

Properties of acid- and aluminum-tolerant *Flavobacterium* ST-3991

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ABSTRACT

Although aluminum (Al) and manganese (Mn) are present in a neutral form in soils, these metals are readily solubilized at low pH and can be toxic to plants and microorganisms. We previously isolated an acid- and Al-tolerant bacterium, *Flavobacterium* ST-3991, which increases the pH of culture medium. In this study, we further examined the growth of strain ST-3991 under acidic and high-Mn conditions to determine the substance increasing the pH, its properties of acid- and Mn-tolerance, and mechanism of increasing pH, and tolerance to Mn^{2+} . The results provide evidence that strain ST-3991 is a unique bacterial strain with both acid and Mn^{2+} tolerance, and raises the pH of culture medium by producing ammonia under acidic conditions.

Aluminum (Al) and manganese (Mn) are present in a neutral form in soils. However, acid rain or other forms of soil acidification increases the solubilization of the Al and Mn present in soils¹⁾, and both metals can be toxic to plants as well as microorganisms. Previously, we isolated an acid- and Al-tolerant bacterium, *Flavobacterium* ST-3991(ST-3991), and reported a few of its properties particularly acid- and Al-tolerance and ability to increase the pH of an acidic culture medium²⁾. However, the properties of acid- and Mn-tolerance and mechanism of increasing pH of strain ST-3991 have not yet been precisely characterized.

In this study, we therefore examined the growth conditions of ST-3991, its tolerance to Mn^{2+} , and the substance increasing the pH and Mn tolerance.

ST-3991 was cultured using an S-LB liquid medium consisting of 1.0 g of bacto-peptone, 0.5 g of bacto-yeast extract, 10 g of NaCl, and 1000 ml of a soil-eluted aqueous solution pH of S-LB liquid medium was adjusted to five different pH values (3.0, 3.3, 3.5, 3.7, and 4.0) for the growth tests. The growth assays of strain ST-3991 in the presence of 0, 0.5, 1.0, 2.0 or 5.0% NaCl and either 0 or 100 mg/l of Al ions were performed using basal medium at pH 3.3 which was incubated at 25°C for 96 h with shaking (100 rpm). To examine the growth of strain ST-3991 at different temperatures (10, 20, 30, 37, or 40°C), Al was added to 1.0 ml of preincubated

Received March 5, 2010

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bacterial cultures pH 3.3 at a final concentration of 0 or 100 mg/l, and the cultures were then incubated without shaking for 96 h.

To evaluate the amount of growth under each condition, the cells were

and expressed as dry cell weights.

To determine the concentration of Al and Mn in bacterial cells, 100 ml of culture medium was suspended in HCl, and centrifuged at 10,000 rpm for 15 min at 4°C at several different time

Table 1. Effects of pH, NaCl concentration, and temperature on the growth of *Flavobacterium* ST-3991

		Dry cell weight (mg/l)	
		Al 0 ppm	Al 100 ppm
pH*	3.0	65	35
	3.3	115	115
	3.5	125	150
	3.7	75	95
	4.0	65	35
NaCl**	0	45	15
	0.5	95	70
	1	115	120
	2	80	35
	5	80	5
Temperature (°C)***	10	45	35
	25	95	55
	30	115	60
	37	105	45
	40	10	35

*Bacterial cells were cultured in 200 ml conical flasks containing 50 ml of S-LB medium at the indicated pH values for 96 h at 25 °C at 200 rpm.

**Bacterial cells were cultured in 200 ml conical flasks containing 50 ml of S-LB medium at pH 3.5 with the indicated concentrations of NaCl for 96 h at 25 °C with shaking at 100 rpm.

***Bacterial cells were cultured at different temperatures in 50 ml of S-LB medium at pH 3.5 and 1.0% NaCl with shaking at 100 rpm for 96 h.

harvested by centrifugation at 10,000 rpm for 20 min at 4°C. The pelleted cells were rinsed twice with 10 ml of 1N HCl medium. The resulting bacterial cell solutions were dried for 2 h at 105°C, and the dried precipitates were weighed

points. The resulting bacterial pellets were resuspended in 50 ml of 1N HCl, shaken for 5 min, and then centrifuged using the above same conditions. The cells were washed twice in 1N HCl. The pellets were treated with a mixture

Table 2. Effects of pH and Mn²⁺ concentration on the growth of ST-3991

pH	Mn ²⁺ concentration (mg/l)				
	0	100	500	1000	2000
3.5	+	+	+	+	+
3.3	+	+	+	+	+
3.2	+	+	+	+	-
3.1	+	+	+	-	-
3	-	+	-	-	-
2.9	-	+	-	-	-
2.8	-	-	-	-	-

The bacterial cells were cultured in 50 ml of S-LB medium (in 200 ml conical flasks) at different pH values and concentrations of Mn²⁺ with shaking at 200 rpm at 25 °C for 7 days. Bacterial growth was measured at 660 nm on a spectrophotometer in 1.0 cm light path after 100-fold dilution of the bacterial cultures. Symbols + and - show growth and no growth, respectively.

consisting of 1.0 ml of concentrated sulfuric acid and 1.0 ml of concentrated nitric acid for 30 min at 25°C. Al and Mn²⁺ extracted from the bacterial cells were measured in terms of ionic Al and ionic Mn using a Hitachi model ICP-8000 flame photometer under standard conditions.

Ammonium ions in the test media were measured by high performance liquid chromatography on a Shimadzu model LC-9A chromatograph equipped with an anion column (4.6 x 250 mm, Dionex Inc., Baltimore, U.S.A.) using a solvent system of 0.13% dimethylglutaric acid at a flow rate of 1.2 ml per min. at 30°C.

When cultured in either the presence of 100 mg/l or absence of Al, the optimal growth of ST-3991 was observed at pH 3.5, 1% NaCl and 30 °C below 100mg/l Al as shown in Table 1. The addition of NaCl also had a positive

effect on bacterial growth at a concentration of 1.0%. Using the optimal growth conditions of 1% NaCl and pH 3.5, growth was evaluated across a temperature range of 10 to 40°C. Under these conditions, strain ST-3991 showed the optimal growth at 30°C.

When ST-3991 was cultured at pH 2.8 ~ 3.5 and 0 ~ 2000mg/l Mn²⁺, the growth was observed above pH 3.1 in the absence of Mn²⁺, and at pH 2.9 and 1000mg/l Mn²⁺. The toxicity of Mn²⁺ increased above 500mg/l Mn²⁺ with increasing pH.

The changes pH and concentration of NH₄-N in the S-LB culture medium during the cultivation of ST-3991 were determined (Fig. 1). In the absence of ionic Al and Mn (control medium), both the pH of the culture medium and the amount of ammonium ions sharply increased after 40 h and reached pH 7.8

and 198 mg per liter, respectively, within 140 h.

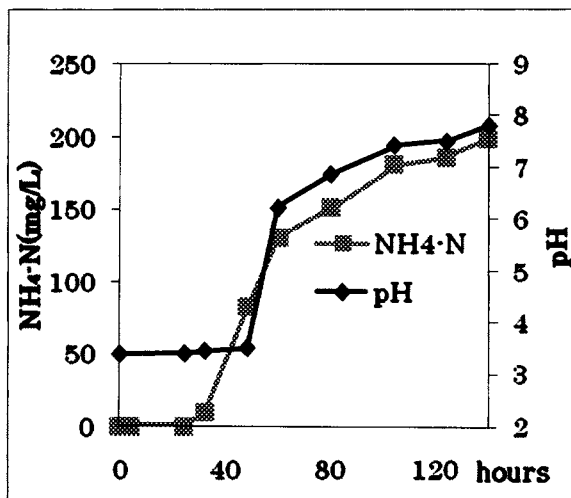


Fig. 1. Changes in pH and NH₄-N concentration in acidic medium without ionic Al and Mn of *Flavobacterium* ST-3991. ◆, pH; ■, NH₄-N.

It is well known that the majority of bacterial species are inactivated or killed at pH 4.0 or below, and acidophiles capable of growth under very intense acidic conditions are not suited to the alkali range³⁾. However, the acid- and Al-tolerant ST-3991 examined in this study was able to grow in a wide range of pH from 3.0 to 7.8, and also displayed resistance to high concentrations of Mn²⁺, which are as toxic as Al ions⁴⁾.

The results of this study indicate that strain ST-3991 has Mn tolerance and produces NH₄-N under acidic conditions. The acid and Al ion tolerance mechanism is likely mediated by cell membrane-associated proteins, and is an interesting topic for future studies.

We would like to express thanks to Mrs. Kuniko Nakatsuji for her help in this study.

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Acknowledgment