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Potential Effects of Microalgae-Supplemented Diets on the Growth, Blood Parameters, and the Activity of the Intestinal Microbiota in *Sparus aurata* and *Mugil cephalus*

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Abstract: This work aimed to assess the suitability of a microalgal blend as a dietary ingredient for feeding juveniles of marine carnivorous and herbivorous teleost, as is the case of *Sparus aurata* and *Mugil cephalus*, respectively, and to isolate microorganisms from different media and characterize them on the base of their enzymatic activities and their antagonism against important fish pathogens. Thirty juveniles of each species (70 ± 3.2 g *S. aurata* mean weight and 47 ± 2.8 g *M. cephalus* mean weight) were distributed in four tanks (15 individuals each) corresponding to four independent dietary treatments (control and microalgae diets designed for each species). Fish were fed their corresponding diets ad libitum for 108 days. At the end of the trial, fish were weighed, and plasma, liver, perivisceral fat, and the entire intestines were obtained for the evaluation of growth performance and metabolic assessment. Furthermore, 117 bacterial strains were isolated in different culture media from the gastrointestinal tract of *S. aurata* fed the microalgae blend and further characterized for their potential use as probiotics in aquaculture. *S. aurata* fed the microalgae-supplemented diet (25% dietary inclusion) showed a significant increase in weight gain, specific growth rate, feed efficiency, hepatosomatic, and intestine length indices. However, growth performance and somatic indices in *M. cephalus* were not affected by the experimental diets. Plasma samples from *S. aurata* fed the microalgal diet revealed higher levels of glucose and triglycerides and a decrease in cortisol levels. No significant differences were found in any biochemical parameters among the experimental diets in *M. cephalus*. In conclusion, both species demonstrated a favorable adaptation to the nutritional formulation employed in this study, and bacterial strains UMA-169 and UMA-216 (both identified as *Bacillus pumilus*) could be considered for use in aquaculture as they might benefit host health by improving digestion and absorption of different energy sources and by minimizing the colonization of pathogenic species.

Keywords: antimicrobial activity; aquaculture; culture medium; enzymatic activity; growth performance; hemolysis; metabolism; *Mugil cephalus*; *Sparus aurata*

Key Contribution: *Bacillus* strains UMA-169 and UMA-216 were isolated from the intestine of *S. aurata* fed the microalgae blend and stood out for their ability to hydrolyze several aquafeed substrates and inhibit important aquaculture pathogens.

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1. Introduction

Due to the high growth population ratio and the subsequent fish protein demand, aquaculture is one of the fastest-growing food-producing sectors [1]. This growth must be in consonance with the Sustainable Development Goals (SDGs) established by the UN in the Agenda 2030, especially in aquaculture nutrition [2]. Traditional ingredients used in the design of aquafeeds for cultured fish, i.e., fishmeal and fish oil, are considered unsustainable due to being derived primarily from fish obtained through extractive fishing, generating wild fish population reliance. In this way, many scientific efforts have focused on exploring alternative protein sources suitable for aquafeed formulation [3]. A nutritional alternative for aquafeed design could be the use of plant protein meals, as they have shown successful application as fish feed ingredients in various studies' assays [4]. However, vegetal protein sources can contain a broad variety of anti-nutritional factors. High levels of these components in feeds can lead to adverse effects on the growth [5] and digestive enzyme activities [6] of cultured fish.

In this context, microalgae could constitute an optimal complement for aquafeed design. They are usually the main feed of some farmed species, such as some mollusks, and critical ingredients in the early stages of some teleost fish species [7]. The recent industrial-scale microalgae production has sparked interest in their application in aquafeeds [8]. Due to their high protein content, good amino acid profile, and high quantity of essential fatty acids, marine microalgae are considered suitable substitutes for fishmeal in several fish species [9]. Thus, the incorporation of marine microalgae into aquafeeds has been assessed in several fish species, demonstrating favorable results in terms of growth performance, physiology, and metabolism [10,11], even at low or very low levels of inclusion [12]. Moreover, including microalgae has promoted positive effects on fish pigmentation [13] or product quality [14], which are particularly important for consumers.

However, some microalgae have a complex cell wall, which may contain anti-nutritional factors, making it difficult for carnivorous fish such as gilthead seabream (*Sparus aurata*) to extract nutrients from them efficiently [15]. Furthermore, other microalgae species possess high recalcitrant cell walls, such as *Chlorella* species [16], or high carbohydrate content that impair the digestive enzymatic activity [17,18]. This impairment can reduce feed digestion and absorption, negatively affecting growth performance [19]. In this sense, the role of microorganisms in the hydrolysis of complex compounds through degradative processes is well known [20], and the reduction in levels of anti-nutritional factors such as phytate, tannins, or protease inhibitors in plant substrates has been verified due to the action of microbial activities [21,22].

Functional diets that include probiotic microorganisms have enormous potential in addressing the present nutritional problems in aquaculture. In this sense, probiotics can aid in the breakdown of various components of aquafeeds, such as carbohydrates, lipids, and anti-nutritional factors, thereby enhancing growth and feed conversion. It is important to note that the enzymatic activity of microorganisms can be modulated by the composition of the medium from which they were isolated or the medium in which they grow. For example, the synthesis of microbial chitinase is controlled through a receptor-inducer system, susceptible to significant effects from cultivation conditions and media components in diverse *Bacillus* strains [23]. Similarly, it was observed that maltose and beef extract served as the most effective carbon and nitrogen sources, respectively, significantly influencing the production of protease enzymes in *Bacillus aryabhattai* Ab15-ES [24]. This approach could provide a valuable strategy for identifying novel enzymes with unique catalytic properties and applications in various biotechnological fields, including aquaculture, food, and pharmaceutical industries.

Gilthead seabream (*S. aurata*) is one of the top-cultured marine fish species in the Mediterranean, including Spain [1], being a perfect model of carnivorous teleost in European aquaculture. Another group of fish species, which are acquiring a special role in the diversification of Mediterranean aquaculture, is the mullets. They have been described as an easily cultured species, making them a promising candidate for aquaculture diversifi-

cation at a low-trophic level [25,26]. Grey mullet (*Mugil cephalus*) is a cosmopolitan fish strongly ligated to estuary water with herbivorous feeding behavior, mostly phytoplankton and detritus, having a digestive system specially adapted to that feeding regime [27].

Feeding fish with microalgae-supplemented diets involves a selection pressure on their intestinal microbiota that can be used to achieve enrichment in bacteria with a set of enzymatic activities capable of metabolizing and mobilizing the components, particularly those enriched with microalgae while also having antibacterial activity against fish pathogens. That way, this piece of research was aimed at assessing the potential of microalgae blend as a dietary ingredient for feeding carnivorous fish, such as gilthead seabream, and herbivorous fish, such as mullet juveniles, and to isolate and characterize probiotic microorganisms from the intestinal microbiota of fish fed with microalgae diets. The microorganisms will be isolated from different media and characterized based on their enzymatic activities, as well as their antagonism against important fish pathogens.

2. Materials and Methods

2.1. Ethical Statement

All experimental procedures involving fish strictly adhered to the guidelines for animal research set forth by the Ethics and Animal Welfare Committee of the University of Cadiz (UCA) and were in strict accordance with the Guidelines established by the European Union (2010/63/UE) and the Spanish legislation (RD 1201/2005 and RD 53/2013) for the use of laboratory animals. Furthermore, the experiments received approval from the Ethical Committee of the Autonomous Andalusian Government (Junta de Andalucía) under reference number 04/04/2019/056.

2.2. Microalgae

Microalgal biomasses (*Chlorella* sp., *Arthrospira* sp., *Tisochrysis* sp., and *Nannochloropsis* sp.) were cultivated at the SABANA facilities at the University of Almería (Almería, Spain). Freshwater strains were cultured in BG11 medium, while seawater strains were cultured in f/2 nutrient medium. The cultures were carefully controlled for light (240 µmol photons m^{-2} s^{-1}, photoperiod 12L:12D) and temperature (25 ± 2 °C), and a continuous supply of 5% CO_2-enriched air was maintained during the light period. After cultivation, the biomass was harvested by centrifugation, freeze-dried, and then milled using a ball mill (RM200 mill, Retsch, Spain) for 20 min to obtain a fine powder with particles below 100 µm in size. This powder was stored in the dark at –20 °C until used as an ingredient in the experimental diets.

2.3. Experimental Feeds and Feeding Trial

Experimental feeds were elaborated at Ceimar-Universidad de Almería facilities (Servicio de Piensos Experimentales, https://www.ual.es/universidad/serviciosgenerales/stecnicos/perifericos-convenio/piensos-experimentales, accessed on 20 November 2022) (Almería, Spain) using standard aquafeed manufacturing procedures [13]. Two control diets were formulated with an ingredient composition within the range of the commercial aquafeeds used these days for feeding gilthead seabream and mullet (CT-SA and CT-MC, respectively). Two microalgae-supplemented diets were also formulated containing a 25% microalgae blend (composed of 25% of each of the strains, M25-SA and M25-MC, respectively). The ingredients and proximate composition of the experimental diets for *S. aurata* and *M. cephalus* are shown in Table 1. The formulation of these experimental feeds was based on our research group's previous studies conducted with gilthead seabream or mullet juveniles [13,28].

Table 1. Ingredients and proximal composition (% dry matter) of the experimental diets for gilthead seabream and grey mullet.

	Sparus aurata		*Mugil cephalus*	
	CT-SA	**M25-SA**	**CT-MC**	**M25-MC**
Ingredients (% dry matter)				
Fishmeal LT94 [1]	25.0	20.0	7.5	7.5
Lysine [2]	1.2	1.2	-	-
Methionine [3]	0.5	0.5	-	-
Squid meal [4]	2.0	2.0	-	-
CPSP90 [5]	1.0	1.0	-	-
Krill meal [6]	2.0	2.0	-	-
Wheat gluten [7]	10.0	9.0	-	-
Soybean protein concentrate [8]	26.0	16.0	17.5	-
Blend of microalgae [9]	-	25.0	-	25.0
Fish oil [10]	8.7	8.0	2.5	2.5
Soybean oil [11]	4.0	4.0	-	-
Soybean lecithin [12]	1.0	1.0	-	-
Wheat meal [13]	14.0	5.7	23.0	15.5
Pea protein [14]	-	-	7.5	7.5
Soybean meal [15]	-	-	18.7	18.7
Corngluten meal [16]	-	-	6.0	6.0
Sunseed meal [17]	-	-	12.7	12.7
Potato starch [18]	-	-	2.3	2.3
Betain [19]	0.5	0.5	-	-
Vitamin and mineral premix [20]	2.0	2.0	0.8	0.8
Vitamin C [21]	0.1	0.1	-	-
Guar gum [22]	2.0	2.0	1.5	1.5
Proximate composition (% dry matter)				
Crude protein	45.3	44.9	39.1	38.8
Crude lipid	16.7	16.5	7.8	7.5
Ash	9.2	8.8	6.8	7.3
Gross energy (kJ/g) [23]	23.5	23.5	21.1	21.0

Dietary codes: CT-SA: microalgae-free diet for *S. aurata*; M25-SA: 25% blend of microalgae-supplemented diet for *S. aurata*; CT-MC: microalgae-free diet for *M. cephalus*; M25-MC: 25% blend of microalgae-supplemented diet for *M. cephalus*. [1] A total of 69.4% crude protein and 12.3% crude lipid (Norsildemel, Bergen, Norway). [2,3] Lorca Nutrición Animal SA (Murcia, Spain). [4,5,6] purchased from Bacarel (UK). CPSP90 is enzymatically pre-digested fishmeal. [7] A total of 78% crude protein (Lorca Nutrición Animal SA, Murcia, Spain). [8] Soycomil, 60% crude protein, 1.5% crude lipid (ADM, Poland). [9] Blend of *Chlorella* sp. (43.2% crude protein, 6.7% crude lipid, 15.4% ash, 34.9% NFE), *Arthrospira* sp. (62.0% crude protein, 6.5% crude lipid, 12.3% ash, 18.5% NFE), *Tisochrysis* sp. (31.3% crude protein, 19.9% crude lipid, 17.6% ash, 31.2% NFE), and *Nannochloropsis* sp. (31.4% crude protein, 21.3% crude lipid, 18.9% ash, 28.4% NFE) (1:1:1:1). [10] AF117DHA (Afamsa, Spain). [11] Soybean oil (Aceites el Niño, Spain). [12] P700IP (Lecico, DE). [13] Local provider (Almería, Spain). [14] Pea protein concentrate, 85% crude protein, 1.5% crude lipid (Emilio Peña SA, Spain). [15] A total of 50% crude protein (Lorca Nutrición Animal SA, Murcia, Spain). [16] A total of 60% crude protein purchased from Bacarel (UK). [17] A total of 32% crude protein (Lorca Nutrición Animal SA, Murcia, Spain). [18] Andres Pintaluba, Spain. [19,22] Lorca Nutrición Animal SA (Murcia, Spain). [20] Lifebioencapsulation SL (Almería, Spain). Vitamins (mg kg^{-1}): vitamin A (retinyl acetate), 2,000,000 UI; vitamin D3 (DL-cholecalciferol), 200,000 UI; vitamin E (Lutavit E50), 10,000 mg; vitamin K3 (menadione sodium bisulfite), 2500 mg; vitamin B1 (thiamine hydrochloride), 3000 mg; vitamin B2 (riboflavin), 3000 mg; calcium pantothenate, 10,000 mg; nicotinic acid, 20,000 mg; vitamin B6 (pyridoxine hydrochloride), 2000 mg; vitamin B9 (folic acid), 1500 mg; vitamin B12 (cyanocobalamin), 10 mg vitamin H (biotin), 300 mg; inositol, 50,000 mg; betaine (Betafin S1), 50,000 mg. Minerals (mg kg^{-1}): Co (cobalt carbonate), 65 mg; Cu (cupric sulfate), 900 mg; Fe (iron sulfate), 600 mg; I (potassium iodide), 50 mg; Mn (manganese oxide), 960 mg; Se (sodium selenite), 1 mg; Zn (zinc sulfate) 750 mg; Ca (calcium carbonate), 18.6%; (186,000 mg); KCl, 2.41%; (24,100 mg); NaCl, 4.0% (40,000 mg). [21] TECNOVIT, Spain. [23] Gross energy was estimated by energetic coefficients (kJ/g): crude protein, 23.6; crude lipid, 38.9; NFE, 16.7.

Juvenile specimens of *S. aurata* and *M. cephalus*, initially weighing 70 ± 3.2 g and 47 ± 2.8 g (mean ± SE), respectively, were obtained from CUPIBAR S. L. (Chiclana de la Frontera, Cádiz, Spain). The feeding trial took place at the Servicios Centrales de Investigación en Cultivos Marinos (SCI-CM, CASEM, University of Cádiz, Puerto Real, Cádiz, Spain; Spanish Operational Code REGA ES11028000312). Fish were cultured in seawater at a temperature of 19 °C and a salinity of 37‰, with continuous control of nitrite, nitrate, and ammonia levels, oxygen saturation, and a 12L:12D photoperiod.

A total of 30 juveniles of each species, which were previously acclimated for 7 days, were distributed into four 200 L cylindrical tanks (15 individuals each), representing four independent dietary treatments (control and microalgae diets specifically designed for each species). The initial fish stock biomass was 5.25 kg/m^3 for *S. aurata* and 3.52 kg/m^3 for *M. cephalus*. Throughout the 108-day feeding trial (March–June 2020), the fish were fed their respective diets to apparent visual satiation (ad libitum) three times daily, ensuring that the amount provided in each experimental unit was fully consumed.

2.4. Fish Sampling

Finally, the remaining six fish of each experimental unit were also weighed and measured to determine the growth performance and biometric parameters described below for the total number of animals assayed.

After 108 days of trial, all fish were subjected to a 24-h fasting period before the final sampling. Then, a final sampling was performed in which nine fish per tank (n = 9) were randomly selected and sacrificed by being deeply anesthetized with a lethal dose of 2-phenoxyethanol (1 mL L^{-1}). Specimens were then individually weighed (wet weight; WW) and measured (total length; TL). Blood was drawn from the caudal vessels with heparinized syringes and centrifuged at 13,000× *g* for 20 min at 4 °C to obtain plasma samples. Subsequently, fish were cervically sectioned to obtain different tissues. Liver and perivisceral fat were removed and weighed from each specimen to obtain different somatic indices. The entire intestine, from the pyloric caeca to the rectum, was removed, and its length was measured. Plasma samples for metabolic assays were snap-frozen in liquid nitrogen and stored at −80 °C for further analysis. Plasma samples for metabolic assays were then snap-frozen in liquid nitrogen and stored at −80 °C until further analysis. The whole intestines of different specimens were placed on ice in independent 15-mL falcon tubes and transported to the University of Málaga within 2 h for bacterial isolation. Finally, the remaining six fish from each experimental unit were weighed and measured to assess the growth performance and biometric parameters, as described below, for the entire cohort of animals analyzed.

2.5. Growth Performance and Somatic Indices

Growth performance was evaluated via analysis of the following parameters (Equations (1)–(4)):

$$\text{Specific Growth Rate (SGR)} = (100 \times (\ln \text{final body weight} - \ln \text{initial body weight})/\text{days} \quad (1)$$

$$\text{Weight Gain (WG)} = (100 \times (\text{body weight increase})/\text{initial body weight} \quad (2)$$

$$\text{Feed Efficiency (FE)} = \text{weight gain}/\text{total feed intake} \quad (3)$$

$$\text{Fulton's Condition Factor (K)} = (100 \times \text{body weight})/\text{fork length}^3, \quad (4)$$

Furthermore, organosomatic indices from the liver, perivisceral fat, and intestine were estimated according to the following Equations (5)–(7):

$$\text{Hepatosomatic Index (HSI)} = (100 \times \text{liver weight})/\text{fish weight} \quad (5)$$

$$\text{Intestine Length Index (ILI)} = (100 \times \text{Li})/\text{Lb}, \quad (6)$$

where Li and Lb are the intestine and fork body length, respectively.

$$\text{Mesenteric index (MSI)} = (100 \times \text{perivisceral fat weight})/\text{fish weight} \quad (7)$$

Due to the intestinal fragility of *M. cephalus*, perivisceral fat was not removed and weighed to avoid intestinal error samples, and MSI was possibly not calculated for this species.

2.6. Biochemical Parameters of the Plasma

Plasma biochemical parameters were evaluated in duplicate using a spectrophotometric method (PowerWave™ 340 microplate spectrophotometer, BioTek Instruments, Winooski, VT, USA), controlled by KCjunior Software for Microsoft® Windows (BioTek Instruments, version 1.4.), according to the methodology described in Molina-Roque et al. [11] and Perera et al. [12].

2.7. Isolation of Strains

Serial 10-fold dilutions were prepared from central intestinal contents of 9 gilthead seabream fed M25-SA diet in Phosphate Buffer Saline (PBS), and 100 µL aliquots were spread on tryptic soy agar supplemented with 2% NaCl (TSAs). Furthermore, 100 µL were spread on minimum media (M9) supplemented with 1.5% agar, 2% NaCl, and 25% microalgae/cyanobacteria mix (*Chlorella* sp., *Arthrospira* sp., *Tisochrysis* sp., and *Nannochloropsis* sp.; MMA). Marine lactic acid bacteria were selectively cultured on De Man, Rogosa, and Sharpe agar (MRS) supplemented with 2% NaCl [29]. Spore-formers were isolated after heat treatment and cultured on TSAs plates, according to Nicholson and Setlow [30]. Plates were incubated at 22 °C (37 °C for lactic acid bacteria) in aerobic conditions for 2–3 days. In total, 117 distinct morphological colonies were individually selected, and pure cultures were obtained by streaking and re-streaking procedures on fresh media. These pure cultures were duplicated and stored in cryo-vials at a temperature of −80 °C.

2.8. In Vitro Screening Assays

2.8.1. Hydrolytic Activity

Protease, collagenase, lipase, and amylase activities were determined by streaking 1–2 colonies from fresh cultures on agar plates containing specific substrates: skimmed milk (2% *w*/*v*); gelatine (1% *w*/*v*); Tween-80 (1% *w*/*v*); and starch (4% *w*/*v*), respectively. Additionally, extra activities such as phytase, tannase, and cellulase were assessed following the method of Kumar et al. [31]. Fresh cultures were streaked on agar plates containing Na-phytate (1% *w*/*v*), tannic acid (2% *w*/*v*), and carboxymethyl cellulose (CMC) (1% *w*/*v*), respectively. The plates were observed for the appearance of clear zones around the colonies, indicating amylase and cellulase activity, after flooding with Lugol and Congo red solution (0.1% *w*/*v*), respectively. The absence of a clear zone was interpreted as no activity.

2.8.2. Antimicrobial Activity

For the antibacterial activity, the agar-well diffusion assay described by García-Márquez et al. [32] was employed. Fish pathogenic bacterial strains *Vibrio anguillarum* (Spanish Type Culture Collection, CECT 522) and *Photobacterium damselae* subsp. *piscicida* [33] were cultured on TSA plates at 23 °C for 24 h. In addition, *Tenacibaculum maritimum* (CECT 4296) was cultured on Flexibacter maritimus medium (FMM) [34] plates supplemented with agar (1.5%) at 28 °C for 48 h. Standardized cultures adjusted to OD600 nm~0.1 were evenly spread onto the surface of the TSAs or FMM plates using sterile swabs. Each plate had six wells (6 mm diameter) filled with 50 µL of freshly cultured isolates (approx. 10^7-10^8 cfu mL^{-1}) grown in tryptic soy broth supplemented with 2% NaCl at 22 °C. Negative control wells were filled with 50 µL of PBS, and positive control wells

contained 50 µL of a suspension of *Vibrio proteolyticus* cells (10^8 cfu mL^{-1}) [35]. Plates were incubated for 24–48 h at 23 °C or 28 °C, depending on the optimal incubation time and temperature for each pathogen. The absence of a clear zone was interpreted as having no antibacterial activity.

2.9. Hemolytic Activity

Hemolytic activity was assessed by streaking 1–2 colonies from fresh cultures on Columbia agar plates containing 5% (*w*/*v*) sheep blood. The plates were then incubated at 23 °C for 24–48 h. Hemolytic activity was determined based on the presence of different signs: α-hemolysis (green zones around colonies or wells), β-hemolysis (clear zones), or γ-hemolysis (no zones) on the plates [36].

2.10. Identification of Isolate Strains

The bacterial genomic DNA was extracted from pure colonies of the selected strains using the GeneJet Genomic DNA purification Kit (Thermo Scientific #K0721, Waltham, MA, USA) according to the manufacturer's protocol. The molecular identification of the strains was performed by PCR amplification of the 16S rRNA gene. The 16S universal primers BACT0008 (5′ AGAGTTTGATCCTGGCTCAG 3′) [37] and BACT1492 (5′ GGTTACCTTGTTACGACTT 3′) [38] were used to obtain sequences with approximately 1400 bp. The reaction mixture contained 2 µL of bacterial genomic DNA, 62.5 U of Taq Accustart II Trough Mix (Boimerieux, Marcy-l'Étoile, France), 20 pmol of BACT0008 primer, and 20 pmol of BACT1492 primer, in a final volume of 20 µL. The PCR steps included initial denaturation at 95 °C for 2 min, 35 cycles of denaturation (95 °C, 30 s), annealing (52 °C, 40 s), extension (72 °C, 90 s), and final extension at 72 °C for 5 min. The results were compared with the NCBI database using the BLAST algorithm [39].

2.11. Statistical Analysis

The results are presented as the mean ± standard error of the mean (SEM). Normality and homogeneity of variance were assessed using Kolmogorov–Smirnov and Levene's tests, respectively, with a significance level of $p < 0.05$. Independent sample *t*-tests were conducted to analyze somatic index data and plasmatic parameters for each species at a significance level of $p < 0.05$. GraphPad Prism 8.0 (GraphPad Software, Inc., San Diego, CA, USA) was used for all statistical analyses.

3. Results

3.1. Growth Performance and Somatic Indices

No mortality occurred during the assay period for both fish species. The initial weight of the specimens was the same for the experimental groups of both fish (Table 2). Furthermore, there were no significant differences in the final biomass of both species based on the diet, indicating an allometric growth pattern (K). However, in the case of *S. aurata*, significant differences were observed between dietary treatment for WG ($p = 0.049$), SGR ($p = 0.032$), and FE ($p = 0.012$) values, showing higher values for fish fed M25-SA diet. For *M. cephalus*, no significant differences were found in the hepatosomatic (HSI; $p = 0.559$) and intestine length (ILI; $p = 0.958$) indexes between fish fed CT-MC and M25-MC diets. However, in the case of *S. aurata*, fish fed the M25-SA diet showed a significant increase in the intestine length (ILI; $p = 0.013$) and a reduction in the hepatosomatic index (HSI; $p = 0.002$) (Table 2).

Table 2. Growth performance and somatic indices of gilthead seabream (*Sparus aurata*) and grey mullet (*Mugil cephalus*) fed with a control diet (CT-SA and CT-MC, respectively) and one supplemented diet with 25% of a blend of microalgae (M25-SA and M25-MC, respectively).

Parameters	*Sparus aurata*			*Mugil cephalus*		
	CT-SA	**M25-SA**	***p*** [a]	**CT-MC**	**M25-MC**	***p*** [a]
Initial body weight (g)	70.11 ± 3.18	70.15 ± 3.22	>0.999	47.28 ± 2.78	47.30 ± 2.76	>0.999
Final body weight (g)	142.70 ± 4.75	146.80 ± 4.24	0.523	62.24 ± 2.95	62.23 ± 3.02	0.997
K [b]	1.86 ± 0.04	1.83 ± 0.02	0.475	1.25 ± 0.01	1.20 ± 0.03	0.147
WG (%) [c]	103.80 ± 2.20	109.70 ± 2.50 *	0.049	32.43 ± 1.55	32.40 ± 1.80	0.962
SGR (%) [d]	0.65 ± 0.01	0.68 ± 0.01 *	0.032	0.26 ± 0.01	0.26 ± 0.01	0.981
FE [e]	0.62 ± 0.01	0.66 ± 0.01 *	0.012	0.32 ± 0.01	0.32 ± 0.01	0.974
HSI (%) [f]	1.36 ± 0.05	1.14 ± 0.04 *	0.002	1.23 ± 0.06	1.31 ± 0.13	0.559
MSI (%) [g]	0.68 ± 0.14	0.52 ± 0.07	0.330	-	-	-
ILI (%) [h]	84.61 ± 4.32	102.40 ± 4.71 *	0.013	277.50 ± 17.43	278.70 ± 13.05	0.958

Dietary codes: CT-SA: microalgae-free diet for *S. aurata*; M25-SA: 25% blend of microalgae-supplemented diet for *S. aurata*; CT-MC: microalgae-free diet for *M. cephalus*; M25-MC: 25% blend of microalgae-supplemented diet for *M. cephalus*. Data of growth indexes are shown as the mean ± SEM of 15 fish. Data on somatic indexes are shown as the mean ± SEM of 9 fish. Asterisks in each row denote intra-species significant differences among dietary treatments based on independent sample Student *t*-test ($p \leq 0.05$). [a] *p*-value resulting from Student *t*-test; [b] Fulton´s condition factor; [c] Weight Gain (%); [d] Specific Growth Rate; [e] Feed Efficiency; [f] Hepatosomatic index; [g] Mesenteric index; [h] Intestine length index.

3.2. Biochemical Parameters of the Plasma

The results of plasma parameters for both fish species are presented in Table 3. In the case of *S. aurata*, there were no significant differences in lactate levels and circulating proteins ($p = 0.125$ and $p = 0.053$, respectively) between the experimental groups. However, a significant increase in plasmatic glucose ($p = 0.019$) and triglycerides ($p = 0.008$) levels was observed in fish fed the M25-SA diet, while cortisol levels showed a reduction ($p < 0.001$) compared to fish fed the CT-SA diet. As for *M. cephalus*, no significant differences in any analyzed biochemical parameters were observed among fish fed the experimental diets.

Table 3. Blood biochemistry of gilthead seabream (*Sparus aurata*) and grey mullet (*Mugil cephalus*) fed with a control diet (CT-SA and CT-MC, respectively) and one supplemented diet with 25% of a blend of microalgae (M25-SA and M25-MC, respectively).

Parameters	*Sparus aurata*			*Mugil cephalus*		
	CT-SA	**M25-SA**	***p*** [a]	**CT-MC**	**M25-MC**	***p*** [a]
Glucose (mg dL^{-1})	46.39 ± 1.51	54.39 ± 2.53 *	0.019	52.21 ± 2.57	48.69 ± 3.48	0.428
Lactate (mg dL^{-1})	12.90 ± 1.10	15.94 ± 1.39	0.125	28.38 ± 3.53	30.12 ± 1.87	0.668
Triglycerides (mg dL^{-1})	98.61 ± 17.38	163.80 ± 13.25 *	0.008	168.60 ± 9.29	168.70 ± 11.22	0.992
Proteins (mg dL^{-1})	49.16 ± 5.43	62.82 ± 3.52	0.053	34.65 ± 3.35	39.37 ± 4.04	0.382
Cortisol (ng mL^{-1})	27.02 ± 1.18	17.46 ± 1.35 *	<0.001	13.53 ± 2.12	13.84 ± 1.45	0.902

Dietary codes: CT-SA: microalgae-free diet for *S. aurata*; M25-SA: 25% blend of microalgae-supplemented diet for *S. aurata*; CT-MC: microalgae-free diet for *M. cephalus*; M25-MC: 25% blend of microalgae-supplemented diet for *M. cephalus*. Data are shown as the mean ± SEM of 9 fish. Asterisks in each row denote intra-specie significant differences among dietary treatments based on independent sample Student *t*-test ($p \leq 0.05$). [a] *p*-value resulting from the Student *t*-test.

3.3. Bacterial Characterization

3.3.1. Hydrolytic, Antimicrobial, and Hemolytic Activity of the Isolated Bacteria

Altogether, 117 strains were isolated (50 from TSAs, 26 from MMA, 11 from MRS, and 30 spore-formers) from *S. aurata* gastrointestinal tract, stored in cryo-vials at −80 °C, and screened for hydrolytic enzyme activities (Table S1). The results indicated that a considerable proportion of the isolated strains exhibited specific enzymatic activities: 48% could hydrolyze proteins; 41% lipids; 77% collagen; and 30% starch (Table 4). Additionally, 46%, 8%, and 57% of the isolates demonstrated the capability to degrade phytate,

tannins, and cellulose, respectively. The enzymatic activity of the isolates was also compared based on the medium they were isolated from. Results showed that the percentage of isolates with protease, tannase, and cellulase activity was highest in TSAs (56%, 10%, and 66%, respectively), followed by MMA (46%, 8%, and 39%, respectively), spore-formers (40%, 7%, and 57%, respectively), and MRS (36%, 0%, and 55%, respectively). Similarly, MMA medium had the highest percentage of isolates with collagenase, lipase, and phytate activity (81%, 46%, and 73%), followed by TSAs (80%, 44%, and 42%, respectively), spore-formers (73%, 33%, and 27%, respectively), and MRS (64%, 36%, and 55%, respectively). Finally, the percentage of amylase activity was higher in spore-formers (40%) than in isolates isolated in other media.

Table 4. Hydrolytic and antimicrobial activities (% of isolates) of culturable bacterial strains isolated from *S. aurata*.

Hydrolytic Activity (% of Isolates)								
Medium	Isolates (N)	Protease	Collagenase	Lipase	Amylase	Phytase	Tannase	Cellulase
TSAs	50	56	80	44	32	42	10	66
MMA	26	46	81	46	23	73	8	39
MRS	11	36	64	36	9	55	0	55
Sporeformers	30	40	73	33	40	27	7	57
Total	117	48	77	41	30	46	8	57
Antimicrobial Activity (% of Isolates)								
Medium	Isolates (N)	*V. anguillarum*	*P. damselae* subsp. *piscicida*		*T. maritimum*			
TSAs	17	29	41		53			
MMA	8	50	63		63			
MRS	3	67	67		100			
Sporeformers	4	25	15		25			
Total	32	38	47		56			

Thirty-two isolates were selected according to their hydrolytic characterization (at least the strain has to be able to hydrolyze ≥ 4 substrates) and screened for their ability to inhibit the growth of several fish pathogens. The inhibition against *V. anguillarum* was detected in 38% of the isolates, while 47% inhibited *P. damselae* subsp. *piscicida* (Table 4). Finally, 56% of the isolates inhibited *T. maritimum*. The antimicrobial activity of the isolates was also compared based on the medium they were isolated from. In this sense, the MRS medium had the highest percentage of isolates with antimicrobial activity against *V. anguillarum*, *P. damselae* subsp. *piscicida*, and *T. maritimum* (67%, 67%, and 100%, respectively), followed by MMA (50%, 63%, and 63%, respectively), TSAs (29%, 41%, and 53%, respectively), and spore-formers (25%, 15%, and 25%, respectively).

Based on the hydrolytic and antimicrobial activities of these 32 strains (Table S2), those strains capable of hydrolyzing ≥ 4 tested substrates and inhibiting the three fish pathogens were selected. The selected strains were UMA-140, UMA-143, UMA-169 (isolated in TSAs), and UMA-216 (isolated in MMA).

Finally, the hemolytic activity of the select strains was evaluated on blood agar plates (Table 5). Strains UMA-140 and UMA-143 showed β-hemolytic activity, while UMA-169 and UMA-216 had γ hemolytic, i.e., negative or no hemolytic activity.

Table 5. Hydrolytic, antimicrobial, and hemolytic activities of the selected strains.

	UMA-140	UMA-143	UMA-169	UMA-216
Hydrolytic activity				
Amylase	-	+	-	-
Collagenase	+	+	+	+
Lipase	-	+	+	-
Caseinase	+	+	+	+
Phytase	+	+	+	+
Tannase	-	-	-	-
Cellulase	+	-	-	+
Antimicrobial activity				
V. anguillarum	+	+	+	+
P. damselae subsp. *piscicida*	+	+	+	+
T. maritimum	+	+	+	+
Virulence factor				
Hemolysis	β	β	γ	γ

3.3.2. Identification of Selected Strains

The four bacterial strains were identified by comparing 16S DNA with the NCBI database. The four strains were identified as the *Bacillus* genus (Table 6). The UMA-143 strain showed similarity to *Bacillus cereus*, while UMA-140, UMA-169, and UMA-216 showed homology with different strains of *Bacillus pumilus*.

Table 6. Selected strains identification.

Strain	Species	Similarity (%)	Accession Number
UMA-140	*Bacillus pumilus*	99.14	MK491037.1
UMA-143	*Bacillus cereus*	98.86	KC969074.1
UMA-169	*Bacillus pumilus*	99.11	MK491030.1
UMA-216	*Bacillus pumilus*	99.35	MK491042.1

4. Discussion

Microalgae are often used as food sources in aquaculture for bivalves and other filter-feeding species as rotifers or artemia, commonly employed as a first feed for larval fish culture due to their nutritional content and their capacity to synthesize and store essential polyunsaturated fatty acids (PUFA) [40]. Although its use as an aquafeed ingredient for the fattening phase of fish is not widespread, in recent years, there has been a growing interest in exploring the potential of microalgae as functional components in aquafeed design for fish, replacing traditional ingredients such as fish and plant protein. These microalgae offer various health benefits, enhance growth, and improve the quality of fish products [11–14,41].

In the present study, we employed a blend of microalgae (25% *Chlorella* sp., 25% *Arthrospira* sp., 25% *Tisochrysis* sp., and 25% *Nannochloropsis* sp.) in aquafeeds. These species have been previously evaluated for their possible use as dietary ingredients because they are a good source of proteins, amino acids, essential fatty acids, vitamins, and minerals [42–45].

During the assay period, no mortality was registered for fish fed with different diets. This could indicate that designed diets cover energy demands and structural components for both fish' correct development and growth. In terms of growth performances, this study shows fish species differences. It is worth noting the lower growth potential of *M. cephalus* with respect to *S. aurata*, as showing lower values of Weight Gain (WG), Specific Growth Rate (SGR), and Feeding Efficiency (FE). This would represent different growth

rhythms and productive performances that carnivorous species [12] showed compared to omnivorous/herbivorous fish species [45].

For *M. cephalus*, there were no significant differences observed in growth parameters (K, WG, SGR, FE), somatic index (HSI and ILI), and plasma biochemical parameters analyzed. These findings align with the results presented by García-Márquez et al. [45], demonstrating that the incorporation of microalgae in the diet did not negatively impact mullet growth performance, feed efficiency, and plasma metabolites. The lack of biochemical and physiological modifications would suppose that the use of microalgae supplemented diet (M25-MC) does not imply a significant change in the nutrient assimilation structures (intestinal absorption surface), energy storage (liver), and transport (plasma) in mullet. This assumption is further reinforced by the lack of significant differences in cortisol plasma levels observed in fish fed both diets (CT-MC and M25-MC). Elevated cortisol levels are associated with increased energy expenditure and reduced growth rate [46]. Consequently, in the absence of relevant results, it could be interesting to analyze quality product parameters to evaluate the suitability of a supplement microalgae diet for the culture of this fish species.

For *S. aurata*, this assay shows that microalgae addition significantly promoted growth performance. Fish fed the M25-SA diet showed higher WG, SGR, and FE values than those fed the CT-SA diet. These results are in accordance with other studies where *S. aurata* fed supplemented microalgae extract diets also showed higher growth values without adverse effects in allometric growth (K) of fish [11,12,41]. This fact could be the cause or the consequence of the significant increase in the intestinal length, with improved feeding efficiency of fish fed diet supplemented with microalgae ingredients compared to those fed a control diet, given that as denoted by Molina-Roque [11] and Perera et al. [12], a higher intake of diets formulated with a high content of vegetable ingredients is associated with a significant increase in the intestine length index, as well as the absorption area of the intestinal microvilli, with improved feeding efficiency of fish fed diets supplemented with plant ingredients compared to those fed without plant ingredients. This would demonstrate the phenotypic plasticity of carnivorous fish, such as *S. aurata*, in adapting to a diet with a higher proportion of plant protein, resulting in improved nutrient processing and assimilation [47]. Furthermore, these observations may suggest the positive impact of microalgae inclusion in the aquafeed formulation on product performance. The lack of significant differences in the MSI and lower HSI values observed in fish fed the M25-MC diet, as compared to the control diet, suggests that there is no additional accumulation of hepatic or perivisceral fat. This finding, in line with the observations of Molina-Roque et al. [11], indicates that the achieved growth is likely associated with fillet yield rather than an increase in perivisceral fat accumulation, which could potentially have adverse effects on consumers.

Regarding intermediary metabolism, a significant increase in plasmatic glucose and triglyceride levels was observed in seabream fed the M25-SA diet compared with fish fed control diet. This suggests that the M25-SA diet significantly enhanced carbohydrate and lipid metabolism, which could suggest a good metabolic condition, as aerobic glycolysis is predominant in obtaining high-energy biomolecules such as ATP and NADH Krebs's cycle [48]. Similarly, triglycerides are associated with mitochondrial energy mobilization, aerobically, next to carbohydrates [49]. The increase in plasma triglycerides in fish fed the M25-SA diet may be linked to higher intestinal bioaccessibility of nutrients, particularly fatty acids from microalgae [11]. Although no significant differences were shown in plasma circulating proteins, the *p*-values ($p = 0.053$) were close to the significance limit of $p < 0.05$. Proteins serve as an energy source and structural component for tissue development and growth [50,51]. Although fish fed the M25-SA diet exhibited the highest mean value, the lack of significant differences in plasma protein concentration between the experimental groups indicates a homeostatic balance at circulating levels. However, it could be interesting to highlight that higher levels of plasmatic proteins could be associated with higher growth parameters shown by fish fed M25-SA diet, resulting in high protein quality of microalgae.

The analysis of growth and metabolic results, together with the lower values of cortisol, a hormone associated with situations of stress prolonged [52] and whose low levels can stimulate protein synthesis resulting in better growth [53], shown by the fish fed M25-SA diet, could denote the suitability of microalgae inclusion in aquafeed for carnivorous cultured teleost, as *S. aurata*, in terms of nutrition and animal welfare.

Finally, it is important to address the impact of the COVID-19 pandemic during the execution of our feeding experiment, which took place between March and June 2020. The pandemic significantly affected our research operations, leading to a reduced workforce and limited access to facilities, making it challenging to include additional biological replicates in this study. While we fully acknowledge the importance of replicates for robust statistical analysis, the unique circumstances imposed by the pandemic made it impractical to incorporate them in this specific study. Despite the absence of biological replicates, we proceeded with this study using sufficient sample size and statistical power to maintain a level of confidence in the results, considering the limitations brought about by the pandemic. We carefully evaluated the experimental conditions and the available resources, and the decision to proceed without biological replicates was made in the interest of conducting this study under the prevailing circumstances. Although the results obtained are promising and provide valuable insights, we are fully aware that biological replicates are critical in scientific research to enhance data reliability and generalizability. The absence of biological replicates may potentially impact the robustness of our conclusions. Therefore, we emphasize the need for further trials with increased replicates to gain a more comprehensive understanding of the beneficial effects of microalgae inclusion on fish.

The gastrointestinal tract's microbial community in fish plays important functions, including vitamin production, nutrient distribution, regulation of innate immunity, and maintenance of intestinal tissue integrity [54]. These functions can be influenced and modulated by the fish's diet [55]. The isolation and characterization of native potential probiotics would enable the detection of positive benefits, e.g., in vitro antagonistic activity towards pathogens or extracellular enzyme production [56]. Probiotics are used in aquaculture as additives to enhance host health by improving feed utilization and digestion, enhancing digestive enzymatic activity, modulating intestinal microbiota, improving the immune system, and controlling fish diseases [57]. Moreover, probiotic bacteria originating from fish (autochthonous) are expected to exhibit superior performance compared to those obtained from terrestrial hosts when used in fish applications [58].

In this way, Chivotiya et al. [59] demonstrated that precise optimization of various components (e.g., urea, peptone, calcium chloride, magnesium sulfate, trace elements) in a specific medium can significantly enhance cellulase production from gut bacteria of tilapia fish. This optimization was achieved using the Plackett Burman design and carboxymethyl cellulose (CMC) as the substrate. Similarly, Yang et al. [60] determined the optimal growth conditions (culture media, temperature, and pH) for lactic acid bacteria (LAB) to optimize the production of bacteriocins, which were significantly different in MRS versus BHI. Therefore, the importance of isolating microorganisms from different culture media to develop an efficient technology for the optimized production of desired enzymatic and antagonistic activities is clear since native probiotics would establish within the original host more efficiently [61].

In this study, we screened 117 bacterial strains isolated from the gastrointestinal tract of seabream fed the M25-SA diet, aiming to identify indigenous candidate probiotics. Probiotics can produce different enzymes of biotechnological interest, in particular in the aquafeed industry as a feed additive for improving the digestion and absorption of different energy sources (e.g., carbon, lipids, proteins), and hydrolyzing different anti-nutritional factors (e.g., phytate, tannins, cellulase) present in the feeds [22,62]. Therefore, including enzyme-producing probiotics in feeds could enhance feed utilization in farmed species. That is the reason for assessing the enzymatic activity of the bacterial isolates.

Out of 117 isolates, 50, 26, and 11 were isolated on TSAs, MMA, and MRS media, respectively. Moreover, 30 isolates were spore formers that grew on TSAs media after

heat treatment. We found that 48% of the strains could hydrolyze proteins, 41% lipids, 77% collagen, and 30% starch, respectively. The hydrolysis of casein and collagen demonstrated the capacity to degrade proteins, in accordance with the reported ability of probiotics to enhance protease activity in various fish species [63,64]. Additionally, amylases, which are enzymes that break down carbohydrates into smaller molecules, such as glucose or maltose, were detected, potentially providing readily available nutrients for the host [65]. Tween-80 is an unsaturated fatty acid employed as a food additive (up to 1%) [66], and its hydrolyzation depends on lipase activity. The lipase activity has been increased by the application of probiotics [67,68]. Furthermore, the ability of the strains to metabolize some anti-nutritional factors was also investigated. The results showed that 46%, 8%, and 57% of isolates were able to metabolize phytate, tannins, and cellulose as only carbon and energy sources, respectively. Therefore, the respective isolates may likely improve the degradation of those anti-nutritional factors in the host's intestine, enhancing fish nutrition. In addition, in relation to the medium used to isolate microorganisms, we found that the percentage of isolates with enzymatic activities was higher in microorganisms isolated from TSAs and MMA. Thus, although the isolated strains in different media had an interesting enzymatic profile, the use of TSAs and MMA as isolation mediums could be the best option to isolate microorganisms with different enzymatic activities.

A relevant criterion in probiotic selection for aquaculture is their antimicrobial activity against fish pathogens [69]. Unlike chemotherapeutics, which have been utilized for disease treatment in aquaculture but often come with undesirable side effects and promote antibiotic-resistant bacteria, probiotics offer an alternative approach. Probiotics can interact with or antagonize other bacteria, resisting colonization and directly suppressing opportunistic infections, thus reducing their incidence [70]. Our study found that 38% of the isolates inhibited *V. anguillarum*; 47% inhibited *P. damselae* subsp. *piscicida,* and 56% of the isolates inhibited *T. maritimum*. The antimicrobial activity of the strains against these pathogens was evaluated due to the incidence of these pathogens in aquaculture and the economic losses they produce [71–73]. Thus, it is suggestive to think that using these isolates in aquaculture may limit chemotherapeutics against those pathogens, avoiding the impacts produced by the overuse of these products. Furthermore, although the number of microorganisms isolated on the MRS medium selected for antimicrobial activity was the lowest (only 3 isolates out of 32), it had the highest percentage of isolates with antimicrobial activity against the three fish pathogens. MRS is a selective growth medium for LAB species [74]. In this sense, some LAB species produce antimicrobial compounds (e.g., lactic acid, bacteriocins, etc.), which have been found to inhibit the growth of important aquaculture pathogens [75].

Considering the extracellular enzyme activity (hydrolysis of $\geq$four substrates) and the antagonism against the three pathogens tested, four bacterial isolates (UMA-140, UMA-143, UMA-169, isolated in TSAs; and UMA-216, isolated in MMA) were characterized as putative probiotics (Table 5). The four bacterial strains were identified as the *Bacillus* genus. The UMA-143 strain showed similarity to *Bacillus cereus*, while UMA-140, UMA-169, and UMA-216 showed homology with different strains of *Bacillus pumilus*. In this sense, the isolation of *B. cereus* and *B. pumilus* from different fish species has been previously reported [75–79]. *Bacillus* species are selected as probiotics to enhance digestive enzyme activities, given their effective metabolism of a diverse range of lipids, proteins, and carbohydrates [80,81]. The improvement in fish digestive enzyme activity following *Bacillus* administration might be associated with enzymes generated by the bacteria, or *Bacillus* could promote the development of indigenous enzymes in the fish [82]. In this sense, *Bacillus* species often modify the digestive enzymes protease, amylase, trypsin, and lipase [83], which is in agreement with the in vitro enzymatic activities exhibited by the four isolates from our study and is consistent with findings from other studies on *B. cereus* and *B. pumilus* strains [84,85]. Furthermore, the isolated strains could hydrolyze the phytate, and two of them the cellulose, which is in line with other *Bacillus* species [86], including *B. pumilus* [87] and *B. cereus* [76]. Thus, it could be suggestive to think that including those

strains in aquafeeds could enhance the feed digestibility and absorption by hydrolyzing those substrates present in the feeds and increasing the digestive enzymatic activity of the fish. Nevertheless, further in vivo experiments are necessary to assess this hypothesis.

Some *Bacillus* species can produce bacteriocins or other bioactive compounds with antagonistic effects against fish pathogens [88]. In this regard, the four *Bacillus* isolates were effective against the three tested fish pathogens. Previous studies found that fish-gut-isolated *Bacillus* species inhibited several fish pathogens, including *Tenacibaculum*, *Photobacterium*, and *Vibrio* species [89–91]. Thus, the antagonism reported in our study expands the potentiality of *Bacillus* species against vibriosis, photobacteriosis, and tenacibaculosis diseases, protecting the host against opportunistic bacteria.

Finally, aside from their functional characteristics, probiotics must undergo a safety examination, such as blood hemolytic activity, before being used in human or animal feeding [92]. Therefore, the hemolytic activity of the four selected strains was assessed on Columbia blood agar plates. Cells from UMA-140 and UMA-143 showed β-hemolytic activity, while cells from UMA-169 and UMA-216 had γ hemolytic, indicating negative or no hemolytic activity. The results are consistent with Cui et al. [93] and Bottone and Peluso [94], who reported hemolytic activity of *B. cereus* and *B. pumilus* strains, respectively. On the other hand, several studies have reported candidate probiotics with no hemolytic activity [95–97], which is crucial in ensuring the safety of probiotic selection. Thus, to ensure the safety of any potential application of the microorganisms, we would not recommend using strains UMA-140 and UMA-143 in any activity involving human or animal feeding.

5. Conclusions

In conclusion, both species demonstrated a favorable adaptation to the nutritional formulation employed in this study, especially *S. aurata*. Furthermore, 117 bacterial strains were isolated in different culture media from the gastrointestinal tract of gilthead seabream fed the microalgae blend and further characterized for their potential use as probiotics in aquaculture. According to our results, bacterial strains UMA-169 and UMA-216 (both identified as *Bacillus pumilus*) could be considered for their use in aquaculture as they might benefit host health by improving the digestion and absorption of different energy sources and by minimizing the colonization of pathogenic species.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes8080409/s1. Table S1. Hydrolytic activity of culturable bacterial strains isolated from *S. aurata*; Table S2. Hydrolytic and antimicrobial activities of selected culturable bacterial strains isolated from *S. aurata*.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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