Planococcus maritimus ML1206 Isolated from Wild Oysters Enhances the Survival of *Caenorhabditis elegans* against *Vibrio anguillarum*

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Method

***1. C. elegans* Length Determination**

Ten synchronized *C. elegans* N2 in L1 stage were transferred to NGM medium coated with OP50 and ML1206, respectively. *C. elegans* were cultured at 20 ℃ to the 2-days adult stage. *C. elegans* were picked to slides covered with agarose, anesthetized with levamisole hydrochloride, and placed under a stereomicroscope to measure body length with a micrometer. The head (oral position) of the *C. elegans* was taken as the starting point, and following the middle part of the body to the tail part of the *C. elegans* was taken as the measurement endpoint. NIS elements software was used to calculate the body of the *C. elegans* body [1]. The test was performed three times.

***2. C. elegans* Motility Determination**

Referring to the methods of Tsalik and Hobert[2], the effect of ML1206 on *C. elegans* motility was tested. Ten synchronized *C. elegans* N2 in L1 stage were transferred to NGM medium coated with OP50 and ML1206, respectively. *C. elegans* were cultured at 20 ℃ to the 2-days adult stage. Then the *C. elegans* were transferred to new NGM plates. After the free movement of *C. elegans* in the first minute, the bending times of the *C. elegans* within 30 s were observed and recorded under a stereoscopic microscope as the indicator of bending frequency. One bending of the body is defined as a change in the movement of the body along the corresponding X-axis during *C. elegans* crawling, assuming that the direction along the pharyngeal pump is the Y-axis[3]. The test was performed three times.

***3. C. elegans* Pharyngeal Pumping Experiment**

Ten synchronized *C. elegans* N2 in L1 stage were transferred to NGM medium coated with OP50 and ML1206, respectively. *C. elegans* were cultured at 20 ℃ to the 2-days adult stage. Then the *C. elegans* were transferred to new NGM plates, the pump activity of *C. elegans* pharyngeal terminal bulb was observed through stereomicroscope (×400), and the number of pharyngeal pump activities within 1 min was recorded, the swallowing rate of *C. elegans* [1]. The test was performed three times.

***4. C. elegans* Reproductive Capacity Determination**

Ten synchronized L1 N2 were selected to NGM medium coated with OP50 and ML1206, respectively. One plate was for one worm and kept *C. elegans* lay eggs at 20 ℃. The parent *C. elegans* were transferred to a new NGM medium coated with OP50 and ML1206 every 12 h, and the number of eggs in each old culture dish was recorded until the *C. elegans* stopped laying eggs. The sum of eggs in plates of each group was the total number of eggs laid by 10 *C. elegans*, and the averages of offspring produced by each *C. elegans* were calculated to characterize the reproductive capacity of the parent *C. elegans*[1]. The test was performed three times.

Results

Table Sl. Stains from the intestine of oyster and perch

number	Stain number	Putative identity
1	M0101	*Pseudoalteromonas haloplanktis* 98.2%

2	M0102	*Marinobacter hydrocarbonoclasticus* 98.8%
3	M0103	*Psychrobacter celer* 98.8%
4	M0104	*Pseudoalteromonas nigrifaciens* 98.1%
5	M0106	*Lacinutrix undariae* 98.0%
6	M0107	*Pseudoalteromonas translucida* 98.2%
7	M1201	*Bacillus siamensis* 98.0%
8	ML1202	*Bacillus tequilensis* 98.9%
9	ML1206	*Planococcus maritimus* 99.2%
10	ML1209	*Pseudoalteromonas fuliginea* 99.2%
11	ML1210	*Sulfitobacter pontiacus* 98.3%
12	ML1211	*Olleya algicola* 99.9%
13	ML1212	*Bacillus flexus* 98.8%
14	ML1216	*Bacillus firmus* 97.5%
15	ML1224	*Pseudoalteromonas neustonica* 97.3%
16	ML1226	*Pseudovibrio japonicus* 99.0%
17	ML1227	*Bacillus altitudinis* 98.4%
18	ML1229	*Ruegeria atlantica* 99.3%
19	YLY02	*Vibrio alfacsensis* 99.5%
20	YLY03	*Sunxiuqinia elliptica* 99.1%
21	YLY04	Maribius pontilimi 95.33%
22	YLY05	*Idiomarina sediminum* 98.9%
23	YLY06	*Vibrio orientalis* 100%
24	YLY07	*Brumimicrobium mesophilum* 95.2%
25	YLY08	*Oceaniglobus indicus* 93.4%
26	YLY09	*Nitratireductor aquimarinus* 98.1%
27	YLY10	*Microbacterium esteraromaticum* 98.1%
28	YLY13	*Bacillus halosaccharovorans* 98.8%
29	YLY14	*Roseovarius pacificus* 99.6%
30	YLY17	*Shewanella indica* 98.6%
31	YLY18	*Vibrio atypicus* 99.9%
32	YLY20	*Ornithinimicrobium kibberense* 95.7%
33	YLY21	*Litoreibacter arenae* 99.8%
34	YLY22	*Halobacillus alkaliphilus* 97.8%
35	YLY23	*Jeotgalibacillus campisalis* 99.0%
36	YLY25	*Marinobacter pelagius* 98.9%
37	YLY26	*Stappia stellulata* 99.1%
38	YLY27	*Idiomarina aestuarii* 99.4%
39	YLY28	*Roseovarius halotolerans* 98.9%
40	YLY29	*Shimia biformata* 97.8%
41	YLY32	*Halomonas aestuarii* 96.8%

Table S2. Hemolysis of strains

Strain number	Hemolysis	Strain number	Hemolysis
M0101	+	YLY06	-
M0102	-	YLY07	-
M0103	+	YLY08	-
M0104	+	YLY09	-
M0106	+	YLY10	-
M0107	+	YLY13	+
ML1201	+	YLY14	+
ML1202	+	YLY17	+
ML1206	-	YLY18	+
ML1209	+	YLY20	-
ML1210	-	YLY21	-
ML1211	+	YLY22	+
ML1212	+	YLY23	+
ML1216	+	YLY25	-
ML1224	+	YLY26	-
ML1226	+	YLY27	-
ML1227	+	YLY28	-
ML1229	-	YLY29	+
YLY02	-	YLY32	-
YLY03	-	SDUM002245	+
YLY04	-	LGG	-
YLY05	-		

Note: "+" means positive; "-"stands means negative

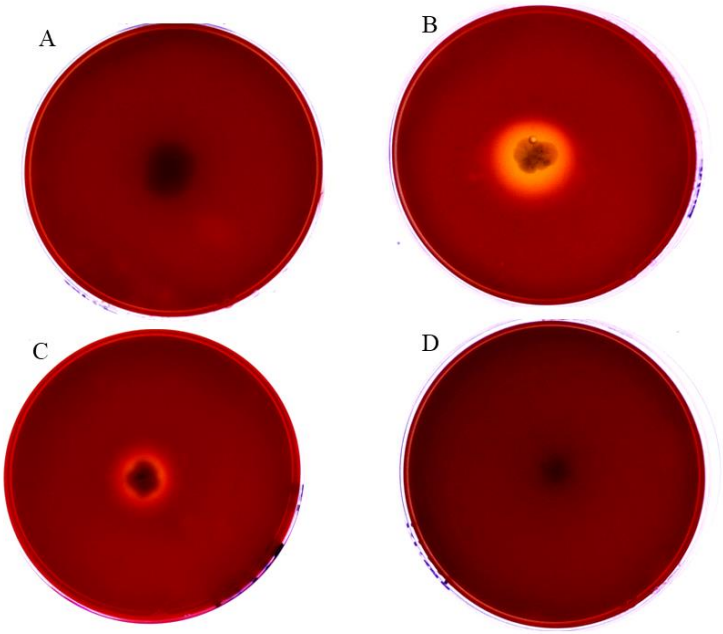

Figure S1. Hemolysis of some strains on 5% sheep blood agar (partial)

Note: A, strain ML1206; B, strain YLY14; C, control (*V. anguillarum*); D, negative control (strain LGG)

Table S3 Low pH tolerance ability of twenty intestinal bacteria (Mean±SD)

Strain number	OD_{600} value		Acid-resistant survival rate（%）
	pH 7.4 (pH5.6 for LGG)	pH3.0	

M0102	0.49±0.03^{cd,A}	0.05±0.05^{gh,B}	10.30±0.02^{gf}
ML1206	0.47±0.03^{cde,A}	0.37±0.02^{c,A}	79.51±0.03^{b}
ML1210	0.41±0.02^{defg,A}	0.07±0.05^{gh,A}	16.33±0.02^{gf}
ML1229	0.68±0.02^{b,A}	0.08±0.01^{gh,A}	11.80±0.02^{gf}
YLY02	0.44±0.04^{deg,A}	0.06±0.00^{gh,A}	13.73±0.01^{gf}
YLY03	0.81±0.02^{a,A}	0.09±0.02^{g,A}	11.12±0.02^{gf}
YLY04	0.38±0.03$^{fg.A}$	0.07±0.01^{gh,A}	18.45±0.04^{gf}
YLY05	0.45±0.02^{de,A}	0.28±0.03^{e,A}	61.14±0.05^{e}
YLY06	0.68±0.01^{b,A}	0.13±0.01^{f,A}	18.69±0.02^{gf}
YLY07	0.39±0.06^{efg,A}	0.06±0.01^{gh,B}	16.67±0.03^{gf}
YLY08	0.20±0.01^{j,A}	0.05±0.01^{i,A}	23.89±0.04^{f}
YLY09	0.38±0.03^{gh,A}	0.21±0.03^{e,A}	54.62±0.04^{d}
YLY10	0.32±0.02^{hi}	0.17±0.01^{e}	53.77±0.05^{d}
YLY20	0.43±0.02^{defg}	0.07±0.01^{gh}	16.91±0.01^{gf}
YLY21	0.45±0.01^{def}	0.05±0.01^{gh}	11.77±0.01^{gf}
YLY25	0.31±0.03^{i,A}	0.26±0.02^{d,A}	85.24±0.04^{b}
YLY26	0.29±0.01^{i,A}	0.07±0.01^{gh,A}	25.02±0.02^{f}
YLY27	0.30±0.02^{i,A}	0.07±0.00^{gh,B}	23.74±0.02^{f}
YLY28	0.44±0.02^{de,A}	0.06±0.00^{gh,B}	13.57±0.01^{gf}
YLY32	0.76±0.06^{a,A}	0.49±0.04^{b,A}	65.56±0.10^{c}
LGG	0.52±0.03^{c,A}	0.62±0.02^{a,A}	118.71±0.02^{a}

Note: different lowercase letters on superscript represent significant differences between different strains ($p< 0.05$); Different capital letters represent significant differences under different pH ($p<0.05$).

Table S4. Bile salt tolerance ability of twenty intestinal bacteria (Mean±SD)

Strain number	D_{600} value		Bile salt tolerance survival rate（%）
	Bile salt 0.0%	Bile salt 0.3%	
M0102	0.41±0.08^{ghij,A}	0.07±0.01^{ghi,B}	17.75±0.03^{efg}
ML1206	0.70±0.02^{c,A}	0.57±0.03^{a,A}	81.84±0.04^{ab}
ML1210	0.46±0.04^{fgh,A}	0.07±0.01^{ghi,B}	15.96±0.01^{efgh}
ML1229	0.46±0.02^{fgh,A}	0.06±0.01^{hi,B}	12.46±0.01^{fgh}
YLY02	0.34±0.02^{jkl,A}	0.20±0.02^{e,A}	57.06±0.06^{c}
YLY03	0.42±0.02^{ghi,A}	0.10±0.01^{g,A}	24.91±0.03^{e}
YLY04	0.57±0.02^{de,B}	0.05±0.01^{hi,B}	9.29±0.01^{h}
YLY05	0.51±0.01^{ef,A}	0.38±0.03^{b,A}	74.45±0.04^{b}
YLY06	0.70±0.02^{c,A}	0.56±0.03^{a,A}	79.60±0.03^{ab}
YLY07	0.32±0.02^{l,B}	0.04±0.01^{hi,B}	13.46±0.02^{fgh}
YLY08	0.32±0.02^{l,B}	0.04±0.00^{hi,B}	12.56±0.01^{fgh}
YLY09	0.43±0.02^{gh,A}	0.27±0.03^{c,A}	63.90±0.07^{c}
YLY10	0.33±0.04^{kl,A}	0.04±0.01^{i,B}	11.32±0.03^{fgh}
YLY20	0.39±0.03^{hijkl,A}	0.05±0.01^{hi,B}	13.01±0.03^{fgh}
YLY21	0.40±0.02^{ghijk,A}	0.08±0.01^{gh,B}	20.16±0.03^{ef}
YLY25	0.45±0.05^{fgh,A}	0.15±0.02^{f,A}	35.12±0.07^{d}
YLY26	0.80±0.01^{b,A}	0.06±0.00^{hi,B}	7.50±0.01^{h}
YLY27	0.60±0.01^{d,A}	0.04±0.00^{hi,B}	6.67±0.00^{h}
YLY28	0.47±0.03^{fg,A}	0.05±0.01^{hi,B}	11.45±0.01^{fgh}
YLY32	0.97±0.03^{a,A}	0.23±0.04^{d,A}	24.10±0.04^{e}
LGG	0.35±0.02^{ijkl,A}	0.30±0.02^{c,A}	87.42±0.05^{a}

Note: different lowercase letters on superscript represent significant differences between different strains ($p < 0.05$);

Different capital letters represent significant differences under different bile salt concentration values ($p<0.05$).

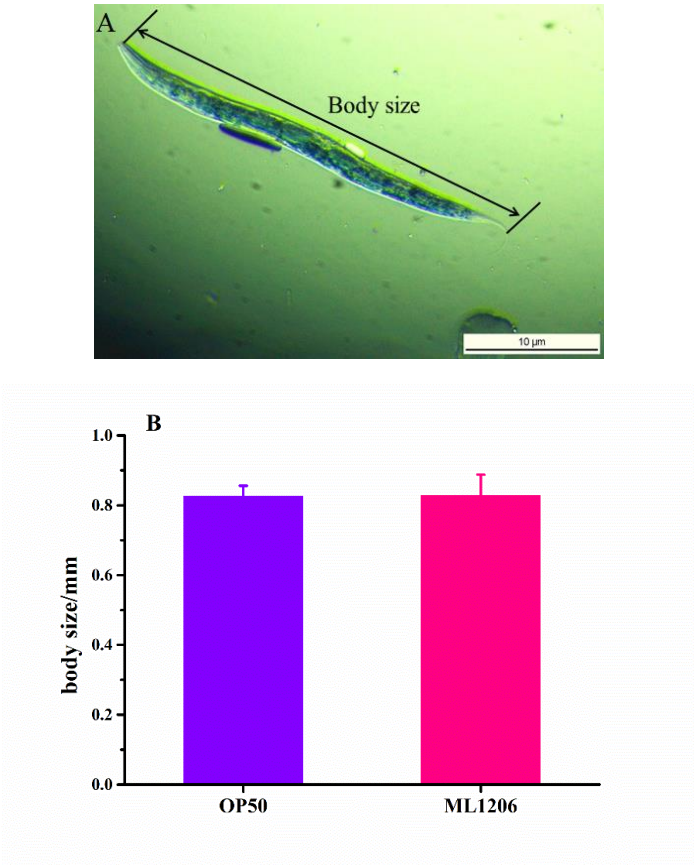

Figure S2. Effect of ML1206 strain on *C. elegans* growth: (a) Schematic diagram of *C. elegans* body length calculated with NIS elements software (b) Worms were grown in the presence of OP50 or ML1206 to the 2-day adult stage *C. elegans*. After anesthesia with levamisole hydrochloride, their body length was measured under a stereoscopic microscope (×400) with a micrometer. Data are representative of three independent experiments and presented as means ± SD.

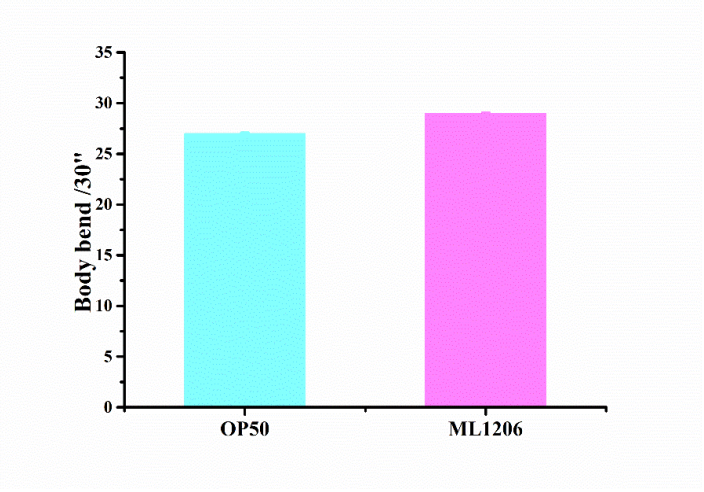

Figure S3. Effect of ML1206 strain on *C. elegans* body bending frequency: Worms were grown in the presence of OP50 or ML1206 to the 2-day adult stage *C. elegans*, and the body bending times of *C. elegans* within 30 S were recorded under a stereoscopic microscope (×400). Data are representative of three independent experiments and presented as means ± SD.

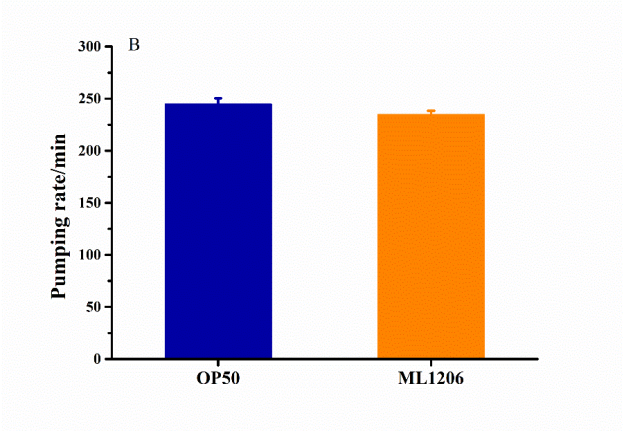

Figure S4. Effect of ML1206 strain on *C. elegans* pharyngeal pumping rate: Worms were grown in the presence of OP50 or ML1206 to the 2-day adult stage *C. elegans*. The worm was recorded within 1 min. Data are representative of three independent experiments and presented as means ± SD.

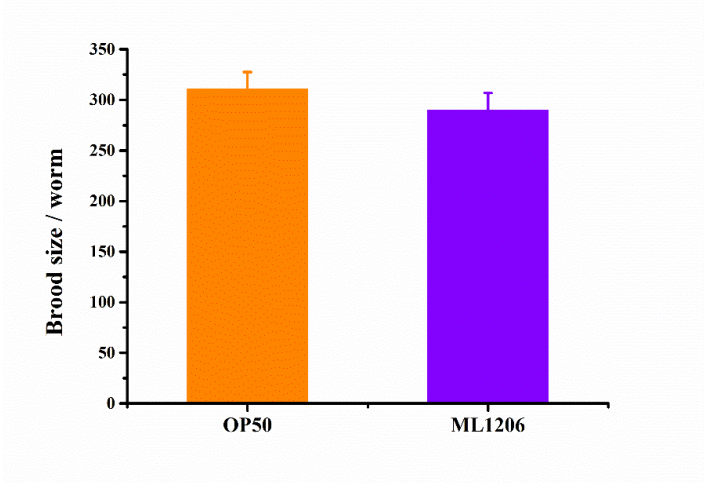

Figure S5. Effect of ML1206 strain on *C. elegans* reproduction ability: Average egg production per worm fed with OP50 or ML1206. Data are representative of three independent experiments and presented as means ± SD.

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