounder [43] and grass carp [44]. (b) The adaptive acquired or specific immune system can be defined by the hormonal immune response through the production of antibodies and by the cellular immune response which is mediated by T-lymphocytes, capable to react with antigens. The normal microbes in the ecosystem affect the fish's immune system that is actually very important to disease control by enhancing physical barriers, hormonal and cellular components. Research in rainbow trout infection, host-isolated probiotics Enterococcus casseliflavus increases resistance against Streptococcus through immune modulation [45]. Cytokines are an essential element of the adaptive and natural immune response, particularly interleukin-lb [46], interferon, tumor necrosis factor-a, transforming growth factor-b and several chemokines influence natural immunity [47]. Probiotics can also influence the unidentified immune response and enzymatic activity [48]. *Bacillus sp.* can activate cellular and hormonal immune security in tiger shrimp that protect against disease [49]. That actually increases phagocytosis and antibacterial function which influences the growth and reduces the early mortality of postlarvae and influences the immune response in shrimp.

3.4. Antiviral Agent

Viruses are one of the great threats to aquaculture. It normally causes random mortality of post larvae. The biochemical substances extract from marine algae and bacterial extra-cellular agents inactive the viruses functioning. But it's mechanism for antiviral effect is not even cleared. A study showed that the strains of *Vibrio sp., Pseudomonas sp., Aeromonas sp.,* and coryneform agents isolated from salmonid acted as an antiviral function against affected hematopoietic necrosis virus with above 50% plaque reduction [50]. Moraxella species is a marine bacterium that affects the activity of poliovirus with plaque reduction percentage is about 62-99%. This was isolated from *P. monodon* hatchery [51].

4. Commercial Preparations

Day by day, the interest for probiotics such as an ecofriendly option is expanding experimentally and scientifically. The worldwide market for probiotics elements, supplements, and feedings is highly demandable. Recently commercial value of probiotics that include at least one live microorganism is rising. Probiotics can be utilized as food additives to the culture farm directly or blended with fish feeds. Aside from laboratory preparation arrangement of microscopic organisms, some commercially accessible items are currently found. At first, Assessments of commercial items focused on a microbial preparation named 'Biostart' that is isolated from Bacillus sp [52]. In 1998 it was first utilized in cultured catfish to test the effects of inoculums absorption. Moriarty (1998) emphasized that the utilization of commercial probiotics strains of Vibrio sp. expanded the quality and reasonability of pond culture of shrimp [53]. In the meantime, it is tested the function of Bacillus tovoi and Enterococcus faecium which was present in Carnival LBC and Toyocerin separately to diminish the European eel mortality rate that ensured more prominent efficiency with Enterococcus faecium [54]. This was utilized to supplement the food strengthen of Nile tilapia getting a significant increase in efficiency [55]. The lactic-acid creating microorganisms have been the focal point of much interest. The human probiotics, Lactobacillus rhamnosus ATCC was utilized as a part of rainbow trout for 51 days to diminish the death rate by Aeromonas salmonicida, to notice the reaction of fish pathogens. "Furunculosis" is one of the major fish diseases. Mortality was lessened from 52.6 to 18.9% when 10^9 cells g-1 were controlled with probiotics fish feed [56]. Experiment with Penaeus vannamei demonstrated that probiotics poly-culture expands survival, encourages transformation, and increases the final production of cultivated shrimp [15]. A study has shown that, poly-culture of bacteria (L. acidophilus, B. subtilis, and C. butyricum) and yeast (S. cerevisiae, B. licheniformis) upgraded unidentified immune expression parameters of tilapia [57] such as, lysozyme action, movement on neutrophils, and plasmatic action, bringing about change of protection from Edwardsiella tarda disease [58]. Additional investigations of probiotics demonstrated their ability to stimulate the activity of microbiota in the higher vertebrate colon [59]. Some commercial aquaculture probiotics such as mannans, glucans, and yucca improve the quality of the product. Recently, the advanced process technique of probiotics has been developed like immobilization and microencapsulation to ensure superlative quality. Right now, commercial items are found as a powder or liquid type in the box or poly packet. The production is carried out in general batch to batch cultures because of the complex mechanism of ongoing systems [60]. Usually, the techniques such as emulsion, shower drying, expulsion, and attachment to starch, have been utilized for microencapsulation of probiotics. Concentrated on the application to aquaculture have effectively exemplified cells of Shewanella putrefaciens in calcium alginate, showing the survival of typified probiotics cells through the intestinal tract in fish. The capacity of transport, dehydration temperature and osmoregulation parameters are crucial to assure the suitability of micro-organisms [61]. It is important that the product must ensure a healthy and bio-security benefit to the host. It is essential to ensure suitable conditions for probiotics organisms during storage and application on the host intestine. The user should be followed the packet labeling properly to ensure the best probiotics storage and safety of cultured species.

5. Probiotics applications for sustainable aquaculture

The requirement for feasible aquaculture modern research is required into the utilization of probiotics fed on aquatic animals. Initially, it was focused on their utilization as growth agents and health improvement but now the investigation has been going on multiplication or stress resilience.

5.1. Growth enhancer agent

Probiotics have been utilized as a part of aquaculture to build the growth development of farming species [62]. Actually, it isn't known whether these items enhance the hunger, or enhance absorbability. A few people are biased to imagine that it could be the two variables. Besides, it is vital to decide if probiotics really taste useful for aquaculture species [63]. It is indicated, probiotics microorganisms can colonize the gastrointestinal tract because they can do higher multiplication than the rate of removal. This likewise relies upon variables, for example, hydrobiont species, body temperature, compound levels, hereditary protection, and water quality. The effect of probiotics on phytoplankton influences the food chain by photosynthetic activities. Inside gatherings of microalgae utilized as a part of aquaculture are recognized focal diatoms as Chaetoceros sp., which is decent live food it may confinements because of the side effects of their healthy prerequisites. Rotifers are one of the essential feed for hatchlings of most refined amphibian species. For example, rotifer B. plicatilis and LABs (Lactococcus casei and Lactobacillus lactis) blend are used to feed the nauplii of marine shrimp to acquire the best outcome [64]. The Bacillus sp. probiotics, isolated from the fish gut into their diet has been studied and it's found a positive approach in increasing size and weight of fishes. The utilization of probiotics

as development promoters of palatable fishes has been accounted for the eating regimen of Nile tilapia [57]. In another study showed that unrefined protein and irregular fish lipid are significantly influenced by probiotics *Streptococcus* strain [65].

5.2. Application in fish/shrimp pathogen control

Probiotics microorganisms can discharge synthetic substances with a bacteriostatic effect on pathogenic microscopic organisms [66] that live in the digestive organ of the host, subsequently constituting an obstruction against the multiplication of entrepreneurial pathogens. The creation of antimicrobials, bacteriocins, siderophores, proteins, hydrogen peroxide, and modification of the intestinal pH are important to kill pathogens [67]. Some researchers demonstrated that suitable probiotics expanded nonspecific invulnerable reaction in salmon and trout fish. In this case, leukocytes number was more prominent than live cells [68]. Bacillus cereus, Paenibacillus polymyxa, and Pseudomonas sp. as bio-control are acted against pathogens from different Vibrio species [69]. Gastrointestinal tract straining probiotic of Amphiprion per*cula* has been utilized to kill some of the pathogens like A.hydrophila and V. alginolyticus. It has been showed that probiotics application in live animals, the main goal is the stock density that permits the generation of antimicrobial metabolites in this way. Antiinfection agents were utilized in aquaculture for disease control up to the harvest. In any case, this creates different issues like anti-toxin environment in living tissues and adverse condition for the gut microbiota of host, which influence host bio-security [70]. At present, it is requested for characteristic items, free of added substances like anti-toxins. Besides, there is an inclination for counteracting ailments as opposed to treating them. Along these lines, the utilization of probiotics referred as an effective treatment for pathogens.

5.3. Change nutrient during digestion

Probiotics can play a vital role in the digestive system in fish. Probiotics strains combine extracellular enzymes like amylases, proteases, and lipases. In addition, it can stimulate vitamins, unsaturated fats, and amino acids by making efficient food supplement with probiotics. In this sense, probiotics have been utilized as a part of edible fish feed in hatchlings of European bass [71]. The probiotics from yeast Debaryomyces hansenii can generate spermine and spermidine, two polyamines engaged with the deviation and development of the gastrointestinal tract in warmblooded organisms. Furthermore, yeast produces trypsin and amylase that guide absorption in sea-bass hatchlings. Concentrates in the juvenile of dentex demonstrated that feed added with 0.5 grams of B. cereus strain expanded fish growth [72]. In white shrimp different strains of Bacillus sp. have been utilized as probiotics feed to increase absorbability of the dry issue, unrefined protein, and phosphorus. Results demonstrated higher growth when the eating routine is supplemented with 50 grams of probiotics [73].

5.4. Change of water quality

To improve culture farm water quality probiotics may be an eco-friendly option. In a few experiments, water quality was recorded amid the expansion of probiotics strains, particularly of Bacillus sp. Most likely since this bacterial gathering is more effective than gramnegative in changing natural issues to CO₂. It is recommended that keeping up irregular amounts of probiotics in ponds, fish ranchers can limit the gathering of natural carbon along with the developing time and this can adjust the creation of phytoplankton [39]. The assumption is not yet confirmed in tests basis on the development of shrimps or catfish. In this way enhancing water quality is limited, with the exclusion of nitrification [67]. In other studies shown for tilapia generation in recycling frameworks, groupings of aggregate ammonia $(NH_4 + NH_3)$ expanded from 4.73 to 14.87mgL-1 / 21-day experiment, in this case, nitrite expanded from 3.75 to 9.77mgL-1. Because of the high convergences of delivered nitrogen mix and the harmful bad-smelling salts, the utilization of probiotics is suggested that it may enhance water quality by using *B. subtilis* and *B. licheniformis* in 17 weeks. Evaluation of water parameters confirmed a satisfactory quality range for fish development: 5.7-6.3mgL-1 for break up oxygen, 0.36–0.42mgL-1 for NH₃ concentration, and pH in the area of 6.3 and 8.2 [55]. The probiotics used in culture farms can help to

improve the water quality by balancing the nitrogen and pH levels of water.

5.5. Stress reliever

Stress in fish's life cycle hampered the total production. The culture species may be weakened and dislike to take food, this is called food phobia. In this situation, for probiotics in culture farm can relieve these types of stress [74]. Research has been accounted for zebra fish (Danio rerio), general dejection on the combination of muscle protein initiated to enlarge stress resistance by utilizing probiotics [75]. One of the fastest formal reports on this area examined the supplementation of Lactobacillus delbrueckii in the eating regimen of E. sea bass, from 25- 59 days. In growing stage hormone cortisol was quantized in fish tissue as a stress marker since it is directly engaged with the host reaction to stress [76]. Another approach to evaluate stress in fish the treated group of probiotics indicated more prominent resilience in the stress test than the control group [77]. The outcomes got so far raise the possibility of good production of fish within time with probiotics treatment, for ordinary aquacultures rehearses that make stress in the animal during transport, change in water temperature, and irregular controls. The probiotic outcomes demonstrated the high possibility of stress prevention in aquatic animals.

5.6. Effect on reproduction of aquatic species

The quality of cultured fish species depends on broodstock quality. Rearing the broodstock it's highly required nutritious feeds containing lipids, proteins, unsaturated fats and vitamins. Besides, the relationships of these elements influence multiplication in production, e.g. probiotics may play an important role to improve food value and hatchlings in ornamental fish [78, 79]. The utilization of these natural fish items frequently doesn't give sufficient levels of supplements required by broodstock fishes. Sometimes pathogens may transfer to the host including parasites, microscopic organisms, and infections. Researchers has shown that the total creation of egg per female and the relative fertility were assessed their outcomes indicated significant deference between the control and probiotics-treated group [79]. So, probiotics as a feed additive or water purifier effective investigation is required before applying in aquaculture.

6. Safety regulation, advantages, and disadvantages of probiotics

It is very important to apply the correct and quality probiotics to the culture farm. Quality probiotics can ensure proper growth and give bio-security in the farming. But if there is a problem with the selection and application of probiotics it may create adverse effects, and the risky species may grow out and make them more resistant against the host. This depends on the selection of probiotics. During selecting probiotics a farmer should know the molecular function of it. Effects of common antibiotics such as guinolone. macrolide, tetracycline and subsequent confirmation of drug resistance genes should be well-analysis before applying probiotics. During feed formulation the ratio should be perfect otherwise it may harm the culture farm. Modern technology can be useful to know it's used and molecular function. So specific labeling with specification should be followed before application. GMP (good management practice) and GLP (good laboratory practice) should be followed during the production of probiotics. The quality probiotics may ensure disease control and sustainable healthier condition of the gut area and the immune system of fish. At present, the application field of probiotics has been applying to the confined level but it should be more affordable. Eco-friendly bacteria can be more beneficial for the aquaculture community to disease control and health improvement. The evidence of probionts efficiency in aquaculture should be more available as possible. The production of probiotics should be increased through effective research. This is a very pleasant approach that FAO has now concern about probiotics using policy for improvement of aquatic environmental quality. Here, we list the advantages and disadvantages of probiotics (table 1).

Advantages	Disadvantages
 Hindrance of pathogens in the gut or elsewhere Enhanced digestibility and microbial adjustment Provide nutrition supplied for aquatic animals Host immune response stimulation to disease Improve water quality and ensure a friendly environ- ment. Reduced blue-green algae load and cleaned the bottom of the pond. Water exchange needed less that reduces water pump- ing costs. 	 Improper application in host sometime may be nega- tively treated. Probiotics work slowly than antibiotics Application in the gut for fish is studied but no more results found in other organs. It cannot reach and main- tain itself in the location where the effect is to be ap- plied. Mislabeling and mishan- dling may cause an adverse condition for the environ- ment. Misguidance may resist the

Table 1: Advantages and disadvantages of probiotics

ment.
6. Misguidance may resist the new pathogens and will be risky for the human being.
7. Advance technology required to prepare probiotics which are the main challenge [80].

7. Conclusion

vibrio loads

8. Reduced green vibrio

counts and reduced overall

9. Reduce ammonia level in

water insuring healthier pro-

duction and increased profits.

The selection of probiotics is essential and this is also a great challenge for the culture species how they adopt this. A good mechanism for applying in the cultured field can be useful in different situations. Recent efforts of research in probiotics showing the positive result in cultured fields. That's why more information should be noted on the host and microbe interactions in life. The technology should be more developed for monitoring tools. For example, a brief understanding of the chemical composition and activity of the native microorganism and their microbial cultures of molecular knowledge is required. The efficiency of competition between species or strains should be more analyzed. Probiotics are beneficial microorganisms for the host though some of these benefits still have to be confirmed using clinically apply. There is however still a problem of preservation of these cultures microbes both in storage and in the gastrointestinal tract. The research for modern technology that can protect and retain the feasibility of probiotics fish cultures is needed. There are also concerns about the negative impacts of probiotics on

receptive consumers but there is insufficient data to support the raised concerns. Probiotics and symbiotic can be alternative mechanisms for increasing the levels of beneficial microorganisms in the gut intestine. Given the health issues of fisheries associated with probiotics, it can be an innovative approach for the future aquatic species.

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