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Efficient production of ectoine using ectoine-excreting strain

Ling-hua Zhang · Ya-jun Lang · Shinichi Nagata

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Abstract Halophilic bacteria strain *Halomonas salina* DSM 5928 was found to excrete ectoine, suggesting its potential in the development of a new method of ectoine production. We performed HPLC and LC–MS analyses that showed that *Halomonas salina* DSM 5928 excreted ectoine under constant extracellular osmolarity. Medium adopting monosodium glutamate as a sole source of carbon and nitrogen was beneficial for ectoine synthesis. The total concentration of ectoine was not affected by NaCl concentration in the range 0.5–2 mol l^{-1}. The total concentration of ectoine and productivity in a 10-l fermentor with 0.5 mol l^{-1} NaCl were 6.9 g l^{-1} and 7.9 g l^{-1} d^{-1}, respectively. These findings show that *Halomonas salina* DSM 5928 efficiently produces ectoine at relatively low NaCl concentration. This research also indicates the potential application of free or immobilized cells for continuous culture to produce ectoine.

Keywords Ectoine · Excretion · *Halomonas salina* DSM 5928 · Excreting strain · Efficient production

Introduction

To rapidly adapt to changes in external media, for example, increasing NaCl concentration, cells accumulate small organic molecules in the cytoplasm to counteract the external osmotic pressure. The largest variety of such osmoregulatory organic molecules is detected among the bacteria. The cyclic amino acid ectoine is often found. It was originally discovered in the anoxigenic phototroph *Ectothiorhodospira halochloris* (Galinski et al. 1985). Ectoine is known to be synthesized by many chemoheterotrophic bacteria (Ventosa et al. 1998; Roberts 2005). Ectoine is valuable because it protects enzymes, DNA, the cell membrane, and intact cells against heat, freezing, drying, and other stresses. Therefore, ectoine has extensive applications in biological and enzyme preparations, the pharmaceutical industry, and other fields (Ventosa and Nieto 1995; Ventosa et al. 1998; Kanapathipillai et al. 2005; Zhang et al. 2006; Lentzen and Schwarz 2006). It has been industrially produced for commercial usage as an additive in cosmetics. For this reason there is great interest in improving the efficiency of ectoine production, and improvements in the production processes are driven by a search for higher yields and lower salt requirements (Onraedt et al. 2005).

Elucidation of the osmoregulation mechanism of ectoine synthesis and accumulation was very important for high-efficiency production of ectoine by halophilic bacteria. A new type of specific ectoine transporter, TeaABC, was found in *Halomonas elongata* DSM 2581^T. Because ectoine exported to the periplasm can be taken back into the cytoplasm from the periplasm by this TeaABC transport system, the exported ectoine does not accumulate in the medium and cannot be harvested (Grammann et al. 2002). Knockout of the ectoine transporter TeaABC led to an ectoine-overproducing mutant of *H. elongata* DSM 2581^T which excreted ectoine into the medium (Grammann et al. 2002; Kunte 2006).

Ectoine productivity of fermentation adopting non-ectoine-excreting strains mainly depended on the concentration of intracellular ectoine, the growth rate, and cell

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L. Zhang (✉) · Y. Lang
Environmental Science and Engineering College,
Dalian Maritime University, 1 Linghai Road,
Dalian 116026, People's Republic of China
e-mail: dlzlh2008@163.com

S. Nagata
Research Center for Inland Seas, Kobe University,
Kobe 658-0022, Japan

density. The concentration of intracellular ectoine depended on the NaCl concentration in the medium. Within the reasonable range of NaCl concentration which was suitable for the ectoine synthesis, higher NaCl concentrations in the medium led to higher concentrations of intracellular ectoine (Roberts 2005). Induction of a relatively high NaCl concentration in the medium is necessary to enhance synthesis; however, high-NaCl medium also aggravates corrosion of the equipment and reduces growth rate and cell density, ultimately leading to lower ectoine production.

In this study we investigated the excretion of ectoine by *Halomonas salina* DSM 5928 and found that this strain excretes ectoine that accumulates in the medium when extracellular osmolarity is constant. The accumulated ectoine was identified and analyzed by high performance liquid chromatography (HPLC) and liquid chromatography–mass spectrometry (LC–MS). These findings may lead to a new method for efficient production of ectoine in media with a low NaCl concentration. Such a method would be very valuable in the commercial preparation of ectoine. This study also investigated the effects of culture conditions on the efficiency of ectoine production from *H. salina* DSM 5928.

Materials and methods

Strains

H. salina DSM 5928 and *H. elongata* DSM 2581^T were obtained from the German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany).

Media

MG medium. L-Monosodium glutamate, 150 mmol l^{-1}; NaCl, 1 mol l^{-1}; inorganic salt mixture, 500 ml l^{-1}. The inorganic salt mixture comprises (%, *w*/*v*) KH_2PO_4, 0.6; K_2HPO_4, 1.8; $MgSO_4.7H_2O$, 0.08; and $MnSO_4.4H_2O$, 0.002. It was sterilized by filtration (0.2-μm pore size) and pH was 7.2.

MM63 medium (mmol l^{-1}). KH_2PO_4, 100; KOH, 75; $(NH_4)_2SO_4$, 15; $MgSO_4$, 1; $FeSO_4$, 0.0039; D-glucose, 22; and NaCl, 1.71 mol l^{-1}; pH 7.2 (Larsen et al. 1987).

Medium A (g l^{-1}). Glucose, 10; $MgSO_4.H_2O$, 1.4; NH_4Cl, 2.3; $FeSO_4.H_2O$, 0.005; Tris, 15; NaCl, 150; pH 7.5 (Sauer and Galinski 1998).

Medium B (g l^{-1}). Monosodium glutamate, 50; yeast extract, 2.5; KH_2PO_4, 3; K_2HPO_4, 9; $MgSO_4.7H_2O$, 0.4; $MnSO_4.4H_2O$, 0.01; $FeSO_4.7H_2O$, 0.01; NaCl, 58.4; pH 7.0 (Onraedt et al. 2005).

Shake-flask culture

These strains were cultivated in the activation medium (0.01 g l^{-1} of yeast extract was added to the MG medium) at 120 rpm and 30°C for 24 h. Shake flasks (300 ml) containing 30 ml fermentation medium on a rotary shaker set at 120 rpm and 30°C were then inoculated with 1% of the cultures.

Batch fermentations

Batch fermentations were performed in a fermentor with a working volume of 10 l. The fermentor was filled with 6 l MG medium (monosodium glutamate, 320 mmol l^{-1}; NaCl, 0.5 mol l^{-1}). The fermentor was inoculated with 300 ml shake-flask cultures of *H. salina* DSM 5928. The fermentor was equipped with a glass electrode to monitor culture pH, which was maintained at pH 7.2 by adding 5 mol l^{-1} HCl. Temperature was maintained at 30°C. The dissolved oxygen level was above 20% saturation, as measured by use of a polarization pO_2 electrode. The antifoam agent Paodi (0.015%, Lüshun Chemical Plant, China) was added to the medium to prevent foaming.

Cell dry weight

Fermentation broth was centrifuged at 4°C and 16,000×*g* for 15 min and the pellets were then washed with KH_2PO_4–K_2HPO_4 buffer (KPi buffer, 100 mmol l^{-1}, pH 7.2) containing NaCl at the same concentration as that in the medium. After centrifugation, the pellets were dried at 105°C until no further change in weight occurred, and were then weighed. Cell density was defined as cell dry weight (CDW) per liter fermentation broth (g l^{-1}).

HPLC analysis of ectoine

Separation of cells from fermentation broth. Centrifugation method: Using MG medium, fermentation broth at the exponential phase of growth (36 h) was centrifuged at 4°C and 16,000×*g* for 15 min, and then the supernatant was used for analysis. Filtration method: Using MG medium, fermentation broth at the exponential phase of growth (36 h) was filtered through a 0.2-μm pore size membrane filter (Advantec, Tokyo, Japan) at 30°C, and the filtrate was then used for analysis.

Intracellular ectoine measurement: Cells were collected by the centrifugation method described above and the pellets were washed with 100 mmol l^{-1} KPi buffer (pH 7.2) containing NaCl at the same concentration as that in the medium. After centrifugation, pellets were extracted with 1 ml

ethanol (80%, v/v), resuspended, then kept at room temperature overnight. The suspension was centrifuged again and the supernatant was then used for HPLC analysis. Here, the ectoine concentration by HPLC analysis was defined as the concentration of intracellular ectoine (mg intracellular ectoine per liter fermentation broth, mg l^{-1} or mg ectoine per gram CDW, mg (g CDW)$^{-1}$).

Extracellular ectoine measurement: Cells were separated from media by the centrifugation (4°C) or filtration (30°C) methods described above then the supernatant or filtrate, respectively, were diluted tenfold with distilled water before HPLC measurement. Here, the ectoine concentration by HPLC analysis was defined as the concentration of extracellular ectoine (mg extracellular ectoine per liter fermentation broth, mg l^{-1} or mg ectoine per gram CDW, mg (g CDW)$^{-1}$).

The total concentration of ectoine was the sum of the concentrations of intracellular and extracellular ectoine.

Excretion rate (%) was expressed as the percentage of the concentration of extracellular ectoine divided by the total concentration of ectoine.

HPLC determination. The concentration of ectoine was measured by HPLC with a TSK-GEL reversed-phase column (Tosoh, Japan) with 50 mmol l^{-1} KPi buffer as mobile phase at 35°C. The flow rate was 1 ml min^{-1} and UV detection at 210 nm was adopted. The retention time of ectoine was determined by using commercially available authentic ectoine ((*S*)-β-2-methyl-1,4,5,6-tetrahydropyrimidine-4-carboxylic acid, purity >97%, Biomol, Hamburg, Germany) (Wang et al. 2006).

LC–MS analysis

Using MG medium, 20 ml fermentation broth at the exponential phase of growth (36 h) was collected and cells were separated by centrifugation. Ethanol (99.5% v/v, 180 ml) was added to the supernatant, and then the mixture was kept at 4°C for 30 min to precipitate and crystallize monosodium glutamate residue in the fermentation broth. The supernatant was used for the LC–MS measurement. HPLC (Waters 2695 separation module) and a mass spectrometer (Quattro Micro Waters Co., USA) were used to identify and quantify ectoine. HPLC conditions: A 2.1 × 150 mm Xterra MS C_{18} reversed-phase column was used for ectoine quantification. Five-microliter samples were eluted with methanol–water solution (80%, v/v) with a flow rate of 0.2 ml min^{-1} at 35°C, and detected by UV absorbance at 210 nm. The effluent from the liquid chromatography column was introduced to the mass spectrometer (Waters, USA). Mass spectrometer conditions: electrospray ionization (ionization mode ES+); source temperature, 120°C; detector, Waters 2996 photodiode array.

Results

Ectoine excretion

H. salina DSM 5928 was cultivated in a shake flask with MM63 medium. The fermentation broth at the exponential phase of growth was collected. The concentrations of intracellular and extracellular ectoine were measured by HPLC. The results showed that *H. salina* DSM 5928 not only accumulated ectoine inside the cells, but also excreted ectoine into the medium, and the concentrations of intracellular and extracellular ectoine were 824.6 and 98.8 mg l^{-1}, respectively. However, when the same experiment was performed with *H. elongata* DSM 2581^T, the concentration of intracellular ectoine was 810.7 mg l^{-1} and no ectoine was detected outside the cells. To determine if extracellular ectoine from *H. salina* DSM 5928 was a result of centrifugation (4°C), cells were separated from fermentation broth by centrifugation (4°C) or filtration (30°C). The concentrations of extracellular ectoine produced by *H. salina* DSM 5928 were not significantly different (*t* test, $P > 0.05$) between centrifugation (4°C) and filtration (30°C) methods. However, no ectoine was detected outside the *H. elongata* DSM 2581^T cells by use of the two methods (Table 1). These results indicated that ectoine release did not result from centrifugation (4°C). *H. salina* DSM 5928

Table 1 Ectoine excretion from *H. salina* DSM 5928

Strain[a]	Concentration of intracellular ectoine (mg l^{-1})[b]	Concentration of extracellular ectoine (mg l^{-1})[c]	
	Centrifugation (4°C)	Centrifugation (4°C)	Filtration (30°C)
H. salina DSM 5928	824.6 ± 4.4	98.8 ± 4.3	99.3 ± 4.9
H. elongata DSM 2581^T	810.7 ± 19.2	—[d]	—

The concentrations of extracellular ectoine produced by *H. salina* DSM 5928 were not significantly different (*t* test, $P > 0.05$) after use of the centrifugation (4°C) and filtration (30°C) methods. The results are averages ± SD from five independent experiments. Relative error of determination is <5.0%

[a] The strains were cultivated in MM63 medium and the fermentation broth at the exponential phase of growth was collected

[b] Intracellular ectoine concentration: cells were collected from the fermentation broth and ectoine extracted from the cells was used for HPLC analysis. The concentration was expressed as mg intracellular ectoine per liter fermentation broth, mg l^{-1}

[c] Extracellular ectoine concentration: cells were separated from the media and the supernatant was used for HPLC analysis. The concentration was expressed as mg extracellular ectoine per liter fermentation broth, mg l^{-1}

[d] Not detectable by HPLC with UV detection

Table 2 Ectoine synthesis and excretion by *H. salina* DSM 5928 in different media

Medium	Concentration of extracellular ectoine (mg l^{-1})[a]	Total concentration of ectoine (mg l^{-1})[b]
MG	1076.9 ± 22.5	1727.9 ± 46.8
A	86.6 ± 3.9	410.7 ± 8.8
B	508.5 ± 12.8	1508.9 ± 38.6

The results are averages ± SD from three independent experiments. Relative error of determination is <4.6%

[a] Extracellular ectoine concentration: cells were separated from media and the supernatant was used for HPLC analysis. The concentration was expressed as mg extracellular ectoine per liter fermentation broth, mg l^{-1}

[b] The total concentration of ectoine was the sum of concentrations of intracellular and extracellular ectoine, mg l^{-1}

was an ectoine-excreting strain of halophilic bacteria whereas *H. elongata* DSM 2581^T was a non-ectoine-excreting strain.

Except for MM63 medium, ectoine excretion of *H. salina* DSM 5928 was also investigated in the media MG, A (Sauer and Galinski 1998), and B (Onraedt et al. 2005). The results showed that *H. salina* DSM 5928 could excrete ectoine into MG, A, and B media (Table 2). Thus, excretion of ectoine by *H. salina* DSM 5928 was not limited to a specific medium. The total concentration of ectoine and the concentration of extracellular ectoine were significantly different (*t* test, $P < 0.01$) among the different types of media. However, the total concentration of ectoine and the concentration of extracellular ectoine from *H. salina* DSM 5928 were maximum in MG medium. Thus, MG medium was adopted in the following experiments.

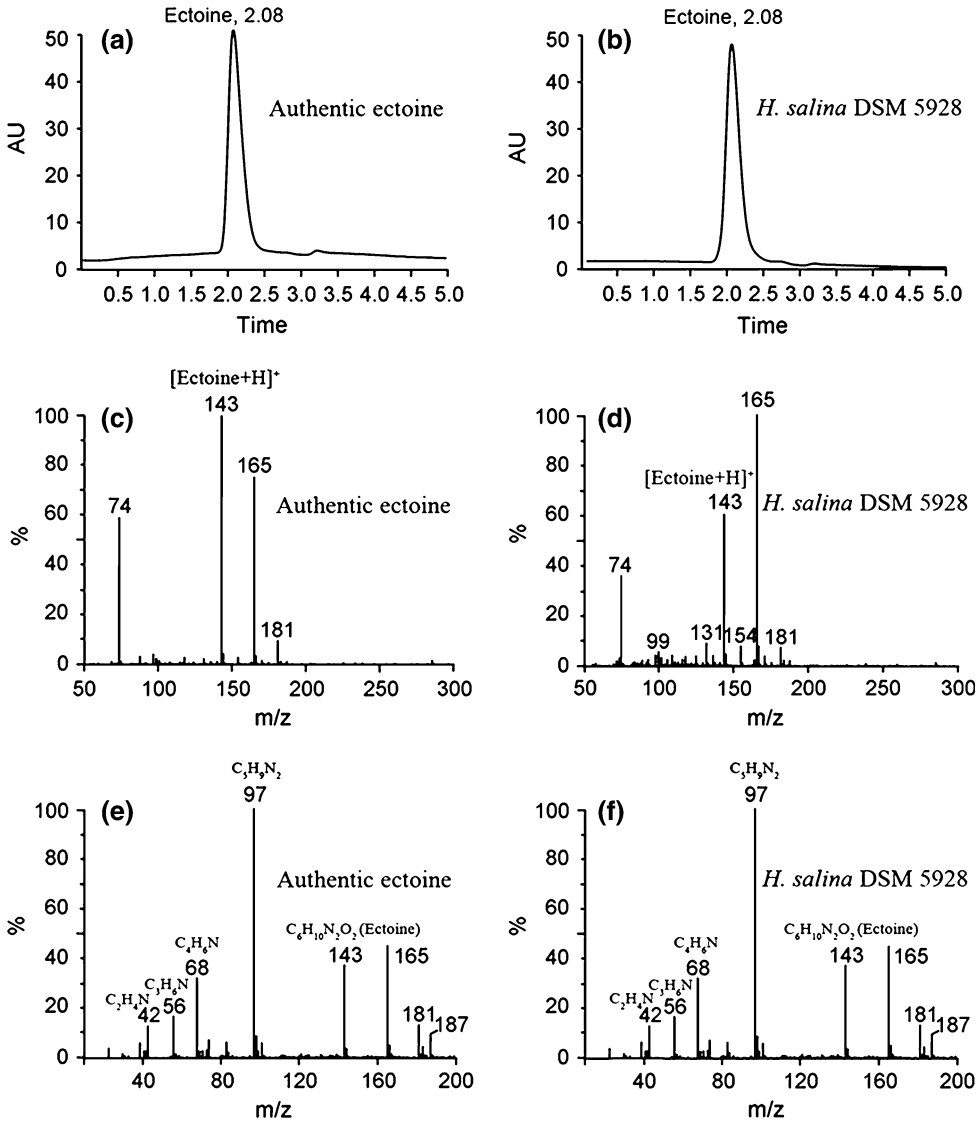

Fig. 1 Results from LC–MS analysis of *H. salina* DSM 5928 fermentation broth. **a**, **b** Chromatograms obtained from authentic ectoine and from fermentation broth of *H. salina* DSM 5928 in LC–MS analysis; **c**, **d** Spectra of the two samples obtained by monopole mass spectrometry; **e**, **f** Spectra of the two samples obtained by tandem mass spectrometry

To confirm the fact that *H. salina* DSM 5928 excreted ectoine, LC–MS analysis was used to identify ectoine in the fermentation broth at the exponential phase of growth in MG medium. The same HPLC retention time was observed for authentic ectoine and the compound present in the fermentation broth (Figs. 1a, b), and the spectra obtained by monopole mass spectrometry were also consistent (Figs. 1c, d); a signal was detected at 143 (*m/z*), which is in good agreement with the molecular weight of ectoine (142). Signals of ectoine and its induced dissociation in the spectra obtained by tandem mass spectrometry occurred at 143, 97, 68, 56, and 42 (*m/z*) (Figs. 1e, f) (Galinski et al. 1985). In conclusion, the compound excreted by *H. salina* DSM 5928 was confirmed to be ectoine. In the follow-up study, HPLC analysis was used to quantify ectoine in the medium.

Optimization of the media

The effect of different concentrations of monosodium glutamate on ectoine synthesis was investigated (Fig. 2).

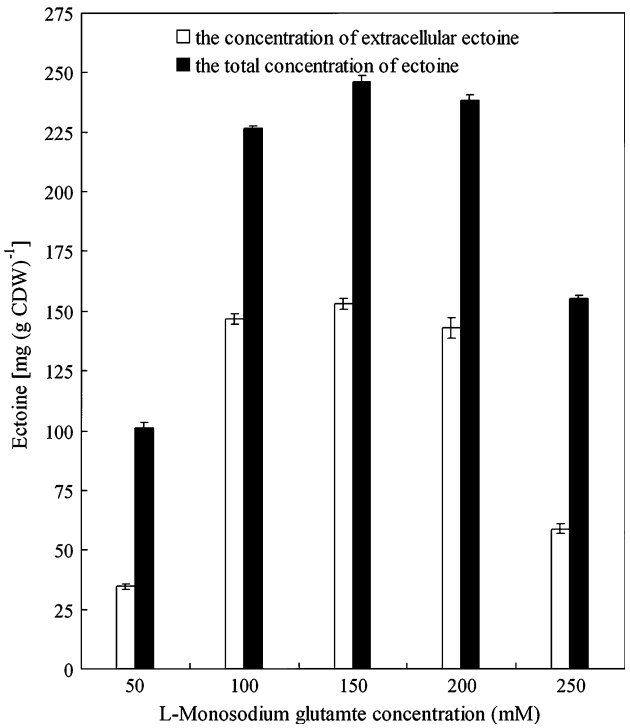

Fig. 2 Effect of monosodium glutamate concentration on ectoine synthesis. *H. salina* DSM 5928 was cultivated in MG medium with different concentrations of monosodium glutamate (shake-flask culture). 1 ml fermentation broth at the exponential phase of growth (36 h) was collected. The concentrations of ectoine were measured by HPLC, [mg (g CDW)$^{-1}$]. *CDW* is cell dry weight. The results are averages ± SD from three independent experiments. Relative error of determination is <3.6%

The maximum total concentration of ectoine and concentration of extracellular ectoine were 245.9 mg (g CDW)$^{-1}$ and 153.3 mg (g CDW)$^{-1}$, respectively, at a monosodium glutamate concentration of 150 mmol l^{-1}. The excretion rate was 62.3% (*t* test, $P < 0.05$).

Compared with the MG medium, an additional 10 g l^{-1} glucose in the MG medium led to no statistically significant differences in the total concentration of ectoine and the concentration of extracellular ectoine (*t* test, $P > 0.05$). However, after adding 2.5 g l^{-1} yeast extract to the MG medium, the total concentration of ectoine and the concentration of extracellular ectoine were reduced by 26.1 and 60.0%, respectively. Adding yeast extract to the MG medium had a significant effect on the total concentration of ectoine and the concentration of extracellular ectoine (*t* test, $P < 0.01$) (Table 3). Yeast extract contains compatible solutes, namely glycine betaine and, most important, choline, the precursor of glycine betaine. Most bacteria can transport choline to the cytoplasm and are able to oxidize choline to glycine betaine (Galinski 1993; Robertson et al. 1990; Kappes et al. 1999), which could inhibit ectoine synthesis.

The effect of NaCl concentration on ectoine synthesis and excretion was investigated (Fig. 3). The concentration of intracellular ectoine was increased, and the concentration of extracellular ectoine was reduced as the concentration of NaCl in the medium was increased, although the total concentration of ectoine was not affected by NaCl concentration in a range 0.5–2 mol l^{-1}. The cell growth rate and cell density of *H. salina* DSM 5928 were maximum at 0.5 mol l^{-1} NaCl (data not shown). Thus, 0.5 mol l^{-1} NaCl was adopted in the following experiments.

Fermentation of *H. salina* DSM 5928

The relationship among ectoine synthesis, excretion, incubation time, and cell growth of *H. salina* DSM 5928 in MG medium was investigated in a 10-l fermentor (Fig. 4). After a lag phase (5 h), *H. salina* DSM 5928 entered the exponential phase of growth and then arrived at stationary phase at 18 h, and ended at 21 h. The maximum cell concentration was 19.4 g l^{-1} at 18 h. Both the total concentration of ectoine and the concentration of extracellular ectoine increased with time until the end of stationary phase, and the maximum values were 6.9 and 4.3 g l^{-1} (21 h), respectively. Corresponding ectoine concentrations per gram dry cell weight were 357.5 mg (g CDW)$^{-1}$ (total) and 220.1 mg (g CDW)$^{-1}$ (extracellular), and excretion rate was 61.6%. The productivity in a 10-l fermentor with 0.5 mol l^{-1} NaCl was 7.9 g l^{-1} d^{-1} (the mass of synthesized ectoine by cells per liter fermentation broth per day).

Table 3 Effect of glucose and yeast extract on ectoine synthesis

Medium	Concentration of extracellular ectoine [mg (g CDW)$^{-1}$]	Total concentration of ectoine [mg (g CDW)$^{-1}$]	Excretion rate (%)
MG	153.3 ± 2.3	245.9 ± 2.9	62.3 ± 3.0
MG + 10 g l^{-1} glucose[a]	136.1 ± 5.6	223.8 ± 2.1	60.8 ± 2.2
MG + 2.5 g l^{-1} yeast extract[b]	61.4 ± 2.7	181.8 ± 2.7	33.7 ± 1.7

The concentrations of ectoine were measured by HPLC and expressed as mg ectoine per gram CDW, i.e. mg (g CDW)$^{-1}$, where CDW is cell dry weight. The results are averages ± SD from three independent experiments. Relative error of determination is <5.1%

[a] 10 g l^{-1} glucose was added to MG medium

[b] 2.5 g l^{-1} yeast extract was added to MG medium

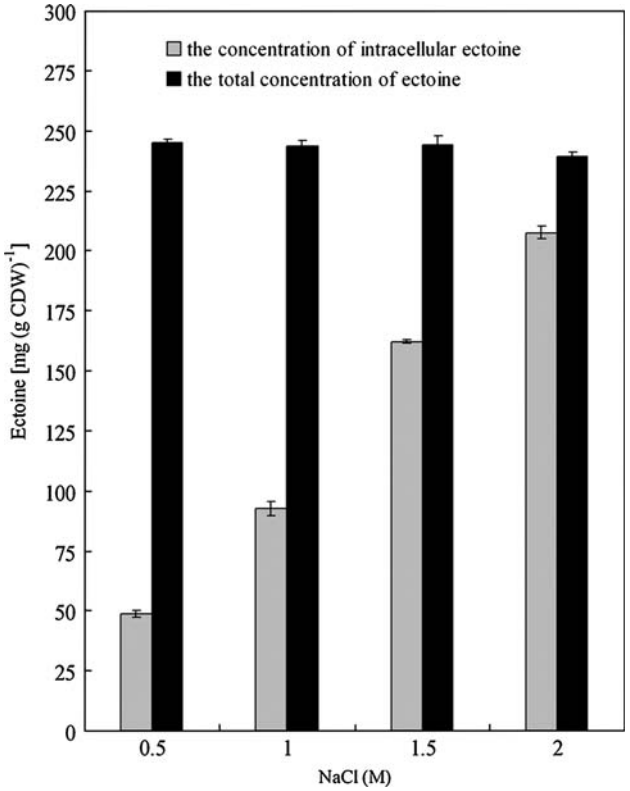

Fig. 3 Effect of NaCl concentration on ectoine synthesis. *H. salina* DSM 5928 was cultivated in MG medium with 0.5–2 mol l^{-1} NaCl (shake-flask culture). 1 ml fermentation broth at the exponential phase of growth (36 h) was collected. The concentrations of ectoine were measured by HPLC, [mg (g CDW)$^{-1}$]. *CDW* is cell dry weight. The results are averages ± SD from three independent experiments. Relative error of determination is <4.0%

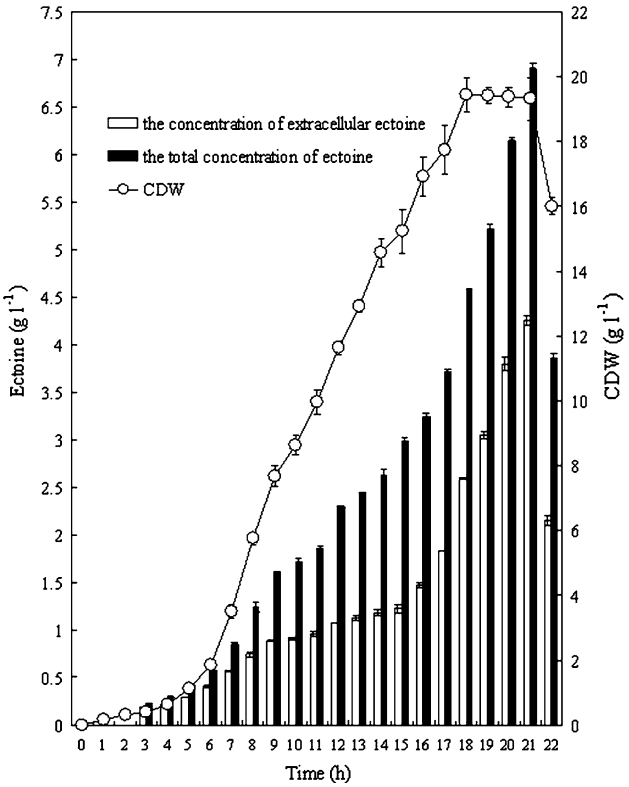

Fig. 4 Time course of ectoine fermentation by *H. salina* DSM 5928. Batch fermentations of *H. salina* DSM 5928 were performed in a fermentor with a working volume of 10 l. The concentrations of ectoine were measured by HPLC. *CDW* is cell dry weight (g l^{-1}). The results are averages ± SD from three independent experiments. Relative error of determination is <5.5%

Discussion

To the best of our knowledge, no previous publication has described an ectoine-excreting wild-type strain of halophilic bacteria. Our findings show that *H. salina* DSM 5928 under salt stress is able to partially excrete ectoine into the medium. This result was confirmed by the HPLC and LC–MS analyses.

During the investigation, we paid particular attention to several points:

1 HPLC analysis of the medium (not inoculated) after filtered sterilization revealed no ectoine peak. Thus, the medium contained no ectoine prior to inoculation.
2 The fermentation broth samples for analysis were sampled at the individual exponential phase of cell

growth; therefore, the autolysis of dead cells was not responsible for the release of ectoine into the medium during that time.

3 Osmotic pressure of the medium was kept constant during fermentation (data not shown), so excreted ectoine from cells was not because of reduced osmotic stress in the environment.

4 Excreted ectoine was continuously found during the fermentation from initial pH 7.2 to final pH 7.9 in the shake flask. When the pH was kept at pH 7.2 during fermentation in a 10-l fermentor, ectoine excreted into the medium was also detected. These results indicated that ectoine could be excreted from *H. salina* DSM 5928 regardless of changes to the pH of the medium.

5 To determine if ectoine excretion from *H. salina* DSM 5928 resulted from centrifugation, cells were separated from fermentation broth by centrifugation (4°C) or filtration (30°C). The values of extracellular ectoine concentrations were not significantly different between the two separation methods. When the same experiment was performed with *H. elongata* DSM 2581^T, no ectoine was detected in the medium. Therefore, the ectoine detected in the medium was not the result of ectoine leakage caused by centrifugation (4°C).

In conclusion, we confirmed that under constant extracellular osmolarity, ectoine synthesized by *H. salina* DSM 5928 is partially excreted by the cells and accumulates in media.

The difference between the ectoine-excreting strain *H. salina* DSM 5928 and the non-ectoine excreting strain *H. elongata* was the dependence on NaCl concentration. The total concentration of ectoine synthesized by *H. salina* DSM 5928 was not affected by the NaCl concentration of the medium in the range 0.5–2 mol l^{-1}. However, to obtain a high yield of ectoine from *H. elongata* required a relatively high NaCl concentration.

The optimum NaCl concentration for ectoine synthesis and excretion from *H. salina* DSM 5928 was 0.5 mol l^{-1}, which is much lower than the concentration needed to synthesize intracellular ectoine by non-ectoine excreting strains *H. elongata* DSM 142^T (2.6 mol l^{-1} NaCl) and *Brevibacterium epidermis* DSM 20659 (1 mol l^{-1} NaCl) (Sauer and Galinski 1998; Onraedt et al. 2005). Relatively low NaCl concentration reduced corrosion of the equipment, was beneficial for cell growth, and increased cell density. Ectoine-excreting strains provide an opportunity for the application of free or immobilized cells for continuous culture to produce ectoine.

Some efficient production processes for ectoine adopting non-ectoine excreting strains have been reported. Application of the "bacterial milking" technique to *H. elongata* DSM 142^T results in productivity of about 3.3 g l^{-1} d^{-1} (Sauer and Galinski 1998). Other production processes described in the literature use *B. epidermis* DSM 20659 (productivity 2 g $l^{-1}d^{-1}$) and anaerobic halophilic denitrifying bacteria (productivity 2 g $l^{-1}d^{-1}$) (Onraedt et al. 2005). In this report, the productivity of ectoine from ectoine-excreting type strain *H. salina* DSM 5928 was 7.9 g l^{-1} d^{-1}, which is the highest level of ectoine productivity yet reported.

It has been reported that the ABC transport system mediating the glucosylglycerol uptake mutant (*ggtA* insertion mutant) of *Synechocystis* sp. loses its glucosylglycerol (GG) uptake ability. After cultivation of the *ggtA* mutant at high salt concentrations, significant amounts of GG are found in the medium (Hagemann et al. 1997); Knockout of the ectoine transporter TeaABC led to a mutant of *H. elongata* DSM 2581^T that excreted ectoine into the medium and also overproduced ectoine (Grammann et al. 2002). The ectoine productivity of the mutant of *H. elongata* DSM 2581^T was about 2.1 g l^{-1} d^{-1} by high-cell-density fermentation (Kunte et al. 2002). *H. salina* DSM 5928 excreted ectoine into the medium where it accumulated; the possible reasons for this accumulation include:

1 the ectoine transporter is defective, or
2 the ectoine transporter is not defective, but the rate of excretion is greater than rate of recovery.

Alternatively, the model for osmoregulation of ectoine biosynthetic pathways in *H. salina* DSM 5928 is probably different from that of *H. elongata* DSM 2581^T. The mechanism of ectoine excretion by *H. salina* DSM 5928 needs further study; however, this strain may have potential for future commercial application in the production of ectoine.

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