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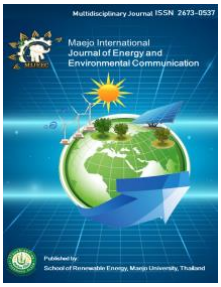

ARTICLE

Alkaline hydrolysis as a simple method for converting chicken manure fertilizer into feedstock for ectoine production by *Halomonas elongata* cell factory

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ABSTRACT

Changes in dietary habits in modern-day Japan have led to a significant expansion in the country's livestock industry. One notable change is the increased consumption of eggs due to their high-quality protein content and affordability. This significantly expanded the poultry industry to the extent that it raised environmental concerns, especially regarding the increased waste production. Major solid waste from the poultry industry is chicken manure (CM), commonly treated through composting into fertilizer. However, there is currently an oversupply of CM fertilizer globally due to a large amount of CM being produced. We address this issue by developing a simple alkaline hydrolysis method to convert the nitrogen-rich CM fertilizer into media for culturing bacterial cell factories. Our result shows that the high-salinity CM-derived media developed here can sustain the growth of *Halomonas elongata* OUT30018. This moderately halophilic bacterium biosynthesizes and accumulates valuable chemicals such as ectoine (ECT) in the cell. We observed that *H. elongata* OUT30018 cultured in a CM-derived medium containing 15% w/v NaCl could accumulate 335 µmol of ECT per g cell fresh weight (CFW), a concentration comparable to that obtained in the cells cultured in common synthetic media. Furthermore, this concentration was increased to 393 µmol/g CFW when the CM-derived medium was supplemented with 4% w/v xylose. The work reported here represents the initial stage of developing new technology for managing CM. Implementation of this technology would improve the sustainability of both the poultry and the fermentation industries.

1. Introduction

Due to changes in dietary habits in present-day Japan, the livestock industry has expanded significantly. One sector that has undergone rapid expansion is the poultry industry due to the increasing demand for high-quality poultry meat and eggs. According to a survey

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conducted by the International Egg Commission (IEC), the average egg consumption in Japan was 337 eggs per capita in 2021 (IEC, 2022). According to the survey done by the Ministry of Agriculture Forestry and Fishery, Japan (MAFF), as of February 1, 2022, there are 1,810 layer farms, which raised approximately 137 million layer chickens and 2,100 broiler farms with approximately 139 million broiler chickens in Japan (Tajima, 2023; MAFF, 2022a; MAFF, 2022b). With an industry of this size, one could imagine its impact on the environment due to the amount of waste generated.

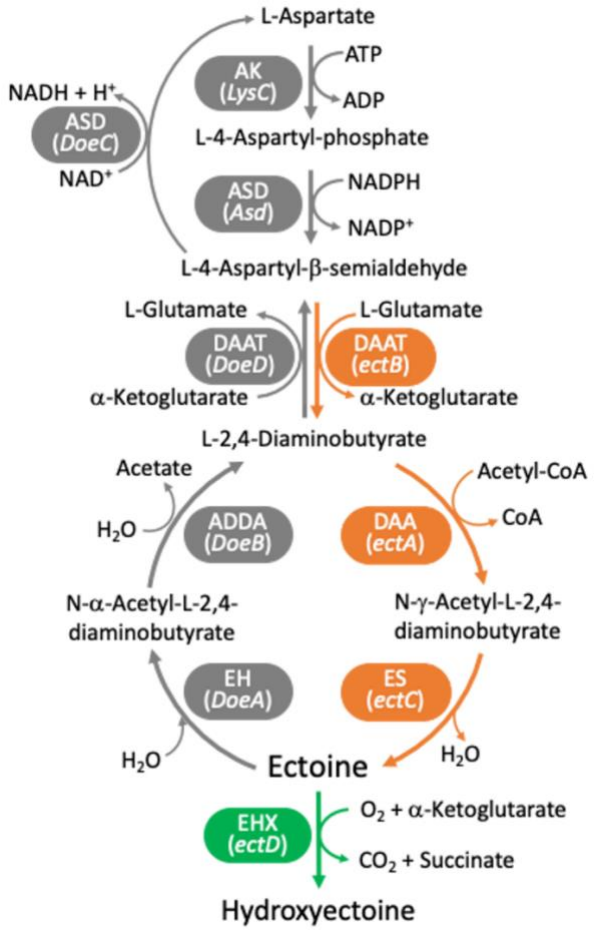

Figure 1. Schematic diagram of ECT metabolic pathway in *H. elongata* OUT30018. *H. elongata* OUT30018 synthesizes ECT in response to high-salinity stress through the activities of the three enzymes of the ECT biosynthetic pathway (orange arrows): L-2,4-diaminobutyrate 4-aminotransferase (DAAT), L-2,4-diaminobutyrate acetyltransferase (DAA), and ectoine synthase (ES). ECT is further hydroxylated to HydECT by ectoine hydroxylase (EHX). ECT can also be recycled to L-aspartate via the ECT catabolic pathway (grey arrow). AK, L-aspartate kinase encoded by the *LysC* gene; ASD, L-aspartate-β-semialdehyde dehydrogenase encoded by the *Asd* or the *doeC* genes; DAAT, L-2,4-diaminobutyrate 4-aminotransferase encoded by the *ectB* or the *doeD* genes; DAA, L-2,4-diaminobutyrate acetyltransferase encoded by the *ectA* gene; ES, ectoine synthase encoded by the *ectC* gene; EHX, ectoine hydroxylase encoded by the *ectD* gene; EH, ectoine hydrolase encoded by the *doeA* gene; ADDA, N2-acetyl-L-2,4-diaminobutanoate deacetylase encoded by the *doeB* gene.

In Japan alone, the estimated amount of waste produced in 2017 was 8 million tons for layer chickens and 5 million tons for broiler chickens (MAFF, 2023). Most of the solid waste generated by the poultry industry is CM, which is a mixture of chicken droppings, waste bedding, waste food, and feathers from the coops. CM contains high nitrogen, phosphorus, and ash content (Hussein et al., 2017). Current CM managing systems include the utilization of CM as biochar for converting waste cooking oil into biodiesel through transesterification (Jung et al., 2018) and hydrolyzation of CM by H_2SO_4 to produce Bio-ethanol (Woldesenbet et al., 2013). The most common method of managing CM in Japan involves composting it as an organic fertilizer. However, the long-term application of CM in agricultural fields can lead to the accumulation of metals in soil that could cause toxicity in some crops and organisms (He et al., 2009). An oversupply issue for CM fertilizer has arisen due to a large amount of CM being produced worldwide. Therefore, it is imperative to implement a new management system for CM.

Halomonas elongata OUT30018 (formerly designated strain KS3) is a moderately halophilic eubacterium isolated from high-salinity soil in Khon Kaen, Thailand (Ono et al., 1998). *H. elongata* OUT30018 thrives in environments with NaCl concentrations ranging from 0.3% to 21% by synthesizing and accumulating ectoine (1,4,5,6-tetrahydro-2-methyl-4-primidinecarboxylic-acid; ECT) and hydroxyectoine (1,4,5,6-tetrahydro-2-methyl-5-hydroxy4-pyrimidinecarboxylic acid; HydECT) as organic osmolytes when grown in high-salinity environments (Cánovas et al., 1997; Ono et al., 1998). ECT and HydECT effectively protect against heat, freezing, and drying (Lippert & Galinski, 1992; Roberts, 2005; Kuhlmann et al., 2008; Vargas et al., 2008). ECT and HydECT are used in cosmetics, moisturizers, and other products. They also can protect healthy cells during chemotherapy and are potential anti-amyloid therapeutics for treating Alzheimer's disease (Sauer & Galinski, 1998; Kanapathipillai et al., 2005). Although there are no differences in the application of ECT and HydECT, which are chemically analogous compounds, it is noteworthy that HydECT exhibits a higher glass transition temperature compared to ECT. Therefore, HydECT is more effective as a desiccation protectant (Tanne et al., 2014; Gracia-Estepa et al., 2006). *H. elongata*, which is recognized as a beneficial microorganism for biotechnological applications (Bourdichon et al., 2012), is being used as a cell factory to produce ECT at the industrial level (Vreeland et al., 1980; Lentzen & Schwarz, 2006; Schiwibbert et al., 2011).

In *H. elongata* OUT30018, three enzymes, L-2,4-diaminobutyric acid (DABA) aminotransferase (DAAT) encoded by the *ect*B gene, DABA acetyltransferase (DAA) encoded by the ectA gene, and ectoine synthase (ES) encoded by the ectC gene, work together in a pathway to biosynthesize ECT from L-4-aspartyl-β-semialdehyde (ASA) (Figure 1) (Ono et al., 1999; Göller et al., 1998; Nakayama et al., 2020). *H. elongata* OUT30018 is distinctive among other bacteria in the same genera due to its ability to utilize unique compounds as substrates for growth, including biomass-derived compounds such as glycerol (Vreeland et al., 1980), xylose (Tanimura et al., 2013), histamine, and tyramine (Nakayama et al., 2020).

Therefore, *H. elongata* OUT30018 and engineered strains derived from it are regarded as high-potential cell factories for the production of valuable compounds such as ECT, HydECT, γ-aminobutyric acid (GABA), and proline (Tanimura et al., 2013; Nakayama et al., 2020;

Zou et al, 2024). Here, we propose an alternative method for managing CM waste by converting CM fertilizer into feedstock for culturing cell factories. As a proof of concept, we also showed that the resulting CM-derived medium can be used as a culturing medium for ECT production by *H. elongata* OUT30018.

2. Materials and methods

2.1 Alkaline hydrolysis of CM fertilizer and preparation of CM-derived medium

The CM fertilizer, fermented to reduce odor, was purchased from Tosho Co., Ltd, Shizuoka, Japan. The package listed the components of the CM fertilizer as follows: total nitrogen (N) of 2.6% w/w, total phosphate (P) of 5.4% w/w, total potassium (K) of 3.3% w/w, and carbon to nitrogen (C:N) ratio of 9.5. As shown in Figure 2, alkaline hydrolysis was used to depolymerize protein and polysaccharide contents of the CM fertilizer to be used as a component of the CM-derived medium. The first step in the process is to suspend 25 g of ground CM fertilizer in 250 mL of 2 M NaOH. This suspension mixture was then autoclaved at 121oC for 60 min to accelerate the alkaline hydrolysis reaction and sterile the mixture.

After the autoclaved hydrolysate had cooled to room temperature, its pH was neutralized to 7.2 with HCl solution. During neutralization, 0.5 mol of NaCl was formed. Therefore, sterile distilled water was added to bring the total volume of the hydrolysate to 500 mL to adjust the salt concentration of the hydrolysate to 1 M (~6% w/v) NaCl. Finally, the hydrolysate was filtered through Qualitative filter paper (ADVANTEC) to remove debris, and the filtrate was used as 5% CM hydrolysate stock for preparing 2.5% or 1.25% w/v CM-derived media that contain 6% or 15% w/v NaCl. For the supplemental carbon (C) source experiment, 4% w/v xylose (Xyl), glycerol (Gly), or glucose (Glu) were added to 2.5% w/v CM-derived medium containing 15% w/v NaCl.

2.2 Bacterial strain and growth condition

H. elongata OUT30018 (Ono et al., 1998) was used in all growth tests. *H. elongata* OUT30018 precultured in 2.5% CM-derived medium containing 6% NaCl until OD600 reaches 0.9 is used as a 5% inoculum for growth tests' main cultures. All cultures were incubated in a 37 °C air incubator with 120 rpm agitation.

2.3 Extraction of major osmolytes accumulated inside *H. elongata* OUT30018 cells

Major osmolytes, which include ECT, HydECT, and glycine betaine (GB), were extracted from the *H. elongata* OUT30018 cells by using a hypo-osmotic extraction method (Zou et al., 2024; Sauer & Galinski, 1998; Cánovas et al., 1997). Briefly, *H. elongata* cells were harvested from the culture medium by centrifugation at 13,000 rpm for 3 min, and the weight of the cell pellet was recorded as CFW. The cell pellets were then suspended in pure water (20 µL per 1 mg CFW) to let the cells export the accumulated major osmolytes into the surrounding pure water. After centrifugation at 13,000 rpm for 3 min, the supernatant containing GB (imported into the cells from the media), ECT, and HydECT was collected as a major osmolyte sample.

2.4 Derivatization of major osmolyte samples for HPLC analysis

The major osmolytes in the sample were derivatized based on the method reported previously (Laryea et al., 1998) with a slight modification. First, 25 µl of 100 mM KH2PO4 was added to a 25 µl aliquot of major osmolyte samples, or ECT, HydECT, and GB standards. Then, 450 µl of derivatizing solution, which contains 2.5 mmol of 18-crown-6 and 50 mmol of 4-bromophenacyl bromide in acetonitrile, was added to each mixture. After mixing by vortex, the final mixture was incubated at 80 °C for 60 min. After the mixture cooled to room temperature, it was mixed again by vortex and centrifuged at 1000 x g for 5 min. Finally, a 450 µL aliquot of the supernatant from each sample was collected and filtered through a filter vial fitted with a 0.2 µm pore-size PTFE membrane (SEPARA® Syringeless filter, GVS Japan K.K., Tokyo, Japan) before being used as an HPLC sample.

2.5 HPLC analysis of the major osmolytes, ECT, HydECT, and GB

Quantification of ECT, HydECT, and GB in the samples was carried out as previously described (Laryea et al., 1998) using a high-performance liquid chromatography (HPLC) system (Shimadzu, Kyoto, Japan) equipped with a UV/VIS detector (SPD-10 A VP), an autosampler (SIL-10 AD VP), a pump (LC-10 AD VP), a degasser (DGU-14A), a system controller (SCL-10A Vp), and a column oven (CTO-10AC VP). The LabSolutions LC software (Shimadzu, Kyoto, Japan) was used to control the system and collect the data. ECT, HydECT, and GB were separated through an analytical SUPELCOSIL™ LC-SCX column (L x ID of 25 × 4.6 mm) with 5 µm particle size (Sigma–Aldrich Corp. St. Louis, MO, USA) equipped with SUPELCOSIL™ LC-SCX Supelguard™ Cartridge C18 guard column (L x ID of 2 cm × 4 mm) with 5 µm particle size (Sigma–Aldrich Corp. St. Louis, MO, USA). Mobile phases contain 22 mmol/L choline in 900 mL/L acetonitrile and 100 mL/L water. The sample injection volume was 10 µL, the flow rate was 1.5 mL/min, and the column temperature was maintained at 28 °C. The signals were detected by the UV/VIS detector at 254 nm.

3. Results and discussion

3.1 Alkaline hydrolysis of CM fertilizer into a feedstock for bacterial growth medium

As CM contains high concentrations of nutrients (Hussein et al., 2017), we saw the potential of using commercially available CM fertilizer as a feedstock for culturing bacterial cell factories. A detailed description of the alkaline hydrolysis process used for preparing CM-derived media from the fertilizer is described in Materials and Methods and Figure 2. Briefly, the CM fertilizer was hydrolyzed in 2 M NaOH solution at a high temperature (121°C for 60 min), and the resulting CM hydrolysate was neutralized to pH 7.2. After adjustment of the CM hydrolysate's salt concentration to 1 M (~6% w/v) NaCl, the CM hydrolysate was used as a 5% w/v CM feedstock for preparing 2.5% and 1.25% CM-derived media containing 6% or 15% w/v NaCl, which were used in the following growth test experiments.

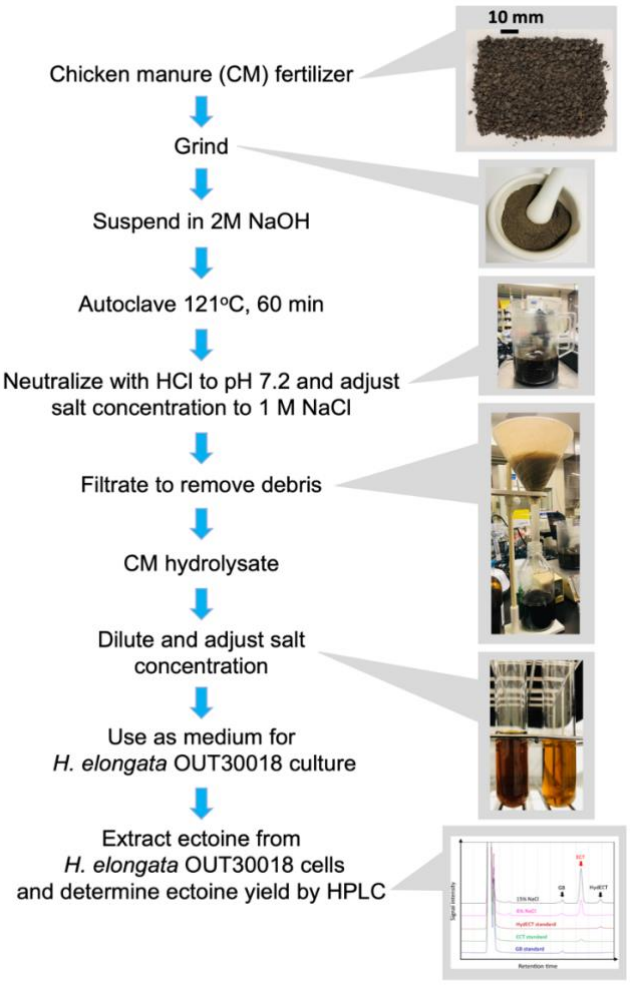

Figure 2. Schematic diagram of the alkaline hydrolysis method for converting CM fertilizer to feedstock to produce ECT and HydECT by *H. elongata* OUT30018. First, the CM fertilizer is ground to a fine powder. Then, 25 g of fine-powder CM is mixed with 250 mL of 2 M NaOH solution, and the mixture is autoclaved at 121°C for 60 min for the complete hydrolyzation to occur. After cooling down, HCl is added to neutralize the CM hydrolysate to pH 7.2; then, the total volume is adjusted to 500 mL with sterile distilled water. Debris is removed from the CM hydrolysate by filtration, and the resulting 5% w/v CM hydrolysate containing 6% w/v NaCl is used to prepare CM-derived media. These CM-derived media are used to cultivate *H. elongata* OUT30018 to produce ECT and HydECT, which are extracted from the cells by a simple osmotic down shock treatment called Bacteria Milking. After removing the cell debris, the amounts of ECT and HydECT in the cell extract are quantified by HPLC. Detailed descriptions of the alkaline hydrolysis method, medium preparation, bacteria culture condition, Bacteria Milking method, and HPLC analysis are described in the Materials and Methods section.

3.2 Effect of CM concentration in the CM-derived media on the growth and the ability to produce ECT and HydECT of *H. elongata* OUT30018

To determine whether the CM feedstock could sustain the growth of bacterial cell factory, we used CM-derived media prepared above to culture *H. elongata* OUT30018, which can produce ECT in high-salinity media containing diverse C and N sources (Vreeland et al., 1980, Tanimura et al., 2013, Nakayama et al., 2020). To find an optimal concentration of CM for *H. elongata* OUT30018's growth and ECT production, we prepared media containing 6% w/v NaCl and either 2.5% or 1.25% w/v CM from the 5% w/v CM, 6% w/v NaCl feedstock and used them to culture *H. elongata* OUT30018. As shown in Figure 3A, *H. elongata* OUT30018 culture grown in 2.5% w/v CM-derived medium reached the OD_{600} value of more than 0.8 within the first 24 hr of cultivation, while those cultured in 1.25% w/v CM-derived medium could not achieve this OD_{600} value even after 72 hr. The concentration of ECT accumulated in the *H. elongata* OUT30018 cells grown in the 2.5% w/v CM-derived medium was also slightly higher (125 µmol/g CFW) than those grown in the 1.25% w/v CM-derived medium (112 µmol/g CFW) (Figure 3B).

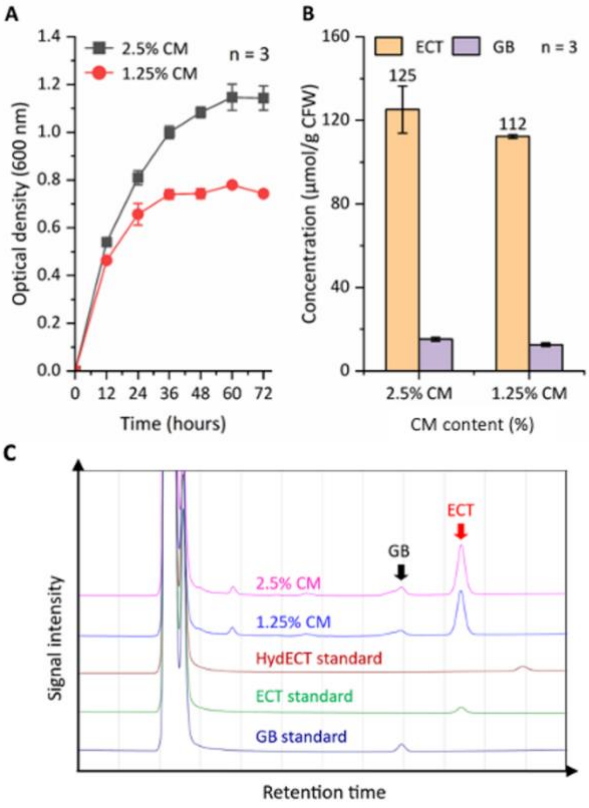

Figure 3. Effect of the concentration of CM hydrolysate in CM-derived media on the growth and major osmolytes accumulation of *H. elongata* OUT30018. *H. elongata* OUT30018 was cultured in CM-derived media containing 6% w/v NaCl and 2.5% or 1.25% w/v CM hydrolysate. Major osmolytes were extracted from the cells grown for 72 hr, and HPLC analyzed concentrations of the major osmolytes. ECT, ectoine; GB, glycine betaine.

A. Growth curves of *H. elongata* OUT30018 cultured in CM-derived media containing 6% w/v NaCl and 2.5% (■) or 1.25% (●) w/v CM hydrolysate.
B. Concentrations of major osmolytes accumulated in the cells of *H. elongata* OUT30018 cultured in CM-derived media containing 6% w/v NaCl and 2.5% or 1.25% w/v CM hydrolysate for 72 hr.

C. HPLC chromatogram showing profiles of major osmolytes accumulated in *H. elongata* OUT30018 cultures.

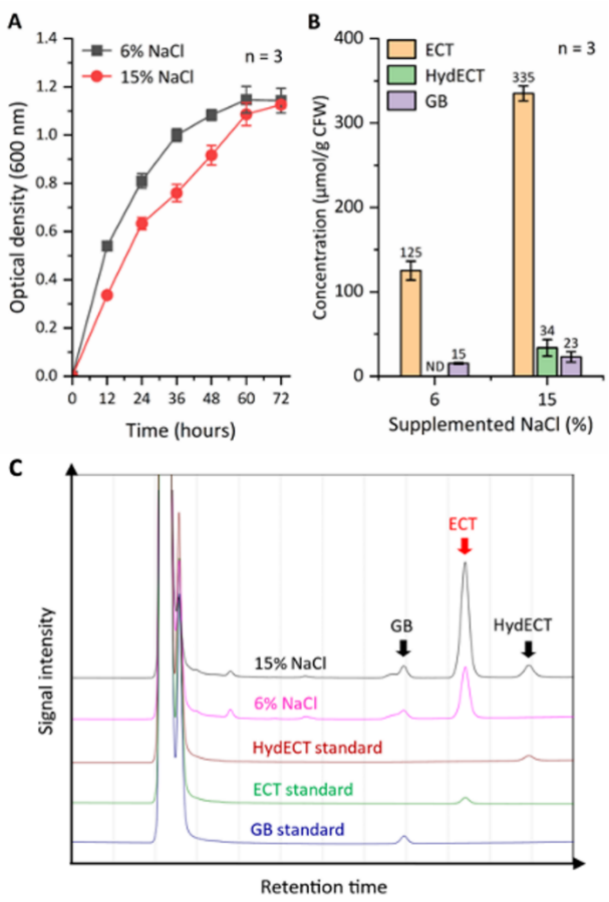

Figure 4. Effect of salinity level of the CM-derived media on the growth and major osmolytes accumulation of *H. elongata* OUT30018.

H. elongata OUT30018 was cultured in CM-derived media containing 2.5% w/v CM hydrolysate and 6% or 15% w/v NaCl. Major osmolytes were extracted from the cells grown for 72 hr, and HPLC analyzed concentrations of the major osmolytes. ECT, ectoine; HydECT, hydroxy ectoine; GB, glycine betaine.

A. Growth curves of *H. elongata* OUT30018 cultured in CM-derived media containing 2.5% w/v CM hydrolysate and 6% (■) or 15% (●) w/v NaCl.
B. Major osmolytes accumulated in the cells of *H. elongata* OUT30018 cultured in CM-derived media containing 2.5% v/v CM extract and 6% or 15% w/v NaCl for 72 hr.
C. showing profiles of major osmolytes accumulated in *H. elongata* OUT30018 cultures.

H. elongata OUT30018 cells grown in the two CM-derived media tested here also accumulated GB in their cells as one of the major osmolytes (Figure 3B). GB is a widely distributed osmolyte found in nature. It can be synthesized from exogenous choline or imported from the environment and accumulated inside the cells in response to high-salinity stress (Warr et al., 1984; Csonka, 1989; Imhoff & Rodriguez-valera, 1984; Cánovas et al., 1998). In Gram-negative bacteria, GB is accumulated in response to osmotic stress in *Escherichia coli* (Lucht and Bremer, 1994), *Salmonella* spp. (Stirling et al., 1989) and *H. elongata* (Wohlfarth and Galinski, 1990, Cánovas et al., 1996). In *H. elongata* OUT30018, GB or significant amounts of other osmolytes were not detected under the high salt conditions, ECT and HydECT were biosynthesized as major osmolytes in the cells grown in glucose-mineral medium (Ono et al., 1998). Therefore, a low concentration of GB in *H. elongata* cells grown in the CM-derived medium suggests the presence of GB in the medium. *H. elongata* OUT30018 cells can import a small amount of GB from the medium and accumulate it as one of the major osmolytes. As the accumulation of other osmolytes could potentially interfere with the production or accumulation of ECT, a strategy to inhibit the uptake of GB could improve ECT production in the *H. elongata* OUT30018 grown in media containing GB. Based on this experiment's growth and ECT production data, 2.5% w/v CM-derived medium was selected for further experiments.

3.3 Effect of salinity level of the CM-derived media on the growth and the ability to produce ECT and HydECT of *H. elongata* OUT30018

Because ECT and HydECT are accumulated in the cells of *H. elongata* OUT30018 as major osmolytes, the level of ECT and HydECT accumulation would change to adjust to the salinity level of the environment. Previously, Tanimura et al. (2013) have shown that the optimal salt concentration for ECT production by H. elongata OUT30018 was 15% w/v NaCl.

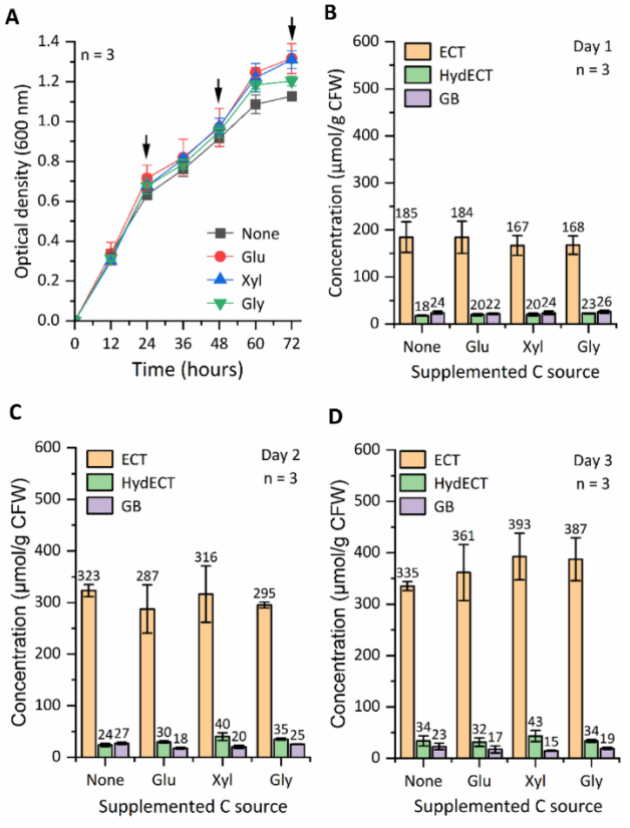

Figure 5. Effect of different C-source supplementations in the CM-derived media on the growth and major osmolytes accumulation of *H. elongata* OUT30018.

H. elongata OUT30018 were cultured in CM-derived media containing 2.5% w/v CM hydrolysate and 15% w/v NaCl, with or without C-source supplementation. Major osmolytes were extracted from the cells grown for 1, 2, or 3 days, and major osmolytes were analyzed and quantified by

HPLC. ECT, ectoine; HydECT, hydroxy ectoine; GB, glycine betaine; Glu, glucose; Xyl, xylose; Gly, glycerol.

A. Growth curve of *H. elongata* OUT30018 cultured in CM-derived media containing 2.5% w/v CM hydrolysate and 15% w/v NaCl, with no carbon-source supplementation (■), or with 4% w/v supplemented Glu (●), Xyl (▲), or Gly (▼). Arrows show sampling points.

B to D. Major osmolytes accumulated in the cells of *H. elongata* OUT30018 grown in CM-derived media containing 2.5% w/v CM hydrolysate and 15% w/v NaCl with different C-source supplementations for 1 (B), 2 (C), or 3 (D) days.

Therefore, we adjusted the salinity level of the 2.5% w/v CM-derived medium to 15% w/v NaCl and compared the growth and the ability to produce and accumulate ECT of *H. elongata* OUT30018 grown in this medium with that of the cells grown in 2.5% w/v CM-derived medium containing 6% w/v NaCl. As shown in Figure 4A, *H. elongata* OUT30018 cells grew slightly better when cultured in the medium containing 6% w/v NaCl. However, the cells grown in 2.5% w/v CM-derived medium containing 15% w/v NaCl accumulated a much higher concentration of ECT (335 µmol/g CFW) than those grown in 2.5% w/v CM-derived medium containing 6% w/v NaCl (125 µmol/g CFW) (Figure 4B). Although a low concentration of HydECT was obtained here, metabolic engineering of *H. elongata* OUT30018's genome to enhance the expression of the *ectD* gene, which encodes an enzyme ectoine hydroxylase (EHX) that converts ECT into HydECT (Liu et al., 2021), would be able to increase HydECT yield. Conversely, ECT yield could be further increased by deleting the *ectD* gene from *H. elongata* OUT30018's genome.

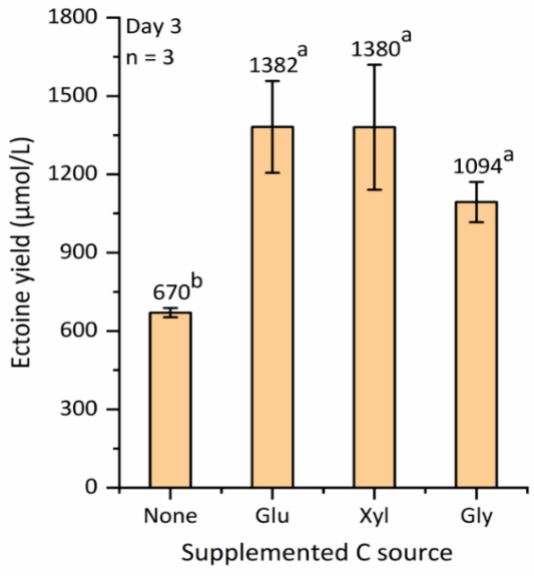

Figure 6. ECT yield was obtained from *H. elongata* OUT30018 cultures grown in CM-derived media containing 2.5% w/v CM hydrolysate, 15% NaCl, and different C-source supplementations for 3 days. ECT yield was calculated from the data shown in Figure 5. Glu, glucose; Xyl, xylose; Gly, glycerol. A one-way factor analysis of variance (ANOVA) was performed to determine significant differences between all treatments. Different letters between all columns indicate a significant difference at a 5% significance level ($p<0.05$) by Duncan's test.

3.4 Effect of carbon (C) source supplementations in the CM-derived media on the growth and ectoine accumulation of *H. elongata* OUT30018

The C:N ratio of the CM fertilizer (9.5) is lower than the C:N ratio commonly found in bacterial media. Therefore, various C sources were tested as supplements to the CM-derived medium, and the effect of C source supplementation on the growth and the ability to produce and accumulate ECT of *H. elongata* OUT30018 was evaluated. In this experiment, 4% w/v Xyl or Gly—both of which can be derived from biomass waste—were added to 2.5% w/v CM-derived medium containing 15% w/v NaCl in comparison to the addition of 4% w/v Glu, which is a C source commonly used in many bacterial culture media. As shown in Figure 5A, all supplemental C sources positively affected the growth of *H. elongata* OUT30018 cultures, with the most significant impact observed after 3 days of culturing. The amount of ECT was also at the highest on the third day (Figure 5D). Consistent with the previous finding of Tanimura et al. (2013), we also observed that Xyl is very effective as a supplementary C-source for the CM-derived medium in the production of ECT by *H. elongata* OUT30018 (Figure 5D). Finally, the results shown in Figure 6 demonstrate that modifying the CM-derived medium with supplemental C sources could significantly enhance the ECT yield from *H. elongata* OUT30018 cultures.

4. Conclusion

To develop an alternative management system for CM, we utilized a simple alkaline hydrolysis method to prepare a bacterial culture medium from CM fertilizer, which is available commercially in Japan. Compared to the primary CM management system currently employed in Japan, which involves fermenting CM into CM fertilizer for agricultural use, our management system took further simple steps to yield higher-value products and does not cause the widespread environmental contamination associated with the repeated application of CM fertilizer in agricultural fields. The potential limitation we encountered from using the alkaline hydrolysis method to prepare CM feedstock is the high salinity of the resulting CM medium. However, bacterial cell factories, which thrive in high-salinity environments such as H. elongata OUT30018, can overcome this limitation. As a proof-of-concept, we tested the ability of *H. elongata* OUT30018 to grow and accumulate valuable compounds, ECT and HydECT, in the resulting high-salinity CM-derived medium. Our results indicate that *H. elongata* OUT30018 could use nutrients in the CM-derived medium for growth and ECT/HydECT biosyntheses as efficiently as the common synthetic media (Ono et al., 1998). CM fertilizer has a low C:N ratio due in part to the degradation of organic C components of CM during the composting process by microorganisms. Therefore, we also evaluate the effect of C source supplementation in the CM-derived medium on the growth and ECT production of *H. elongata* OUT30018. By supplementing the CM-derived medium with 4% w/v Glu, Xyl, or Gly, we could significantly increase ECT yield from *H. elongata* OUT30018 cultures. The components of the CM-derived medium developed here can easily be adjusted to accommodate the growth of different halophilic and halotolerant microbial cell factories. Upon successful implementation, CM-derived media will significantly contribute to advancing the sustainability of both the poultry and the fermentation industries.

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Author Statement

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Huynh Cong Khanh: Data curation, Formal analysis, Software, Visualization, Investigation, Validation, Writing – original draft. **Pulla Kaothien-Nakayama**: Supervision, Writing – original draft, review and editing. **Hideki Nakayama**: Conceptualization, Project administration, Funding acquisition, Resources, Methodology, Data curation, Formal analysis, Investigation, Supervision, Validation, Writing-original draft, Writing – review and editing.

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