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ENVIRONMENTAL MICROBIOLOGY

The Organosulfur Compound Dimethylsulfoniopropionate (DMSP) Is Utilized as an Osmoprotectant by *Vibrio* Species

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ABSTRACT Dimethylsulfoniopropionate (DMSP), a key component of the global geochemical sulfur cycle, is a secondary metabolite produced in large quantities by marine phytoplankton and utilized as an osmoprotectant, thermoprotectant, and antioxidant. Marine bacteria can use two pathways to degrade and catabolize DMSP, a demethylation pathway and a cleavage pathway that produces the climate-active gas dimethylsulfide (DMS). Whether marine bacteria can also accumulate DMSP as an osmoprotectant to maintain the turgor pressure of the cell in response to changes in external osmolarity has received little attention. The marine halophile *Vibrio parahaemolyticus* contains at least six osmolyte transporters, namely four betaine carnitine choline transport (BCCT) carriers (BccT1 to BccT4) and two ATP-binding cassette (ABC) family ProU transporters. In this study, we showed that DMSP is used as an osmoprotectant by *V. parahaemolyticus* and by several other *Vibrio* species, including *Vibrio cholerae* and *Vibrio vulnificus*. Using a *V. parahaemolyticus proU* double mutant, we demonstrated that these ABC transporters are not required for DMSP uptake. However, a *bccT* null mutant lacking all four BCCTs had a growth defect compared to the wild type (WT) in high-salinity medium supplemented with DMSP. Using mutants possessing only one functional BCCT in growth pattern assays, we identified two BCCT family transporters, BccT1 and BccT2, that are carriers of DMSP. The only *V. parahaemolyticus* BccT homolog that *V. cholerae* and *V. vulnificus* possess is BccT3, and functional complementation in *Escherichia coli* MKH13 showed that *V. cholerae* VcBccT3 could transport DMSP. In *V. vulnificus* strains, we identified and characterized an additional BCCT family transporter, which we named BccT5, that was also a carrier for DMSP.

IMPORTANCE DMSP is present in the marine environment, produced in large quantities by marine phytoplankton as an osmoprotectant, and is an important component of the global geochemical sulfur cycle. This algal osmolyte has not been previously investigated for its role in marine heterotrophic bacterial osmotic stress response. *Vibrionaceae* species are marine species, many of which are halophiles exemplified by *V. parahaemolyticus*, a species that possesses at least six transporters for the uptake of osmolytes. Here, we demonstrated that *V. parahaemolyticus* and other *Vibrio* species can accumulate DMSP as an osmoprotectant and show that several BCCT family transporters uptake DMSP. These studies suggest that DMSP is a significant bacterial osmoprotectant that may be important for understanding the fate of DMSP in the environment. DMSP is produced and present in coral mucus, and *Vibrio* species form part of the microbial communities associated with corals. The function of DMSP in these interactions is unclear, but it could be an important driver for these associations, allowing *Vibrio* proliferation. This work suggests that DMSP likely has a more important role in heterotrophic bacteria ecology than previously appreciated.

KEYWORDS BCCT transporters, dimethylsulfoniopropionate, osmolyte, osmotic stress

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Compatible solutes (osmolytes) are accumulated by bacteria to maintain the turgor pressure of the cell in response to high external osmolarity (1–3). Known compatible solutes of bacteria include glycine betaine, ectoine, proline, glutamate, glycerol, and trehalose (2, 4–9). Compatible solutes, as the name suggests, are compounds that can be accumulated to high levels and are compatible with the molecular machinery and processes of the cell. These osmolytes allow organisms to continue to grow and divide in unfavorable environments that may be characterized by changes in osmolarity and temperature shifts (10–19). The uptake and biosynthesis of compatible solutes in response to osmotic stress has been studied extensively in *Escherichia coli* and *Bacillus subtilis*; both species can biosynthesize glycine betaine from exogenous choline and can uptake this and glycine betaine, among other osmolytes (10, 20–32).

Organisms that live in marine environments have adapted to grow optimally in high salinity and have also evolved mechanisms to maintain cellular homeostasis to cope with fluctuations in salinity that they encounter. Members of the family *Vibrionaceae* have the ability to grow optimally at 0.5 to 1.0 M NaCl, but regularly encounter fluctuations in osmolarity ranging from 0.1 to 1.5 M NaCl (33). One of the key responses of *Vibrio* species to fluctuations in osmolarity is the accumulation of compatible solutes, either through biosynthesis of the osmolytes ectoine and glycine betaine or the rapid uptake of these osmolytes from the environment using as many as six osmolyte transporters (33–35). *Vibrio parahaemolyticus*, a halophile, biosynthesizes glycine betaine from exogenously supplied choline and ectoine from aspartic acid *de novo*, and it can uptake at least 14 different compatible solutes (33–36). This species contains two osmolyte ATP-binding cassette (ABC) family transporters, ProU1 (VP1726 to VP1728) and ProU2 (VPA1112 to VPA1114), and four betaine carnitine choline transporter (BCCT) family transporters, BccT1 (VP1456), BccT2 (VP1723), BccT3 (VP1905), and BccT4 (VPA0356) (33). All BCCTs described have 12 predicted transmembrane (TM) α-helical domains, TM1 to TM12, which is a defining feature in their classification (21, 37–41). In addition to the 12 TM domains, these proteins contain hydrophilic N- and C-terminal tails of various lengths (21, 39–41). Some species, such as *Vibrio cholerae*, *Vibrio vulnificus*, and *Aliivibrio fischeri*, contain only a single BccT, a homolog of BccT3 (33). We have previously demonstrated that in *Vibrio parahaemolyticus*, BCCTs can transport glycine betaine, choline, dimethylglycine (DMG), ectoine, and proline, with some redundancy in the substrates transported by each BCCT (35, 36).

Dimethylsulfoniopropionate (DMSP) is an organosulfur compound abundant in marine surface waters that is produced by phytoplankton and some halophytic vascular plants in large quantities and used by these primary producers as an osmoprotectant, thermoprotectant, and antioxidant (42–47). Particulate DMSP levels can range from nanomolar amounts in surface water to micromolar amounts during phytoplankton blooms, and intracellular concentrations of DMSP in marine phytoplankton can range from 120 mM to 1 M (48–50). It was reported previously that DMSP is also produced in significant quantities by bacteria present in marine and estuarine sediments, as well as in seawater (51, 52). DMSP is an important component of the global geochemical sulfur cycle as a precursor for dimethylsulfide (DMS). DMS is a climate-active gas that is produced from the degradation of oceanic DMSP, releasing sulfur-containing aerosols into the atmosphere (44, 45, 53–57). Many bacteria and phytoplankton catabolize DMSP as a source of reduced sulfur and carbon using two pathways, a demethylation pathway and a cleavage pathway that produces DMS (43, 58–62). DMSP-catabolizing bacteria are mainly confined to marine *Alphaproteobacteria* taxa such as SAR11, SAR116, and *Roseobacter*, and some *Gammaproteobacteria* taxa (63–72). Given that species of *Vibrionaceae* interact and associate with DMSP producers, it is surprising that there are no studies on the use of DMSP as an osmoprotectant in these bacteria or in marine bacteria in general (3, 73–75), although studies have shown that DMSP is assimilated by cyanobacteria *Prochlorococcus* and *Synechococcus* (45, 76). The first direct evidence that DMSP can be used as an osmoprotectant for bacteria came from a study in *E. coli*, which does not encounter this compound naturally. Osmotically stressed *E. coli* bacteria responded to DMSP, and the ABC family ProU transporter was shown to take up DMSP to relieve NaCl stress (73). More recently, the Gram-positive bacterium *Bacillus* was shown to utilize DMSP as an

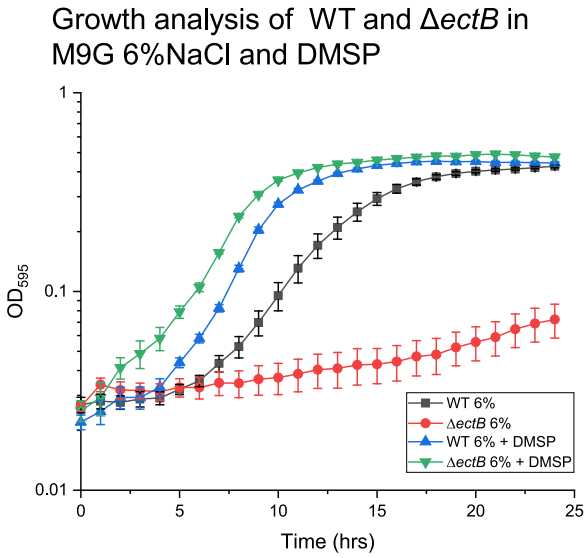

FIG 1 Growth analysis of wild-type (WT) *Vibrio parahaemolyticus* RIMD2210633 and an *ectB* mutant was conducted in M9 minimal medium supplemented with glucose (M9G) supplemented with 6% NaCl and 500 μM DMSP. Optical density at 595 nm (OD_{595}) was measured every hour for 24 h; means and standard errors of at least two biological replicates are displayed.

osmoprotectant by uptake via the ABC family transporters OpuC and OpuF (74, 77). The BCCT family transporter DddT, which is linked to DMSP cleavage pathways in *Halomonas* sp. HTNK1, was demonstrated to take up DMSP in a heterologous *E. coli* background (70, 75). Outside of these studies, little is known about bacterial utilization of DMSP as an osmolyte or the bacterial response to this abundant marine compound.

In this study, we examined *Vibrio* species for their ability to utilize DMSP as an osmoprotectant. A *V. parahaemolyticus ectB* mutant, which cannot grow in high NaCl in the absence of an exogenous osmolyte, was rescued in M9 minimal medium supplemented with glucose (M9G) 6% NaCl supplemented with DMSP. The effectiveness of DMSP as an osmoprotectant was determined in several *Vibrio* species, including *V. cholerae*, which has only a single known osmolyte transporter, and *V. vulnificus*. *Vibrio parahaemolyticus* has six osmolyte transporters, two ProUs, and four BCCTs. We examined a *proU* double mutant and a *bccT* null mutant for defects when grown under high-salt conditions with DMSP as an osmolyte. The ProU transporters did not play a significant role in DMSP uptake. The role of each of the BCCTs in DMSP uptake was investigated using four triple mutants that each contained a single *bccT* gene, showing that two BccTs were responsible for DMSP uptake. *Vibrio cholerae* contained only a single BCCT family transporter, a BccT3 homolog, and we determined its ability to take up DMSP. In *V. vulnificus*, an additional BCCT family transporter that could uptake DMSP, named BccT5, was identified.

RESULTS

***Vibrio parahaemolyticus* can utilize dimethylsulfoniopropionate as a compatible solute.** As a halophile, *V. parahaemolyticus* grows optimally in 0.5 M NaCl (~3% NaCl) and can grow in salinities up to 1.5 M (~9%) (34). We examined the ability of *V. parahaemolyticus* to grow in high-salinity conditions using DMSP as an osmolyte. The *V. parahaemolyticus ectB* deletion mutant (Δ*ectB*) cannot grow under high-NaCl growth conditions in the absence of exogenous osmolytes (34, 36). The wild type (WT) and the *ectB* mutant were grown in M9 minimal medium supplemented with glucose and 6% NaCl (M9G 6%NaCl), in the presence and absence of 500 μM DMSP at 37°C for 24 h. Growth pattern analysis showed that the lag phase of the wild-type strain without exogenous compatible solutes is ~6 h with a growth rate of 0.042 h^{-1}, while the Δ*ectB* mutant did not grow (Fig. 1). However, in M9G 6% NaCl supplemented with DMSP, the Δ*ectB* mutant grew similarly to the wild type, with growth rates of 0.082 h^{-1} and 0.074

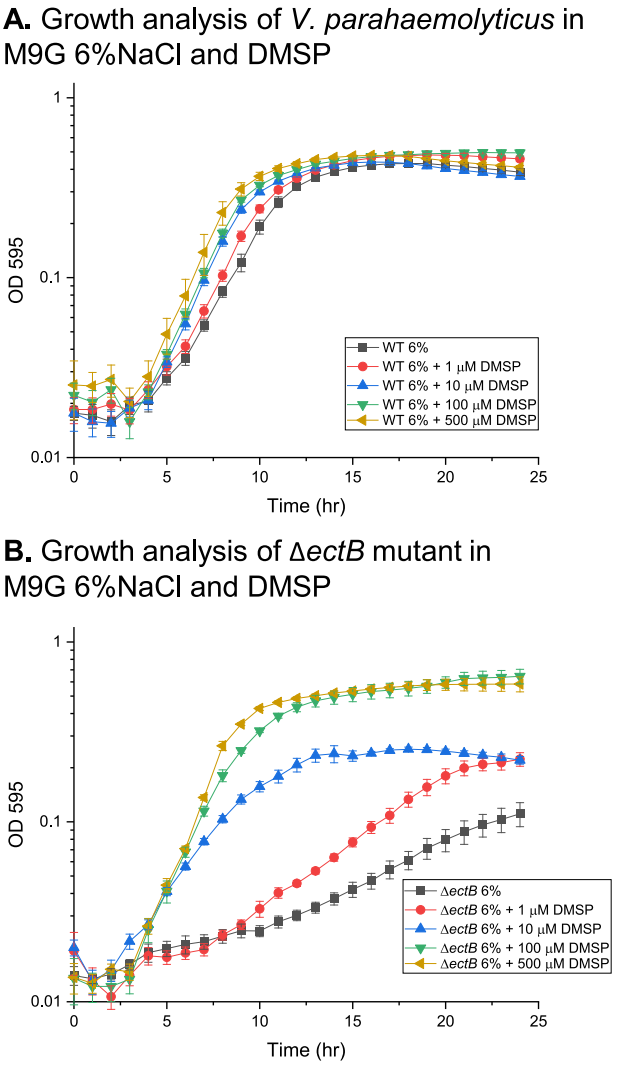

FIG 2 (A) Growth analysis of wild-type (WT) *V. parahaemolyticus* RIMD2210633 in M9G supplemented with 6% NaCl (M9G6%) and 1 μM to 500 μM DMSP. (B) Growth analysis of the Δ*ectB* mutant in M9G6% supplemented with 1 μM to 500 μM DMSP. Optical density (OD_{595}) was measured every hour for 24 h; means and standard errors of at least three biological replicates are displayed.

h^{-1}, respectively. Lag phases were reduced to ~3 h for the wild-type strain and to <1 h for the Δ*ectB* mutant (Fig. 1). It is likely that the Δ*ectB* mutant has a shorter lag phase than that of the wild type in the presence of DMSP because it does not produce ectoine, which is energetically costly (78–80). The data demonstrate that *V. parahaemolyticus* can uptake and utilize DMSP as an osmolyte.

Since DMSP levels in the environment can vary from nanomolar to micromolar amounts in algal blooms, we examined the growth of *V. parahaemolyticus* in a range of DMSP concentrations (Fig. 2). At DMSP micromolar concentrations (1, 10, 100, and 500 μM), in the wild type a shift in the growth curves was observed for all four concentrations, indicating that DMSP is taken up and utilized as an osmolyte (Fig. 2A). Similarly, we examined the rescue of the Δ*ectB* mutant and found that all four DMSP concentrations rescued the mutant, with the higher concentrations being more effective (Fig. 2B). At DMSP nanomolar concentrations (10 nM to 500 nM), a shift in the lag phase in the wild type is observed, with the greatest shift at 500 nM DMSP (see Fig. S1 in the supplemental material). The Δ*ectB* mutant was not rescued under nanomolar concentrations (data not shown). These data demonstrate that *V. parahaemolyticus* responded to physiologically relevant DMSP concentrations.

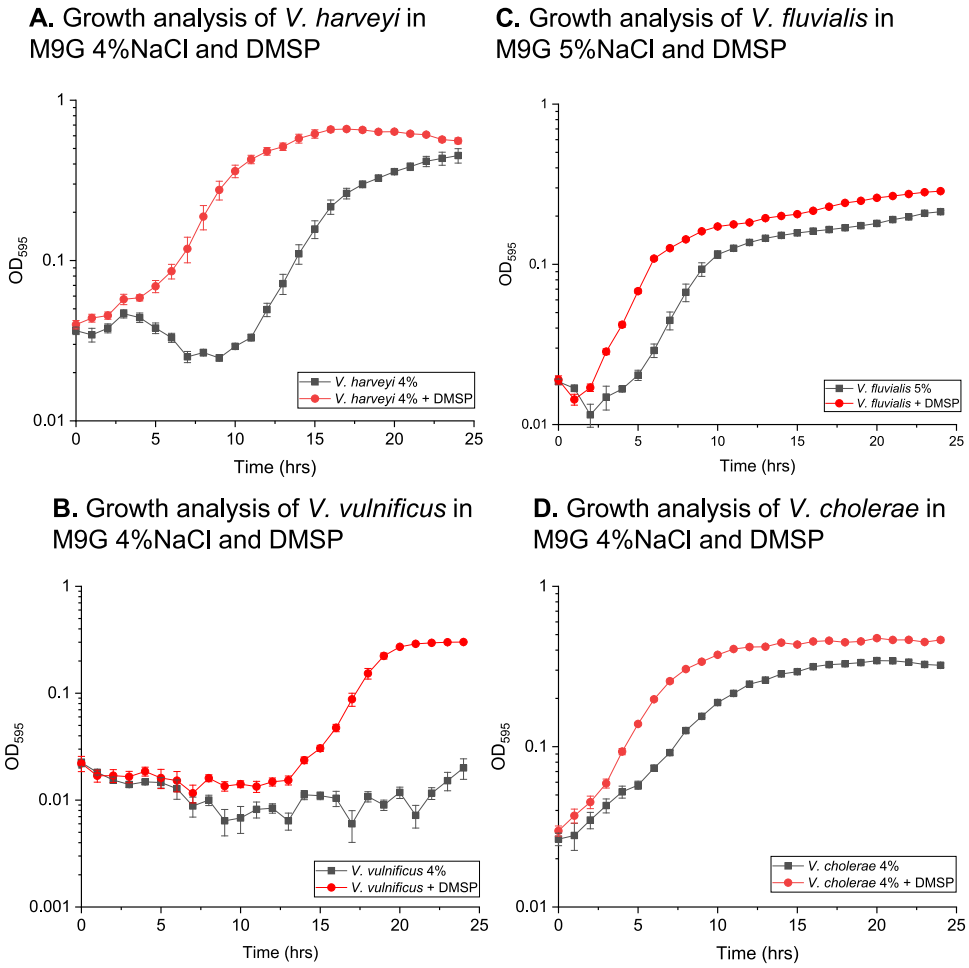

FIG 3 Growth analyses of (A) *Vibrio harveyi* 393, (B) *Vibrio vulnificus* YJ016, (C) *Vibrio fluvialis* ATCC 33809, and (D) *Vibrio cholerae* N16961 was conducted in M9G 4% NaCl (5% NaCl for *V. fluvialis*) with and without 500 μM DMSP. Optical density (OD_{595}) was measured every hour for 24 h. Means and standard errors of two biological replicates are shown.

Next, we compared the effectiveness of DMSP and glycine betaine as osmoprotectants in the wild-type and Δ*ectB* mutant strains. We compared the growth patterns of both strains in M9G 6% NaCl in 100 μM DMSP or 100 μM glycine betaine (see Fig. S2A and B in the supplemental material). Both strains responded to glycine betaine more effectively than to DMSP, exhibiting shorter lag phases and higher growth rates in glycine betaine (Fig. S2A and B).

DMSP is utilized as an osmolyte by other *Vibrio* species. To determine whether other *Vibrio* species can utilize DMSP as an osmolyte, we examined *Vibrio harveyi* 393, *V. vulnificus* YJ016, *V. cholerae* N16961, and *Vibrio fluvialis* ATCC 33809. These species are members of divergent *Vibrionaceae* clades, and each contains a different complement of osmolyte transporters. The growth of *V. harveyi* 393, *V. vulnificus* YJ016, and *V. cholerae* N16961 was examined in M9G 4% NaCl and that of *V. fluvialis* ATCC 33809 in M9G 5% NaCl, with 500 μM DMSP (Fig. 3A to D). The growth of *V. harveyi* 393 showed a 10-h lag phase with a growth rate of 0.06 h^{-1} in the absence of DMSP; however, the addition of DMSP resulted in a lag phase of less than 2 h, with a maximum growth rate of 0.088 h^{-1}. *Vibrio vulnificus* did not grow in M9G 4% NaCl, but the addition of DMSP rescued growth with a 13-h lag phase and a growth rate of 0.065 h^{-1} (Fig. 3B). *V. fluvialis* had a growth rate of 0.026 h^{-1} with a lag phase of ~4 h when grown without DMSP, which was reduced to ~2 h and the growth rate increased to 0.041 h^{-1} in the presence of DMSP (Fig. 3C). *Vibrio cholerae* cells had a reduced lag phase, from 2 h to less than 1

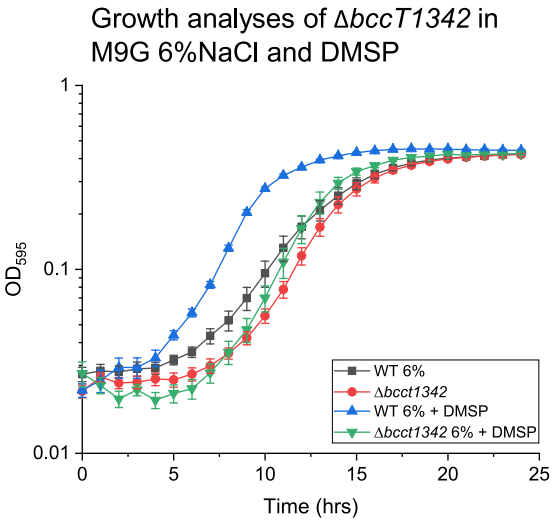

FIG 4 Growth analysis of wild-type (WT) *V. parahaemolyticus* RIMD2210633 and a Δ*bccT1* Δ*bccT3* Δ*bccT4* Δ*bccT2* mutant was conducted in M9G6% and 500 μM DMSP. Optical density (OD_{595}) was measured every hour for 24 h; means and standard errors of at least two biological replicates are displayed.

h, and an increased growth rate, from 0.034 h^{-1} to 0.059 h^{-1}, in the presence of DMSP (Fig. 3D). These data demonstrated that all *Vibrio* species tested can utilize DMSP as an osmolyte.

Some marine bacteria are able to catabolize DMSP, although this has never been shown in any *Vibrio* species (71, 72, 81). To ensure that the reduced lag phase in each *Vibrio* species tested was not due to the use of DMSP as a carbon source, we grew each strain in M9 minimal medium with DMSP as the sole carbon source, utilizing M9G (glucose as the sole carbon source) as a control. None of the *Vibrio* species tested grew with DMSP as the sole carbon source, and all grew on M9G, which indicated that they cannot utilize DMSP as a carbon source (see Fig. S3 in the supplemental material). In addition, *in silico* analysis of the published genomes of these species did not identify any known DMSP catabolism genes. These data demonstrated that DMSP appears to be a *bona fide* compatible solute for the *Vibrio* species tested.

BCCT transporters required for DMSP uptake. Previous studies in *E. coli* and *B. subtilis* showed that DMSP was transported into cells by an ABC family osmolyte transporter (73, 74). *Vibrio parahaemolyticus* contains two ABC-type osmolyte transporters, ProU1 (VP1726 to VP1728) and ProU2 (VPA1112 to VPA1114). Growth analyses with a double *proU* mutant (Δ*proU1* Δ*proU2*) were performed to determine whether either is required for DMSP uptake. In these assays, no difference between the mutant and the wild type in the absence or presence of DMSP was seen (see Fig. S4 in the supplemental material). These data demonstrated that ProU transporters were not required for uptake of DMSP in *V. parahaemolyticus*. Next, we examined whether any of the four BccTs in *V. parahaemolyticus* were responsible for the uptake of DMSP into the cell. To accomplish this, the wild type and a *bccT* null strain were grown in the presence or absence of DMSP in M9G 6% NaCl. The lag phase of the wild-type strain was reduced from ~6 h to ~3 h when grown in the presence of DMSP, which indicates that DMSP is an osmoprotectant for *V. parahaemolyticus* (Fig. 4). However, the *bccT* null mutant did not exhibit a reduced lag phase in the presence of DMSP (Fig. 4). This indicated that at least one of the BCCTs is responsible for transport of DMSP into the cell and confirms that neither ProU plays a significant role in uptake of DMSP.

To determine which of the BCCTs were responsible for uptake of DMSP, four triple *bccT* deletion mutants were utilized, each containing only one functional *bccT* gene. The Δ*bccT2* Δ*bccT3* Δ*bccT4* mutant, which expresses only *bccT1* (VP1456), had a slight reduction in the lag phase and had a higher growth rate through the exponential

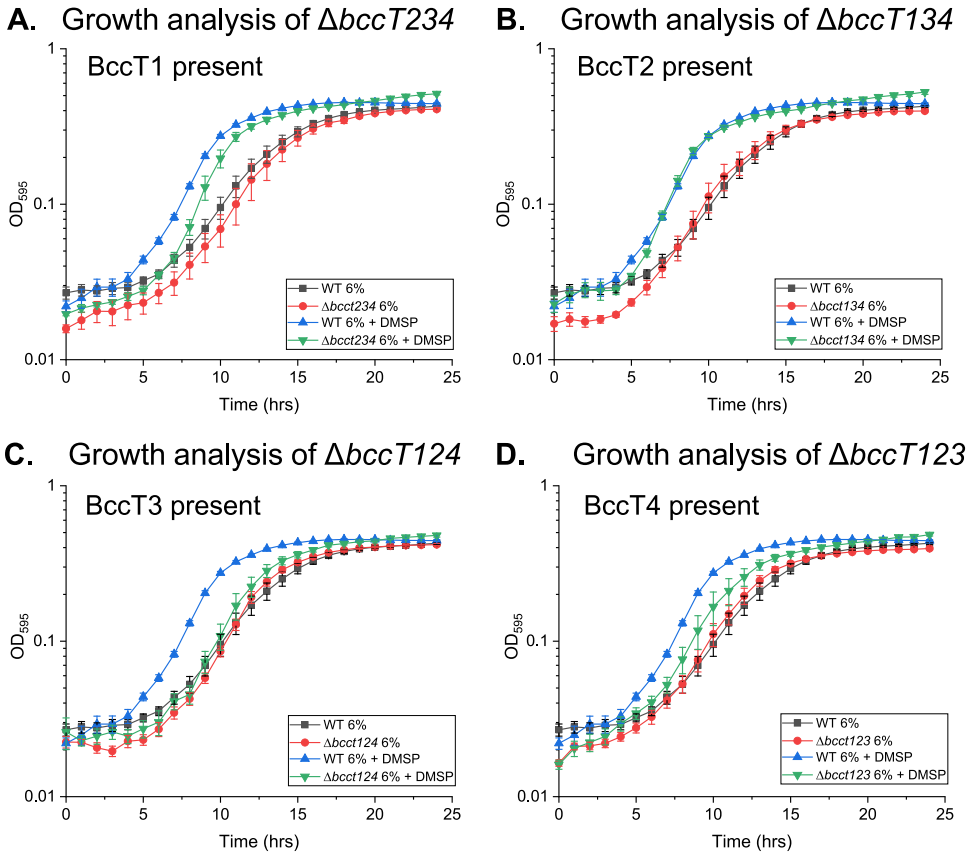

FIG 5 DMSP uptake analysis of wild-type (WT) *V. parahaemolyticus* RIMD2210633 and (A) Δ*bccT2* Δ*bccT3* Δ*bccT4*, (B) Δ*bccT1* Δ*bccT3* Δ*bccT4*, (C) Δ*bccT1* Δ*bccT2* Δ*bccT4*, or (D) Δ*bccT1* Δ*bccT2* Δ*bccT3* in M9G6% with and without the addition of 500 μM DMSP. Optical density (OD_{595}) was measured every hour for 24 h; means and standard errors of at least two biological replicates are displayed.

phase when grown in the presence of DMSP. This suggests that BccT1 can transport DMSP with low efficiency (Fig. 5A). The Δ*bccT1* Δ*bccT3* Δ*bccT4* mutant, which expresses only *bccT2* (VP1723), showed a nearly identical reduction in the lag phase as that in the wild-type strain, which indicated that it also transports DMSP into the cell (Fig. 5B). The Δ*bccT1* Δ*bccT2* Δ*bccT4* mutant, which expresses only *bccT3* (VP1905), and Δ*bccT1* Δ*bccT2* Δ*bccT3*, which expresses only *bccT4* (VPA0356), had slight reductions in the lag phase in the presence of DMSP (Fig. 5C and D), which indicated that BccT3 and BccT4 do not play a significant role in DMSP transport in *V. parahaemolyticus*.

Next, we examined the DMSP transport capabilities of each BccT using functional complementation in *E. coli* MKH13, a mutant strain that has deletions in *betIBA-betT*, *proU*, *proP*, and *putP* and cannot grow in M9G 4% NaCl (82). Strains were grown in M9G 4% NaCl in the presence of 500 μM DMSP, with an arabinose-inducible expression plasmid, pBAD33, harboring a full-length copy of a single *bccT*. We measured the optical density at 595 nm (OD_{595}) after 24 h for each complemented strain and compared the values to that of the empty vector strain. Only the BccT2-complemented strain (pBAVP1723) could grow in the presence of DMSP (Fig. 6A). The *E. coli* BccT1-complemented strain (pBAVP1456) was unable to grow in the presence of DMSP (Fig. 6A). In *V. parahaemolyticus* expressing only BccT1, DMSP was taken up, though the shift in growth was not as significant as that when BccT2 was expressed, suggesting that it is not an efficient DMSP transporter (Fig. 5A). To confirm that *bccT1* is expressed and produces a functional protein in *E. coli*, we examined the ability of BccT1 to take up glycine betaine, a known substrate for this transporter. In this assay, *E. coli* pBAVP1456 grew with the addition of glycine betaine, which indicated that BccT1 is functional (Fig. 6B).

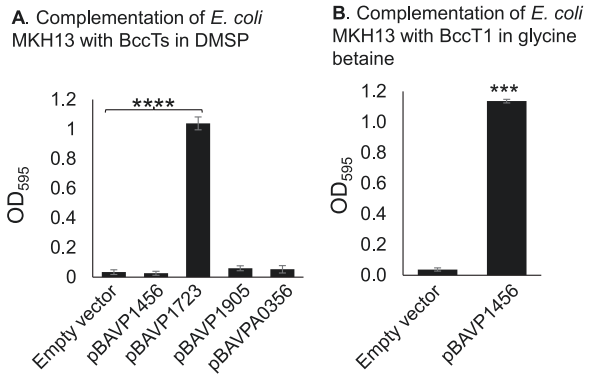

FIG 6 *E. coli* strain MKH13, which has deletions in all compatible solute transporters, was grown in M9G supplemented with 4% NaCl and functionally complemented with *V. parahaemolyticus* RIMD2210633 (A) *bccT1* (pBAVP1456), *bccT2* (pBAVP1723), *bccT3* (pBAVP1905), or *bccT4* (pBAVPA0356). Strains were grown for 24 h in the presence of 500 μM DMSP and the final optical density (OD_{595}) was compared to that of a strain harboring empty pBAD33. (B) *E. coli* MKH13 complemented with *bccT1* was grown in the presence of 500 μM glycine betaine and compared to a strain harboring empty pBAD33. Means and standard errors of at three biological replicates are shown. Statistics were calculated using Student's *t* test (***, $P < 0.001$; ****, $P < 0.0001$).

It is important to note that functional complementation assays do not provide information about efficiency of transport of a particular substrate, only that the substrate in question is able to be imported into the cell. Here, we have shown that only BccT2 is capable of transporting DMSP in the *E. coli* background.

BccT3 from *V. cholerae* is a DMSP transporter. Of the four additional *Vibrio* species that used DMSP as an osmolyte, *V. harveyi* and *V. fluvialis* contain homologs of BccT1 and BccT2, which transport DMSP in *V. parahaemolyticus*. However, *V. vulnificus* contains homologs of only BccT3 and ProU2, while *V. cholerae* possesses only a BccT3 homolog and no ProU transporter. Given that *V. vulnificus* and *V. cholerae* can utilize DMSP as an osmolyte and contained only a BccT3 homolog, we cloned *V. vulnificus bccT3* (VV2103 and VVbccT3) and *V. cholerae bccT3* (FY484_RS06475 and VCbccT3) homologs into the expression plasmid pBAD33 and performed functional complementation assays in *E. coli* strain MKH13. We tested the growth of MKH13 in the presence of glycine betaine to determine whether these transporters were functional and found that both were glycine betaine carriers, as evidenced by growth of the MKH13 strain in M9G4% (Fig. 7A). Next, we examined DMSP uptake by these transporters and showed that VCBccT3 functionally complemented MKH13, while VVBccT3 did not (Fig. 7B). Thus, the *V. cholerae* VCBccT3 is functional and can transport DMSP into the cell.

BccT5, an additional BCCT transporter present in *V. vulnificus*. Since VVBccT3 did not transport DMSP, we reexamined the *V. vulnificus* genome and identified an additional BCCT family transporter that shared <32% homology with BCCTs from *V. parahaemolyticus*. This BCCT, which we named BccT5 (VV0783), was annotated as a 637-amino acid protein. It was demonstrated via hydropathy profile analysis to have 12 TM domains, a feature of all BCCT transporters (see Fig. S5 in the supplemental material). *E. coli* MKH13 complemented with VVBccT5 was unable to grow in the presence of 500 μM DMSP and grew poorly in the presence of 500 μM glycine betaine (data not shown) in M9G 4% NaCl. Further analysis identified an alternative start codon in VV0783, which resulted in a larger 672-amino-acid protein, which was used in functional complementation of *E. coli* MKH13 and resulted in growth in the presence of either glycine betaine or DMSP (Fig. 7). It is interesting to note that the N-terminal tail of BccT5 appears to be required for full functionality of the transporter. Overall, the data show that *Vibrio* species have at least four BCCT family transporters that are capable of DMSP uptake.

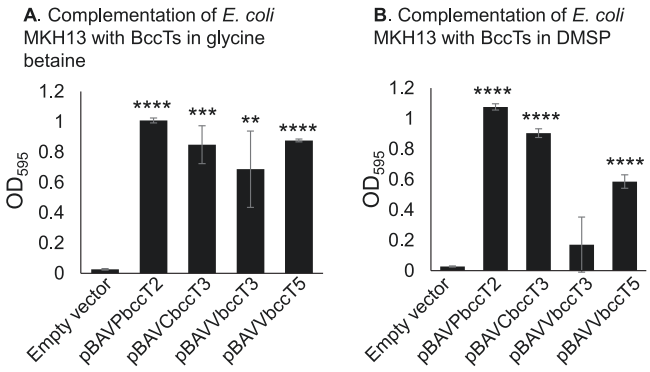

FIG 7 *E. coli* strain MKH13 was grown in M9G supplemented with 4% NaCl and functionally complemented with VP*bccT2* (pBAVP1723), VC*bccT3*, VV*bccT5*, or VV*bccT5*. Strains were grown for 24 h in the presence of (A) 500 μM glycine betaine or (B) 500 μM DMSP, and the final optical density (OD_{595}) was compared to that of a strain harboring empty pBAD33 expression vector. Means and standard errors of three biological replicates are shown. Statistics were calculated using Student's *t* test (**, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$).

DISCUSSION

Bacteria have adapted to hyperosmotic conditions by changing the level of osmolytes in their cells to maintain turgor pressure. The most prevalent and energetically effective method to maintain turgor pressure is the uptake of osmolytes from the surrounding environment. DMSP is produced in vast quantities by marine phytoplankton and utilized as an osmoprotectant for these primary producers. The concentrations of DMSP in the environment reach micromolar amounts during algal blooms, and many marine bacteria associate with phytoplankton during these blooms. The contribution of DMSP to the bacterial osmotic stress response is largely unknown. Our studies demonstrate that DMSP can play a significant role in adaptation to osmotic stress by the uptake of DMSP as an osmolyte. Additionally, we demonstrated that the BCCT family of transporters are important components of DMSP uptake for use as an osmolyte, and BccT transporters are widespread among *Vibrionaceae*.

Here, we have shown that marine bacteria in the genus *Vibrio* can use DMSP as an osmolyte demonstrating the ecological significance of this compound to marine bacteria. Our studies demonstrate a more widespread use of DMSP as an osmolyte among marine bacteria that was previously unrecognized. BCCT family transporters were solely responsible for the uptake of DMSP by *V. parahaemolyticus*, and among *Vibrio* species, four different BCCTs could uptake DMSP. The ABC family transporters ProU1 and ProU2 did not contribute significantly to DMSP uptake, suggesting that BCCTs likely play a major role in DMSP uptake in *Vibrio* species. BCCT family proteins were highly prevalent among *Vibrio* with most species containing multiple BccT proteins.

Recently, an increased incidence of algal blooms has led to increased *V. parahaemolyticus* and *V. vulnificus* proliferation (83–85). Our data show that *V. parahaemolyticus* does not utilize DMSP as a carbon source, but it can grow and divide rapidly in high salinity when exogenous DMSP is present. We speculate that the ability of *V. parahaemolyticus* and other *Vibrio* species to utilize DMSP as a compatible solute increases their ability to proliferate in algal blooms that occur both under high salinity conditions and in warmer months. Dinoflagellates are some of the main producers of DMSP, and *V. parahaemolyticus* proliferation has been found in conjunction with dinoflagellate algal blooms (84, 85). Likewise, *V. vulnificus* can grow and divide rapidly in higher salinity when exogenous DMSP is present, likely contributing to its fitness. Coral reefs are often described as DMSP hot spots, and DMSP production has been linked to the coral stress response (86). Several *Vibrio* species are associated with coral disease such as *Vibrio coralliilyticus* that infects reef-building corals and *Vibrio mediterranei* (*Vibrio shilonii*) associated with coral deaths worldwide (87–90). Our *in silico* analysis of the *V. coralliilyticus* genome sequences identified five BccT proteins, which included a copy present on a plasmid prevalent among strains. This suggests a mechanism of horizontal transfer of

TABLE 1 Strains and plasmids used in this study

Strain or plasmid	Genotype or description[a]	Reference(s) or source
Strain		
Vibrio parahaemolyticus		
RIMD2210633	O3:K6 clinical isolate, Str^r	15, 98
Δ*ectB*	RIMD2210633 Δ*ectB* (VP1721), Str^r	33
SOYBCCT124	RIMD2210633 ΔVP1456 ΔVP1723 ΔVPA0356, Str^r	35
SOYBCCT123	RIMD2210633 ΔVP1456 ΔVP1723 ΔVP1905, Str^r	35
SOYBCCT134	RIMD2210633 ΔVP1456 ΔVP1905 ΔVPA0356, Str^r	35
SOYBCCT234	RIMD2210633 ΔVP1723 ΔVP1905 ΔVPA0356, Str^r	35
SOYBCCT1342	RIMD2210633 ΔVP1456 ΔVP1723 ΔVP1905 ΔVPA0356, Str^r	35
Δ*proU1*	RIMD2210633 Δ*proU1*, Str^r	33
Δ*proU1*Δ*proU2*	RIMD2210633 Δ*proU1* Δ*proU2*, Str^r	This study
Vibrio harveyi 393	Isolated from barramundi in Australia	99
Vibrio fluvialis ATCC 33809	[NCTC 11327][606] Clinical isolate	100
Vibrio vulnificus YJ016	Clinical isolate	101
Vibrio cholerae N16961	O1, El Tor strain, Bangladesh, clinical, 1975	102
Escherichia coli		
DH5α λpir	Δ*lac pir*	
β2155 λpir	Δ*dapA*::*erm pir* for bacterial conjugation	103
MKH13	MC4100 (Δ*betTIBA*) Δ(*putPA*)*101* Δ(*proP*)*2* Δ(*proU*); Sp^r	82
Plasmids		
pBAD33	Expression vector; *araBAD* promoter; Cm^r; p15a origin	94
pBAVP1456	pBAD33 harboring full-length VP1456 (*bccT1*)	36
pBAVP1723	pBAD33 harboring full-length VP1723 (*bccT2*)	35
pBAVP1905	pBAD33 harboring full-length VP1905 (*bccT3*)	35
pBAVPA0356	pBAD33 harboring full-length VPA0356 (*bccT4*)	36
pBAVCBccT3	pBAD33 harboring full-length BccT3 from *V. cholerae*	This study
pBAVVBccT3	pBAD33 harboring full-length BccT3 from *V. vulnificus*	This study
pBAVVBccT5	pBAD33 harboring 637-amino-acid BccT5 from *V. vulnificus*	This study
pBAVVBccT5long	pBAD33 harboring 672-amino-acid BccT5 from *V. vulnificus*	This study

[a]Str, streptomycin; Sp, spectinomycin; Cm, chloramphenicol; ^{r}, resistance.

BccT among *Vibrio*. In addition, in *V. coralliilyticus* CN52H-1, a strain recovered from the surface mucus layer of the coral *Colpophyllia natans*, we identified a BccT protein that clustered with a putative DMSP lyase gene, suggesting a potential for this species to metabolize DMSP. In the *V. mediterranei* genome sequences, we identified up to eight different BCCTs, which included homologs of BccT1 to BccT5, suggesting that these species have the ability to take up and utilize DMSP as an osmolyte that could enhance their association with corals. Indeed, it has been proposed that the concentrations of DMSP and DMS in corals drives the structure of the microbial community associated with them (91). In fact, it was reported that DMSP alone was sufficient to elicit a chemotactic response from *V. coralliilyticus*, and this response was increased at higher temperatures, when heat-stressed corals emit five times as much DMSP (92). This highlights the importance of DMSP in marine communities, and our work suggests that the BCCT family transporters play a significant role in these interactions. It will be interesting to learn whether *Vibrio* species associated with coral, such as *V. coralliilyticus*, can also use DMSP as an osmolyte and/or for thermoprotection.

MATERIALS AND METHODS

Bacterial strains, media, and culture conditions. All strains and plasmids used in this study are listed in Table 1. A streptomycin-resistant *V. parahaemolyticus* RIMD2210633 was used as the wild-type (WT) strain. *Vibrio parahaemolyticus* were grown either in lysogeny broth (LB) (Fisher Scientific, Fair Lawn, NJ) with 3% (wt/vol) NaCl (LB3%) or M9 minimal medium (47.8 mM Na_2HPO_4, 22 mM KH_2PO_4, 18.7 mM NH_4Cl, and 8.6 mM NaCl; Sigma-Aldrich) supplemented with 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, and 20 mM glucose as the sole carbon source (M9G) and NaCl (wt/vol), as indicated. Dimethylsulfoniopropionate (DMSP) was used at a final concentration of 20 mM when supplied as a carbon source. *E. coli* strains were grown in either LB supplemented with 1% NaCl (LB1%) or M9G supplemented with 1% NaCl (M9G1%). All strains were grown at 37°C with aeration. Chloramphenicol (Cm) was used at a final concentration of 25 μg/ml as necessary.

TABLE 2 Primers used in this study

Primer name	Sequence (5′–3′)[a]	Length (bp)[b]
VCBCCT3F	tgggctagcgaattcgagctTGATTAAAAAACCGAAAATGCTTTG	1,675
VCBCCT3R	ggatccccgggtaccgagctTTAAGATTGGTGAAGACGCTTG	
VVBCCT3F	tgggctagcgaattcgagctGGCTGTATTTTCAAAATTAGTTAG	1,630
VVBCCT3R	ggatccccgggtaccgagctTTATTGCTTCTCGGTGCG	
VVBCCT5F	tgggctagcgaattcgagctTATCGAACAATCGGTATTTCATAC	1,966
VVBCCT5R	ggatccccgggtaccgagctTTAACTGAGTGATTCTGACTGAAG	
VVBCCT5Flong	tgggctagcgaattcgagctAAGCAATTACTCACCAACCAATTTAG	2,069
VVBCCT5Rlong	ggatccccgggtaccgagctTTAACTGAGTGATTCTGACTGAAG	
VPA1109A	GCATGCTGCCATTGCCGCACCAATC	355
VPA1109B	CAGCTGAGATCTGGTACCGTCCATGATTAAGCCTCCT	
VPA1109C	GGTACCAGATCTCAGCTGGCTTAGTTGAGATAAACGT	380
VPA1109D	GAGCTCCTTCAACGGTCATTGGAC	
VPA1109FL-F	CATTGTACCGGGACT	2,008
VPA1109FL-R	AGCAGATATGTACAACACC	

[a]Lowercase letters indicate complementary regions for Gibson assembly.
[b]Length (bp) indicates length of PCR product for each primer pair.

Mutant strain construction. The Δ*proU1* Δ*proU2* double mutant was constructed by creating an in-frame deletion of *proU2* in a *proU1* background (33). Splicing by overlap extension PCR and allelic exchange were performed as previously described (93). *V. parahaemolyticus* RIMD2210633 genomic DNA template and splicing by overlap extension (SOE) primers listed in Table 2 were used in the SOE PCR amplification to create a truncated *proU2* PCR fragment of 750 bp in size by removing ~1,150 bp from the wild-type *proU2* gene (1,900 bp). The truncated *proU2* fragment was subsequently cloned into a pDS132 suicide vector, transformed in *E. coli* β2155 λpir DAP auxotroph, and mobilized into a recipient wild-type *V. parahaemolyticus* RIMD2210633 strain by conjugation (33). The generated *V. parahaemolyticus* mutant strain harboring a truncated version of the *proU2* gene was designated Δ*proU2*. To create the double mutant Δ*proU1* Δ*proU2* strain, a *V. parahaemolyticus* Δ*proU2* strain was used as a background, and a previously created Δ*proU1* inserted into the Δ*proU2* background by conjugation and homologous recombination. All deletions were confirmed by PCR and sequencing.

Growth analysis of mutant strains in DMSP. *V. parahaemolyticus* RIMD2210633 or an in-frame deletion mutant of *ectB* were grown overnight in M9G1%. Cultures were subsequently diluted 1:50 into fresh medium, grown for 5 h, and used as previously described for analysis of DMG as an osmolyte (36). These strains, the *proU* double mutant, and a quadruple Δ*bccT1* Δ*bccT3* Δ*bccT4* Δ*bccT2* mutant (*bccT* null) strain exponential culture were then diluted 1:40 into 200 μl of M9G6% medium with and without exogenous compatible solutes in a 96-well microplate and grown at 37°C with intermittent shaking for 24 h. Dimethylsulfoniopropionate (DMSP) was added to a final concentration of 500 μM. Growth analysis was repeated following the above procedure with each of four triple *bccT* deletion mutants, Δ*bccT2* Δ*bccT3* Δ*bccT4*, Δ*bccT1* Δ*bccT3* Δ*bccT4*, Δ*bccT1* Δ*bccT2* Δ*bccT4*, and Δ*bccT1* Δ*bccT2* Δ*bccT3*, to determine which BCCT was responsible for transport of DMSP.

Functional complementation of *E. coli* strain MKH13. The 1,623-bp *opuD* gene (*bccT3* homolog) was amplified from the *V. cholerae* N16961 genome using the primers listed in Table 2. The 1,572-bp *bccT3* homolog, the 1,914-bp *bccT5*, and the 2,016-bp *bccT5* gene were amplified from the *V. vulnificus* YJ016 genome using primers listed in Table 2. All primers were purchased from Integrated DNA Technologies (Coralville, IA). Gibson assembly protocol using NEBuilder HiFi DNA assembly mastermix (New England Biolabs, Ipswich, MA) was followed to ligate the amplified fragments with the expression vector pBAD33 (94), which had been linearized with SacI. Regions of complementarity for Gibson assembly are indicated by lowercase letters in the primer sequences in Table 2. The resulting expression plasmids, pBAVCbccT3, pBAVVbccT3, pBAVVbccT5, and pBAVVbccT5long, were transformed into *E. coli* Dh5α cells for propagation. Plasmids were then purified, sequenced, and subsequently transformed into *E. coli* strain MKH13, which has large deletions that include compatible solute transporters (*putP*, *proP*, and *proU*) and the choline uptake and glycine betaine biosynthesis loci (*betT-betIBA*) (82).

E. coli MKH13 strains containing pBAD expressing a single *bccT* gene were grown overnight in minimal medium supplemented with 1% NaCl and 20 mM glucose (M9G1%) with chloramphenicol and subsequently diluted 1:100 into M9G supplemented with 4% NaCl (M9G4%) and 500 μM the indicated compatible solute and chloramphenicol. Expression of each BCCT was induced with 0.01% arabinose, and functional complementation was determined by measuring OD_{595} after 24 h of growth at 37°C with aeration. Growth was compared to that of an MKH13 strain harboring empty pBAD33, which cannot grow in M9G4% without exogenous compatible solutes. Statistics were calculated using Student's *t* test.

Bioinformatics analyses. Transmembrane helix probabilities of *V. parahaemolyticus* BccT1 (GenPept accession number Q87PP5.1) and *V. vulnificus* BccT5 (GenPept accession number BAC93547.1) were generated using OCTOPUS and aligned via the AlignMe program (http://www.bioinfo.mpg.de/AlignMe) (95–97).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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