Lactic acid bacteria increase the resistance of turbot larvae, *Scophthalmus maximus*, against pathogenic vibrio

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Abstract

Characteristics of Lactic Acid Bacteria (LAB) isolated from rotifers, *Brachionus plicatilis*, resembled those of *Lactobacillus plantarum* or *Carnobacterium* sp. It was investigated whether the artificial maintenance of a high concentration of LAB in rotifers might increase their dietary value for turbot larvae, particularly when the fish were infected with pathogenic *Vibrio* sp. One LAB strain was cultivated, then introduced daily into the enrichment medium of rotifers. In this way, LAB were retrieved in large amounts in turbot, and a significant limitation of larval mortality rate was observed when turbot were challenged with pathogenic vibrio at day 9. The inoculum concentration of LAB had a decisive effect on survival rate, and the optimum was between 10^7 and 2×10^7 Colony Forming Units (CFU) daily added per ml of the enrichment medium (53 % survival rate after 72 h of challenge, versus 8 % for the infected control group without LAB).

Keywords: Lactic acid bacteria, vibrio, pathogen, rotifer, experimental infection, larval fish culture, turbot, Pleuronectiformes.

Des bactéries lactiques améliorent la résistance des larves de turbot, Scophthalmus maximus, *contre un vibrion pathogène.*

Résumé

Les caractéristiques de bactéries lactiques isolées dans des rotifères, *Brachionus plicatilis*, ressemblent à celles décrites pour *Lactobacillus plantarum* ou *Carnobacterium* sp. La concentration de ces bactéries a été maintenue artificiellement élevée dans les rotifères, en vue d'évaluer leur effet sur des larves de turbot nourries avec ces animaux-proies, en particulier en cas d'infection par un vibrion pathogène. Une souche de bactérie lactique a été cultivée et introduite quotidiennement dans le milieu d'enrichissement des rotifères. De cette façon, les bactéries lactiques ont été retrouvées en grande quantité dans les larves, et cet enrichissement bactérien a permis de limiter significativement le taux de mortalité des larves soumises au jour 9 à une infection expérimentale avec un vibrion pathogène. La concentration des bactéries lactiques a un effet décisif sur le taux de survie, et l'effet optimum a été obtenu pour une addition quotidienne comprise entre 10^7 et 2×10^7 unités formant des colonies par ml de milieu d'enrichissement (53 % de survie 72 h après le début de l'infection, contre 8 % pour le groupe de contrôle infecté, mais n'ayant pas été enrichi avec les bactéries lactiques).

Mots-clés : Bactéries lactiques, vibrion, pathogène, rotifère, infection expérimentale, larve de turbot, Pleuronectiformes.

INTRODUCTION

Pathogenic Vibrionaceae may hinder mass production in fish hatchery. For instance, Kusuda *et al.* (1986) described the experimental infection of black sea bream fry by *Vibrio alginolyticus*, and several strains of vibrio have been reported to cause tissue necroses in flatfish larvae, e.g. enteritis in Japanese flounder (Masumura *et al.*, 1989), and damage in heart and skeletal muscle of halibut (Bergh *et al.*, 1992). In addition, rotifers have been suspected of contaminating first feeding larvae, and antibacterial treatments of live food organisms have been suggested (Gatesoupe, 1982; Tanasomwang and Muroga, 1989). The introduction of selected bacterial strains into the food chain have been proposed as an alternative treatment. Commercial preparations of live *Lactobacillus plantarum* and *Bacillus* spores decreased the amount of Vibrionaceae in rotifers fed with these additives (Gatesoupe, 1991*a*, 1993). *Bacillus* spores also improved the survival rate of turbot larvae experimentally infected with Vibrionaceae (Gatesoupe, 1993). Native bacteria with inhibitory effect against vibrios are especially promising, since they could colonize the gut of turbot (Olsson *et al.*, 1992). However, their proliferation must be kept under control, and good probiotic candidates might be Lactic Acid Bacteria (LAB) which have seldom been isolated from fish (Ringø *et al.*, in press). Thus, *L. plantarum* isolated from cod decreased and changed the flora associated with cod larvae (Strøm and Ringø, 1993). LAB have also been isolated in rotifers, and the aim of the present study therefore was to investigate their ability to improve bacterial environment and the resistance of turbot larvae against pathogen. In practice, a single bacterial strain was artificially maintained at high concentration in rotifers fed to turbot. Then bacteria were counted in turbot, and the larvae were challenged with a pathogenic vibrio, according to a new procedure (Gatesoupe, 1994).

MATERIAL AND METHODS

Lactic acid bacteria

LAB were isolated as a non-dominant part of the microflora of rotifers, *Brachionus plicatilis*. Under sterile conditions, rotifers were harvested on 80 μm net, rinsed and suspended in half-salinity water (18 $^{0}/_{00}$; aliquot volumes of distilled- and sea-water), according to Santos *et al.* (1993) who have used aged seawater (20 $^{0}/_{00}$) as diluent for marine bacteria. Then the rotifers were crushed in a glass homogenizer and the suspension was diluted with half-salinity water. The 10^{-3} and 10^{-4} dilutions were cultivated for 48 h at 30°C under anaerobic conditions on the lactobacillus agar of de Man, Rogosa and Sharpe (MRS agar, AES Laboratoire). Among Gram-positive, catalase-negative, non-sporing, and non-motile bacilli, one strain was selected because of its high growth rate on MRS medium. The carbohydrates fermented by this strain were determined with the Api 50 CHL system (Bio Mérieux; 48 h of incubation at 30°C). The strain was cultivated on a special broth designated as LMRS (from Diagnostics Pasteur, in g/l: peptone Pastose 10, peptone Pastone 10, yeast extract 5; from Merck, in g/l: D(+)-lactose 20, dipotassium hydrogen phosphate 2, sodium acetate 5, ammonium acetate 2, magnesium sulphate 0.2, manganese sulphate 0.05, Tween-80 1.08). Agar was added for counting plates (Pastagar B, Diagnostics Pasteur, 20 g/l). LMRS was derived from MRS, but lactose substituted for glucose, with a view to improving its selectivity, which was good when the plates were incubated for 48 h at 30°C under anaerobic conditions.

Larval rearing

Three batches of one-day-old turbot larvae, *Scophthalmus maximus*, were reared till day 10 after hatching in 150 l cylindrical tanks with conical bottoms (experiments 1-3). The rearing salinity was 35 $^{0}/_{00}$. The initial stocking density was 19, 25, and 26 larvae/l, in the three consecutive experiments, respectively. The initial temperature (14°C in experiments 1 and 3, 16°C in experiment 2) was gradually increased to 18°C by day 5 after hatching. The larvae were fed with rotifers from day 3 till day 9, at the daily rates of 20, 50, 75, 100, 150, 200 and 250 rotifers per initial larva, corresponding to the seven consecutive days of feeding.

Bacteria were counted in eight-day-old turbot larvae, after four days of incubation at 20°C on "Petrifilm Aerobic Count Plates" (3M Health Care), according to the method already described (Gatesoupe, 1994). They were also counted on LMRS agar (48 h of anaerobic incubation at 30°C) and Thiosulphate-Citrate-Bile Salt agar (TCBS, AES Laboratoire) dissolved in half-salinity water (48 h of incubation at 20°C).

The resistance of turbot was determined at day 9 by a challenge with a pathogen designated as vibrio P. The pathogenic strain corresponded to that previously described as "*Aeromonas hydrophila*" (Gatesoupe, 1991*b*) according to its Api 20 E profile (Bio Mérieux). However, it should be better designated as *Vibrio* sp. according to Santos *et al.* (1993), since it was sensitive to O/129 (2,4-diamino-6,7-di-*iso*propyl-pteridine, Diagnostics Pasteur), while it required NaCl for growth. Considering that the strain was arginine-dihydrolase-positive, lysine-decarboxylase-negative, and ornithine-decarboxylase-negative, it would belong to the third group described by Alsina and Blanch (1994), but this strain could not be further identified with the keys proposed by these authors. The challenge method was already described (Gatesoupe, 1994). Fifty larvae were gently poured into each challenge bottle, which contained 500 ml of seawater at 18°C. One bottle was used per tank, as a control without adding bacteria. Vibrio P was

inoculated in another bottle per tank in experiment 1, at the rate of 2×10^6 CFU/ml. This experimental infection was performed in duplicates per tank in experiment 2, at the rate of 10^6 CFU/ml. Triplicates were used in experiment 3 for the infection, at the rate of 4×10^5 CFU/ml. The survivors were counted after 48 h of incubation in experiment 1, and after 72 h in the two other experiments.

Rotifers

The rotifers were cultured in seawater, according to the method already described (Gatesoupe, 1991*a*). They were fed with baker's yeast, menhaden oil, soybean lecithin, DL-α-tocopherol, and vitamin premix (Spyridakis *et al.*, 1988), at the daily rates of 60.8, 7.7, 0.7, 0.015 and 0.7 mg/l, respectively, on a dry matter basis. When LAB were added to the rotifer culture medium, an inoculum of about 4×10^5 CFU/ml was introduced. In practice, 15 ml of the bacterial culture incubated for 24 h at 30°C in LMRS broth were poured once a day into the 150-l rotifer tank, after its daily renewal of seawater.

For feeding the larvae, rotifers were transferred daily from their culture medium into clean seawater tanks (200 rotifers/ml), where they were enriched with spray-dried fish autolysate, menhaden oil, soybean lecithin, and DL-α-tocopherol, at the rates of 15, 7.7, 0.7 and 0.015 mg/l, respectively, on a dry matter basis. Meanwhile, LAB inoculum was added once a day to the rotifer enrichment media for experimental treatments. It corresponded to an initial LAB concentration in these enrichment media decreasing from 4×10^7 to 3×10^6 CFU/ml during the seven days of feeding in experiment 1, whereas this initial concentration decreased from 2×10^7 to 10^6 CFU/ml in experiment 2 (*fig.* 1). The effect of this initial concentration was investigated in the five treatments of experiment 3, where the lowest level of this concentration was between 6×10^6 and 10^7 CFU/ml (treatment 1), and the highest one was between 3×10^7 and 5×10^7 CFU/ml (treatment 5). The rotifers were continuously distributed to turbot with a peristaltic pump, the flow rate of which was adjusted so that the whole volume was carried through in 24 h.

Experimental design

In the first two experiments, five replicates were fed with rotifers cultured, then enriched with LAB

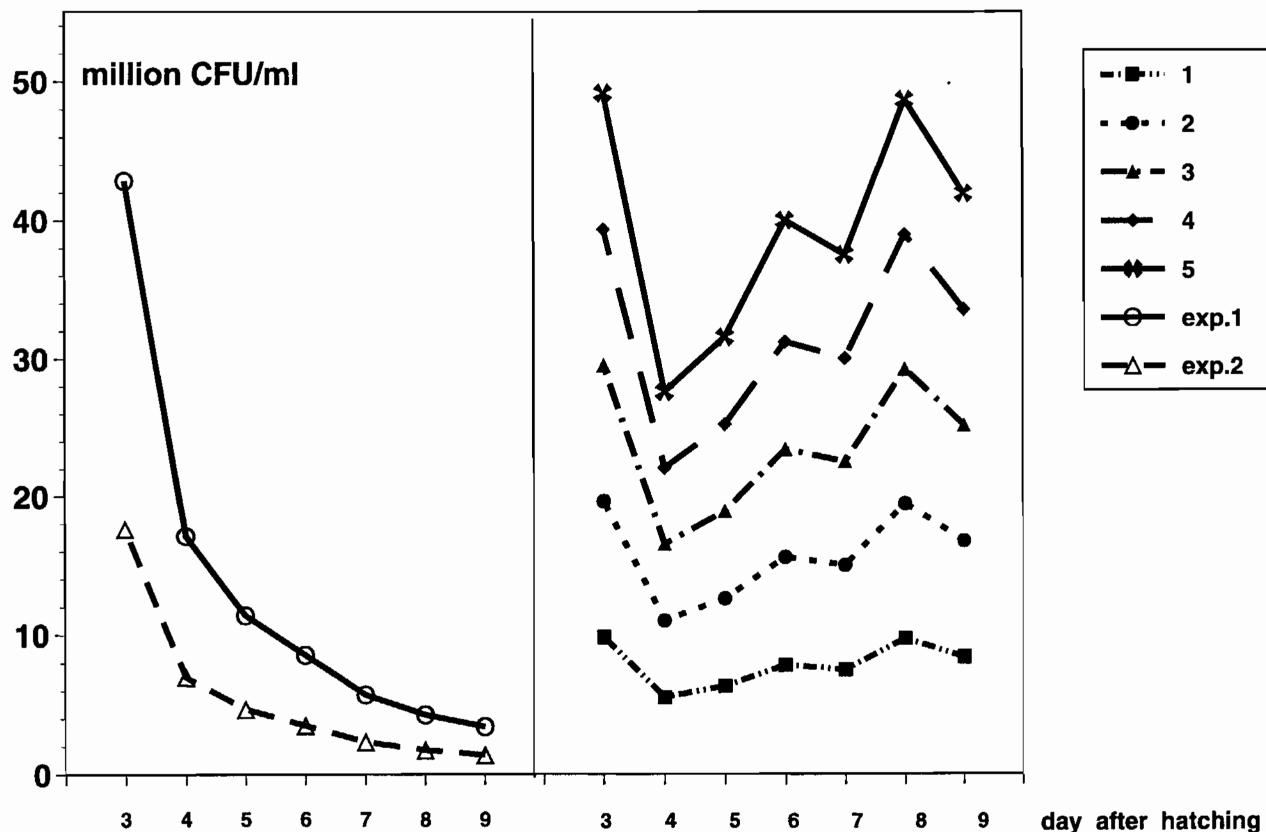

Figure 1. – Daily amounts of lactic acid bacteria, LAB (million CFU) introduced per ml of rotifer enrichment medium for treatments BB and CB of experiments 1-2 (left), and treatments 1-5 of experiment 3 (right).

(treatment BB), whereas five other tanks were given the control rotifers without LAB (treatment CC). In experiment 2, four other replicates were given rotifers cultured with LAB, but enriched without them (treatment BC), while the last four tanks were fed with rotifers enriched with LAB, but cultured without them (treatment CB). In the third experiment, treatment CC was compared in triplicates to five other groups of triplicates, where the rotifers were not cultured with LAB, but enriched with five different levels of the bacteria (treatments 1-5 in *fig.* 1). The data obtained in each experiment were compared by analysis of variance, and when there was a significant difference, the effects of the individual treatments were assessed by an a priori test (Sokal and Rohlf, 1969).

RESULTS

The lactic acid bacterium fermented L-arabinose, ribose, galactose, D-glucose, D-fructose, D-mannose, rhamnose, mannitol, sorbitol, α-methyl-D-mannoside, N-acetyl-glucosamine, amygdalin, arbutin, esculin, salicin, cellobiose, maltose, lactose, melibiose, saccharose, trehalose, melezitose, D-raffinose, β-gentiobiose, and gluconate. This Api 50 CH profile could correspond to *Lactobacillus plantarum*, and this strain might be closely related to some others, already isolated from fish (Schrøder *et al.*, 1980). However, considering that the growth of this strain was depressed on a special MRS agar, the pH of which was adjusted at 5.5 with acetic acid, it might be designated as *Carnobacterium* sp., based on Holzapfel (1992).

The introduction of LAB into the enrichment medium of the rotifers increased by a hundred times the colonies isolated from the larvae and counted on LMRS agar in the first two experiments, but these counts were higher in experiment 1 than in experiment 2 (*table* 1). No colony was detected on LMRS agar for the control group of experiment 3 (*i.e.* less than 9 CFU per larva), whereas amounts increasing from 10^4 to 10^5 CFU per larva were retrieved in the groups fed with the increasing levels of LAB. The effects of treatments on other bacterial counts were not significant (about 10^5 and 10^4 CFU per larva counted on Petrifilm and TCBS plates, respectively). The amount of LAB was at the same range as that of the vibrios growing on TCBS agar dissolved in half-salinity water in the experimental group of the first experiment. This was not observed in the second experiment, where the concentration of LAB introduced into the enrichment medium was very low. In the experimental groups of experiment 3, the lowest LAB level was again at the same range as the TCBS-growing colonies, while the highest level was at the same range as Petrifilm aerobic flora.

The addition of LAB had no significant effect on growth and survival rates of turbot reared in normal conditions. However, it was possible to observe significant differences in the survival rates of the challenge tests (*tables* 2, 3 and 4). In the last two experiments, the mortality peak caused by the vibrio was observed later than in the first experiment (72 versus 48 h). The enrichment of the rotifers with LAB (treatments CB, BB, and 1-5) improved significantly the survival rate of turbot challenged with vibrio P, and the maximum effect was observed with treatments 2-5, where about 50 % of turbot survived after 72 h of challenge. Consequently, it seemed that LAB concentrations greater than 10^7 CFU per ml of enrichment medium (*fig.* 1) were necessary to obtain the maximum protection effect. Lastly, the survival rate of the non-infected groups was significantly decreased with the highest LAB concentrations (treatments 3-5), in comparison with treatment CC in experiment 3 (*table* 4). The optimum LAB concentration corresponded therefore to treatment 2 in experiment 3 (*i.e.* between 10^7 and 2×10^7 CFU/ml of enrichment medium).

Table 1. – Bacteria counted in eight-day-old turbot larvae on Petrifilm, TCBS and LMRS plates (CFU/larva; n.d.: not detected).

Treament*	Petrifilm	TCBS	LMRS
Experiment 1			
CC	9×10^4	2×10^4	3×10^2
BB	8×10^4	2×10^4	2×10^4
Experiment 2			
CC	2×10^5	5×10^4	20
BC	2×10^5	9×10^4	50
CB	2×10^5	5×10^4	4×10^3
BB	2×10^5	7×10^4	5×10^3
Experiment 3			
CC	10^5	7×10^3	n.d.
1	6×10^4	7×10^3	2×10^4
2	7×10^4	2×10^4	4×10^4
3	7×10^4	2×10^4	6×10^4
4	10^5	10^4	5×10^4
5	7×10^4	10^4	9×10^4

* CC: control without LAB, BB: rotifers cultured then enriched with LAB, BC: rotifers cultured but not enriched with LAB, CB: rotifers enriched but not cultured with LAB, 1-5: same treatment as CB but with five levels of LAB inoculum (see *fig.* 1).

Table 2. – Survival rates after 48 h of nine-day-old turbot larvae challenged with vibrio P in experiment 1 (%; ± standard errors of the 5 replicates; superscripts indicate significant differences; $p < 0.001$).

Treament*	CC	BB
not infected	$90^a \pm 4$	$98^a \pm 1$
infected	$13^c \pm 4$	$28^b \pm 6$

* CC: control without LAB, BB: rotifers cultured then enriched with LAB.

Table 3. – Survival rates after 72 h of nine-day-old turbot larvae challenged with vibrio P in experiment 2 (%; ± standard errors; n = number of replicates; superscripts indicate significant differences; $p < 0.001$).

Treament*	CC	BC	CB	BB
not infected	$88^{a} \pm 2$ ($n = 5$)	$92^{a} \pm 3$ ($n = 4$)	$93^{a} \pm 2$ ($n = 4$)	$94^{a} \pm 2$ ($n = 5$)
infected	$19^{c} \pm 1$ ($n = 10$)	$17^{c} \pm 2$ ($n = 8$)	$22^{b} \pm 2$ ($n = 8$)	$23^{b} \pm 2$ ($n = 10$)

* CC: control without LAB, BC: rotifers cultured but not enriched with LAB, CB: rotifers enriched but not cultured with LAB, BB: rotifers cultured then enriched with LAB.

Table 4. – Survival rates after 72 h of nine-day-old turbot larvae challenged with vibrio P in experiment 3 (%; ± standard errors; n = number of replicates; superscripts indicate significant differences; $p < 0.001$).

Treament*	CC	1	2	3	4	5
not infected ($n = 3$)	$87^{a} \pm 4$	$81^{ab} \pm 6$	$81^{ab} \pm 7$	$79^{b} \pm 3$	$74^{b} \pm 2$	$74^{b} \pm 2$
infected ($n = 9$)	$8^{e} \pm 1$	$17^{d} \pm 1$	$53^{c} \pm 3$	$55^{c} \pm 3$	$46^{c} \pm 2$	$55^{c} \pm 2$

* CC: control without LAB, 1-5: rotifers enriched with five levels of LAB inoculum (see *fig.* 1).

DISCUSSION

It is important to compare the survival rates obtained with the experimental infection to those obtained with a simple transfer of the larvae. This transfer treatment may be considered as an "activity test" (also called "handling test"; Kitajima *et al.*, 1979), although the larvae were not netted out from water, but gently poured into seawater bottles. Indeed in the third experiment, 72 h after transfer without vibrio, the survival rate of the control group without LAB was greater than those obtained with the highest three LAB levels in the enrichment medium. This is an indication that the artificial maintenance of LAB at the highest concentration in turbot might be detrimental when the larvae were transferred into a closed medium. However, the most important result is the improvement of the survival rate of turbot challenged with vibrio P when the rotifers were enriched with LAB. This improvement depended also on LAB concentration in the enrichment medium, and there was a narrow margin for the optimum concentration, which should be greater than 10^7 CFU/ml for maximum resistance against vibrio, and less than 2×10^7 CFU/ml for maximum survival in closed medium.

The native LAB were maintained in turbot larvae by the continuous distribution of enriched rotifers. This was different from what was observed with alien *Bacillus*, which were quickly inactivated by rotifers, while only a few colonies were retrieved in turbot larvae (Gatesoupe, 1993). The amount of LAB in turbot might be as high as that of TCBS-growing vibrios, or even Petrifilm aerobic flora, depending on the inoculum range. The hypothesis that LAB would act as barrier flora against pathogenic vibrio may therefore be considered. However, no decrease of the larval flora was observed with the experimental treatments, at variance with the results obtained by Strøm and Ringø (1993) with non-fed cod larvae.

The artificial maintenance of LAB did not affect the total counts of the vibrios growing on TCBS agar, but it may be thought that it curbed the invasion of turbot by the pathogen. There are many reports about the antibacterial activity of LAB, but most against Gram-positive bacteria (reviewed by Piard and Desmazeaud, 1992). Nevertheless, Lewus *et al.* (1991) have shown the activity against *A. hydrophila* of 19 LAB strains including *Carnobacterium piscicola* and *L. plantarum*. The latter species has been also reported to inhibit the growth of "gut bacteria" in dead fish, and to decrease the respiration rate of *Vibrio* sp. (Schrøder *et al.*, 1980). Besides the production of antimicrobial substances, a great variety of mechanisms have been proposed for the action of probiotics (Montes and Pugh, 1993), *e.g.* competition for adhesion receptors in the intestine, competition for nutrients, and immunostimulation. Further investigation is required before speculating on the actual mechanisms in the present case.

There was no further advantage to introduce LAB into the culture medium of the rotifers. In particular, the mean weight of turbot was not improved by this treatment, contrary to what was observed with commercial preparations (Gatesoupe, 1991*a*). Finally, since the LAB strain was selected in the present study only because it was easy to cultivate, others should be tested for their probiotic effects.

REFERENCES

Alsina M., A. R. Blanch, 1994. A set of keys for biochemical identification of environmental *Vibrio* species. *J. Appl. Bacteriol.*, **76,** 79-85.

Bergh O., G. H. Hansen, R. E. Taxt, 1992. Experimental infection of eggs and yolk sac larvae of halibut, *Hippoglossus hippoglossus* L. *J. Fish Dis.*, **15,** 379-391.

Gatesoupe F. J., 1982. Nutritional and antibacterial treatments of live food organisms: the influence on survival, growth rate and weaning success of turbot (*Scophthalmus maximus*). *Ann. Zootech.*, **31,** 353-368.

Gatesoupe F. J., 1991*a.* The effect of three strains of lactic bacteria on the production rate of rotifers, *Brachionus plicatilis*, and their dietary value for larval turbot, *Scophthalmus maximus. Aquaculture*, **96,** 335-342.

Gatesoupe F. J., 1991*b.* Experimental infection of turbot, *Scophthalmus maximus* (L.), larvae with a strain of *Aeromonas hydrophila. J. Fish Dis.*, **14,** 495-498.

Gatesoupe F. J., 1993. *Bacillus* sp. spores as food additive for the rotifer *Brachionus plicatilis*: improvement of their bacterial environment and their dietary value for larval turbot, *Scophthalmus maximus* L. *In*: Fish Nutrition in Practice, S. J. Kaushik, P. Luquet, eds., Institut National de la Recherche Agronomique, Paris, Les Colloques, **61,** 561-568.

Gatesoupe F. J., 1994. Early assessment of the quality of turbot larvae, *Scophthalmus maximus* L.: bacterial aspects. *In*: Measures for Success, J. Muir and F. Sevila, eds., European Aquaculture Society, Oostende, Belgium, Spec. Publ., **21,** 124-125.

Holzapfel W. H., 1992. Culture media for non-sporulating Gram-positive food spoliage bacteria. *Int. J. Food Microbiol.*, **17,** 113-133.

Kitajima C., S. Fujita, F. Ohwa, Y. Yone, T. Watanabe, 1979. Improvement of dietary value for red sea bream larvae of rotifers *Brachionus plicatilis* cultured with baker's yeast *Saccharomyces cerevisiae. Bull. Jpn. Soc. Sci. Fish.*, **45,** 469-471.

Kusuda R., J. Yokoyama, K. Kawai, 1986. Bacteriological study on cause of mass mortalities in cultured black sea bream fry. *Bull. Jpn. Soc. Sci. Fish.*, **52,** 1745-1751.

Lewus C. B., A. Kaiser, T. J. Montville, 1991. Inhibition of food-borne bacterial pathogens by bacteriocins from lactic acid bacteria isolated from meat. *Appl. Environ. Microbiol.*, **57,** 1683-1688.

Masumura K., H. Yasunobu, N. Okada, K. Muroga, 1989. Isolation of a *Vibrio* sp., the causative bacterium of intestinal necrosis of Japanese flounder larvae. *Fish Pathol.*, **24,** 135-141.

Montes A. J., D. G. Pugh, 1993. The use of probiotics in food-animal practice. *Vet. Med.*, **88,** 282-288.

Olsson J. C., A. Westerdahl, P. L. Conway, S. Kjelleberg, 1992. Intestinal colonization potential of turbot (*Scophthalmus maximus*)- and dab (*Limanda limanda*)-associated bacteria with inhibitory effects against *Vibrio anguillarum. Appl. Environ. Microbiol.*, **58,** 551-556.

Piard J. C., M. Desmazeaud, 1992. Inhibiting factors produced by lactic acid bacteria. 2. Bacteriocins and other antibacterial substances. *Lait*, **72,** 113-142.

Ringø E., E. Strøm, J. A. Tabachek, *in press.* Intestinal microflora of salmonids. *Aquac. Fish. Manage.*

Santos Y., J. L. Romalde, I. Bandin, B. Magarinos, S. Nunez, J. L. Barja, A. E. Toranzo, 1993. Usefulness of the Api-20E system for the identification of bacterial fish pathogens. *Aquaculture*, **116,** 111-120.

Schrøder K., E. Clausen, A. M. Sandberg, J. Raa, 1980. Psychotrophic *Lactobacillus plantarum* from fish and its ability to produce antibiotic substances. *In*: Advances in Fish Science and Technology, J. J. Connell ed., Fishing News Books Ltd, Farnham, Surrey, UK, 480-483.

Sokal R. R., F. J. Rohlf, 1969. Biometry, Freeman and Co., San Francisco, CA, USA, 776 p.

Spyridakis P., J. Gabaudan, R. Métailler, J. Guillaume, 1988. Digestibilité des protéines et disponibilité des acides aminés de quelques matières premières chez le bar (*Dicentrarchus labrax*). *Reprod. Nutr. Dév.*, **28,** 1509-1517.

Strøm E., E. Ringø, 1993. Changes in the bacterial composition of early developing cod, *Gadus morhua* (L.) larvae following inoculation of *Lactobacillus plantarum* into the water. *In*: Physiological and Biochemical Aspects of Fish Development, B. T. Walther and H. J. Fyhn, eds., Univ. Bergen, Zoological Institute, Bergen, Norway, 226-228.

Tanasomwang V., K. Muroga, 1989. Effects of sodium nifurstyrenate and tetracycline on the bacterial flora of rotifers (*Brachionus plicatilis*). *Fish Pathol.*, **24,** 29-35.