Comparative genomics and proteomics of *Eubacterium maltosivorans*: functional identification of trimethylamine methyltransferases and bacterial microcompartments in a human intestinal bacterium with a versatile lifestyle

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Summary

*Eubacterium maltosivorans* YIT is a human intestinal isolate capable of acetogenic, propionogenic and butyrogenic growth. Its 4.3-Mb genome sequence contains coding sequences for 4227 proteins, including 41 different methyltransferases. Comparative proteomics of strain YIT showed the Wood–Ljungdahl pathway proteins to be actively produced during homoacetogenic growth on H2 and CO2 while butyrogenic growth on a mixture of lactate and acetate significantly upregulated the production of proteins encoded by the recently identified lctABCDEF cluster and accessory proteins. Growth on H2 and CO2 unexpectedly induced the production of two related trimethylamine methyltransferases. Moreover, a set of 16 different trimethylamine methyltransferases together with proteins for bacterial microcompartments were produced during growth and deamination of the quaternary amines, betaine, carnitine and choline. Growth of strain YIT on 1,2-propanediol generated propionate with propanol and induced the formation of bacterial microcompartments that were also prominently visible in betaine-grown cells. The present study demonstrates that *E. maltosivorans* is highly versatile in converting low-energy fermentation end-products in the human gut into butyrate and propionate whilst being capable of preventing the formation of the undesired trimethylamine by converting betaine and other quaternary amines in bacterial microcompartments into acetate and butyrate.

Introduction

The human intestinal tract is a natural reservoir of dynamic and diverse microbial communities with important developmental, nutritional and protective functions that are tightly linked to health and disease. The vast majority of the intestinal species are anaerobic bacteria which salvage nutrients and energy by fermenting complex carbohydrates into short-chain fatty acids (SCFAs), other organic acids such as formate and lactate, and gases, including H2 and CO2 (Rajilić-Stojanović and de Vos, 2014; Louis and Flint, 2017).

Lactate is another intermediate of carbon metabolism, produced by lactic acid and other abundant bacteria in the human intestinal tract (Smith and Macfarlane, 1997; Barcenilla et al., 2000). Lactate accumulation could lead to detrimental consequences, such as acidosis and neurotoxicity whilst increased colonic levels are associated with colitis in patients with inflammatory bowel disease (Hove et al., 1994; Hove and Mortensen, 1995; Mortensen and Clausen, 1996; Kowlgi and Chhabra, 2015). Therefore, both isomers of lactate must be efficiently depleted by intestinal microbes. Lactate can be utilized by sulfate- or sulfite-reducing bacteria like *Desulfovibrio*,

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Bilophila and Clostridium sp. resulting in elevated hydrogen sulfide levels, which could potentially cause colonic pain and gastrointestinal discomfort in both infants and adults (Chassard et al., 2012; Feng et al., 2017; Pham et al., 2017). Lactate can also be consumed by butyrate-producing bacteria in presence of acetate as isotope labelling studies have identified lactate and acetate as important precursors of butyrate production in mixed gut communities (Bourriaud et al., 2005; Muñoz-Tamayo et al., 2011). Intestinal bacteria that can perform this low energy metabolic conversion mostly belong to Clostridium cluster XIVa, including Anaerostipes spp. and Anaerobutyricum spp. (previously known as Eubacterium hallii) (Duncan et al., 2004; Shetty et al., 2020). The pathway of butyrate production from lactate and acetate has been recently elucidated in Anaerobutyricum and Anaerostipes spp. (Shetty et al., 2020).

Acetate, propionate and butyrate – the most abundant SCFAs – can fuel the intestinal epithelium cells and confer health benefits via a variety of pathways (Tremaroli and Bäckhed, 2012; Fan and Pedersen, 2021). Moreover, all intestinal SCFAs are known to signal to G-protein coupled receptors, such as free fatty acid receptor 2 (FFAR2) also known as G-protein coupled receptor 43, and have specific health-promoting effects, among others via the production of anti-oxidative effects (Hinnebusch et al., 2002; Klampfer et al., 2003). Notably, butyrate-producing bacteria have gained increasing attention as butyrate plays an important role in maintaining gut homeostasis and intestinal epithelial integrity (Hamer et al., 2008). Butyrate is also the major substrate for colonocytes and is reported to have anti-carcinogenic, anti-inflammatory and anti-oxidative effects (Hinnebusch et al., 2002; Klampfer et al., 2003). Interestingly, decreased amounts of butyrogenic bacteria have been observed in inflammatory bowel diseases, colon cancer and type 2 diabetes (Qin et al., 2010; Flint et al., 2012; De Vos and Nieuwdorp, 2013; Rajlić-Štojanović et al., 2013).

Propionate has high affinity for FFAR2 of all SCFAs and its role in promoting health effects as well as increasing satiety has been described (Chambers et al., 2015). Propionate can be produced from sugars via various pathways, but several intestinal species can produce this C3 compound from 1,2-propanediol (1,2-PDO), generated from methylpentoses such as fucose or rhamnose (Louis and Flint, 2017). The conversion of 1,2-PDO proceeds through a pathway that generates toxic intermediates that are encapsulated in self-assembling organelles termed bacterial microcompartments (BMCs) that are separated from the cytosol (Kerfeld et al., 2018). These BMCs are typically produced when needed and are thought to include a respiratory electron acceptor and a vitamin B12 cofactor (Cheng et al., 2011; Thompson et al., 2014).

Besides carbohydrate fermentation in the human intestinal tract the metabolism of many other compounds present in our diet is highly relevant since some may generate undesired products (Fan and Pedersen, 2021). These include quaternary amines such as betaine, choline and l-carnitine, which are commonly found in vegetables, fruits, meat and seafood and are well-known bacterial osmoprotectants (Sleator and Hill, 2002; Zeisel et al., 2003; Demarquoy et al., 2004). Several gut bacteria can convert these quaternary ammonium ions into trimethylamine (TMA), which is converted in the liver into trimethylamine-N-oxide (TMAO) that has been implied in atherosclerosis and cardiovascular risks (Brown and Hazen, 2014). Hence there is considerable interest in understanding the metabolism of these quaternary ammonium ions into compounds that do not lead to TMA or other TMAO precursors.

Recently, we isolated and described a novel human intestinal species Eubacterium maltosivorans YT2 affiliated with the Clostridium cluster II, which is capable of converting H2 and CO2, as well as methanol and formate with weak growth, into acetate (Feng et al., 2018). Besides homoacetogenic growth, it was able to ferment a range of monomeric sugars and disaccharides, including maltose, with acetate and butyrate as the main end products. Moreover, E. maltosivorans can also convert lactate and acetate into butyrate and 1,2-PDO into propionate. These properties are unique for the gut ecosystem and link three important metabolic processes, homoacetogenesis, propionigenes and butyrogenesis in a single human species. Some of these features were predicted by genomic characterization of the phylogenetically closely related Eubacterium limosum strain ATCC 8486 isolated from human colon (Song et al., 2017), strain KIST612 isolated from an anaerobic digester (Roh et al., 2011), and strain SA11 obtained from sheep’s rumen (Kelly et al., 2016), the latter being considered to belong to the new species of E. maltosivorans (Feng et al., 2018). Eubacterium limosum and E. maltosivorans strains are capable of using a variety of C1 compounds, such as methanol, CO, as well as H2, with CO2, and their demethylating activity is high (Gentner et al., 1981; DeWeerd et al., 1988). These activities in the intestinal tract have been involved in co-degradation of dietary components such as pectin that generates methanol (Rode et al., 1981). Moreover, dietary lignans and (iso) flavonoids that produce methoxylated aromatics can be converted by E. limosum strains into enterolactone and a range of other beneficial products (Jin et al., 2007; Possemiers et al., 2008; Struijs et al., 2009). Similarly, vitamin B12 corrinoid-dependent TMA methyltransferases were recently identified in E. limosum and found to catalyze the demethylation of carnitine and betaine, thereby limiting the TMA formation and TMAO accumulation.
(Koeth et al., 2013; Picking et al., 2019; Kountz et al., 2020). Based on their potential health-promoting properties, *E. limosum*-related bacteria have been suggested to be among the next-generation beneficial microbes (Kanauchi et al., 2006), and bacteria related to *E. limosum* have been associated with longevity and health in a cohort of centenarians (Biagi et al., 2010).

In the present study, we describe the complete genome sequence of the aforementioned *E. maltosivorans* YIT, providing a detailed comparative genomic analysis and use a proteome approach to identify the enzymes and pathways involved in acetogenesis (from H₂ and CO₂), butyrogenesis (from lactate and acetate and various sugars) and propionogenesis (from 1,2-PDO) as well as the conversion of quaternary amines (betaine, carnitine and choline), which is associated with the induction of unique TMA methyltransferases and proteins involved in the production of BMCs.

**Results and discussion**

**Complete genome of *E. maltosivorans* YIT and comparative genomics of prophages, CRISPR systems and relevant features**

*Eubacterium maltosivorans* YIT affiliates the *Eubacteriaceae* family of *Clostridium* cluster II, sharing the highest 16S rRNA genes similarity to *E. limosum* SA11 (99.7%), *E. limosum* KIST612 (98.1%), *E. callanderi* AMC0717 (97.5%) and *E. limosum* ATCC 8486T (97.3%), the latter being the type species of the *Eubacterium* genus (Wade, 2006) (Fig. 1). The complete genome of *E. maltosivorans* YIT consisted of a single circular chromosome of 4,337,501 bp with its general features being listed in Table 1. Many genomic features were shared with the related *E. limosum* strains ATCC 8486T, KIST612 and SA11, for which complete genomes have been reported (Roh et al., 2011; Kelly et al., 2016; Song et al., 2017).

The genome of *E. maltosivorans* YIT revealed five prophages sequences, of which four are intact and one is incomplete (Fig. 2). The prophages in strain YIT had similar G + C content: 47.70% (33.3 kb), 41.61% (38.1 kb), 44.95% (53 kb) and 48.59% (44.8 kb) for intact prophage one to four respectively; and 46.47% (22.9 kb) for the incomplete prophage (Fig. S1A). These prophages were unique as no sequences were shared with the single 58.8-kb prophage sequence with a reduced G + C content (40.86%) in strain SA11 that is closest to strain YIT (Fig. S1B), indicating exposure to different prophages in human colon and in sheep rumen. In terms of prophage defence systems, both strains possessed CRISPR/Cas system in their genomes. Strain YIT has one cluster of a 225 bp CRISPR array that contained a 30 bp long direct repeat (5-GTTGAAGCTTAACATGAGATGTATTTAAAT-3) with three spacers. Strain SA11 has a 2,328 bp CRISPR system with the same direct repeat with 35 spacers. No spacers were found to be shared between these two strains, indicating previous viral infections were different. Besides, strain SA11 has another 1675 bp CRISPR array, which contained another 30 bp long repeat sequence (5-GTTGAAGTTAACATGAGATGTATTTAAAT-3) with 25 spacers.

The genome of *E. maltosivorans* YIT (hereafter *E. maltosivorans*) is predicted to contain 41 *mttB* genes encoding TMA methyltransferases (*MttB*, COG05598), all of which are predicted to encode proteins in similar size of 458–487 amino acids with one exception of 54 amino acids. These *mttB* genes in *E. maltosivorans* clustered at four major locations in the genome, while in *E. limosum* strains they are more scattered (Fig. 2). Similar to *E. limosum* strains, the *mttB* genes in *E. maltosivorans* are also often associated with genes for cobalamin B12-binding proteins, BCCT (betaine/carnitine/choline transporter) and MFS family transporters and GntR family transcriptional regulators (Kelly et al., 2016; Picking et al., 2019; Kountz et al., 2020).

![Fig. 1. Phylogenetic affiliation of *E. maltosivorans* YIT and related *E. limosum* strains. Neighbour-joining tree based on 16S rRNA gene sequence similarities calculated with Jukes–Cantor correction was adapted from Feng et al. (2018), with accession numbers of the deposited complete genomes of *E. maltosivorans* YIT and related *E. limosum* and *E. callanderi* strains.](image-url)
Table 1. General genome features of *E. maltosivorans* YIT, *E. limosum* ATCC 8486T, SA11, KIST612 and *E. callanderi* AMC0717.

| Strain   | YI CP029487.1 | SA11 CP011914.1 | ATCC 8486T CP019962.1 | KIST612 CP002273.2 | AMC0717 JACCKS000000000 |
|----------|---------------|-----------------|-----------------------|---------------------|--------------------------|
| Host     | Human         | Sheep           | Human                 | Anaerobic digester  | Human colonic Mucosa     |
| Genome size (bp) | 4 337 501 | 4 150 332 | 4 422 837 | 4 316 707 | 4 327 770 |
| GC content (%) | 47.8     | 47.4            | 47.2                  | 47.5                | 47.5                     |
| rRNA     | 16            | 16              | 17                    | 16                  | 11                       |
| tRNA     | 61            | 60              | 60                    | 57                  | 53                       |
| Prophage | 5             | 1               | 4                     | 4                   | 1                        |
| No. subsystems | 351     | 347             | 354                   | 358                 | 245                      |
| No. CDs  | 4227          | 4044            | 4303                  | 4284                | 4539                     |
| No. *mttB* genes | 41       | 39              | 42                    | 33                  | 31                       |

*mttB*: TMA methyltransferases.

Fig. 2. Genome map of *E. maltosivorans* YIT compared to the close-related strain KIST612 (purple), SA11 (pink) and ATCC8486T (green), with trimethylamine methyltransferases indicated in each strain (yellow).
Comparative proteomics of E. maltosivorans grown on different carbon sources

Since E. maltosivorans is highly versatile and capable of growing on a variety of different substrates (Feng et al., 2018), we focused first on growth with H2/CO2 (further referred to as homoacetogenic growth) and lactate supplemented with acetate (further referred to as butyrogenic growth). Next, we tested the growth of E. maltosivorans with 1,2-PDO (further referred to as propionogenic growth), as well as demethylation growth of quaternary amines including betaine, carnitine and choline. For this purpose, actively growing E. maltosivorans cells pre-cultured in glucose were washed and inoculated in basal medium containing either H2/CO2, 20 mM lactate plus 15 mM acetate, 20 mM glucose, or 20 mM 1,2-PDO. When E. maltosivorans was grown with 20 mM quaternary amines, an extra 2.5 g L−1 PEP was supplied in basal medium. The carbon recovery of homoacetogenic, butyrogenic and propionogenic growth were 57.1%, 88.2%, and 80.5% respectively, while that on its preferred substrate glucose was 72.8% (Table 2). The missing carbon can be attributed to biomass production and intermediates that could not be detected by high-performance liquid chromatography at mid-log phase, such as amino acids.

Table 2. Substrate utilization and product formation by E. maltosivorans at the end exponential phase.

| Consumption substrate (mM) | Acetate (mM) | Butyrate (mM) | Lactate (mM) | Propionate (mM) | Propanol (mM) |
|---------------------------|-------------|--------------|--------------|-----------------|---------------|
| Glucose                   | 11.6 ± 0.49 | 13.5 ± 1.16  | 0.6 ± 0.57   | 5.4 ± 1.10      | <0.1          | <0.1          |
| Lactate                   | 9.3 ± 0.95  | 4.7 ± 0.77   | 3.8 ± 1.0    | <0.1            | <0.1          | <0.1          |
| H2 (CO2)                  | 45.5 ± 0.85 | 6.5 ± 0.58   | <0.1         | <0.1            | <0.1          | <0.1          |
| 1,2-PDO                   | 17.5 ± 0.98 | <0.1         | <0.1         | 7.5 ± 0.63      | 6.6 ± 0.30    |

Values are average of triplicates and their standard deviation. 1,2-PDO: 1,2-Propanediol.

Proteomics-guided identification of the active DL-lactate utilization gene cluster and butyrogenic pathway in E. maltosivorans

Lactate is one of the most important metabolites for the growth of anaerobic bacteria in the human intestinal environment (Duncan et al., 2004). It must be efficiently reused to avoid potential negative consequences, such as acidosis, neurotoxicity and cardiac arrhythmia.

During butyrogenic growth on lactate supplied with acetate, various proteins showed highly increased abundance (over 1000-fold compared to homoacetogenic growth and glucose fermentation), including lactate dehydrogenase (Ldh, encoded by CPZ25_RS02230), electron transfer flavoprotein alpha subunit (EtfA, encoded by CPZ25_RS02225), ETF beta subunit (EtfB, encoded by CPZ25_RS02230) and lactate racemase (LctF1, encoded by CPZ25_RS18455) (Fig. 4A; Table S1). The lactate racemase LctF1 ensures both D- and L-lactate promoted the growth of E. maltosivorans, which is in line with the substrate utilization since both DL-lactate were provided. In the genome of E. maltosivorans, downstream of the Idh gene, there is a gene encoding a potential lactate permease (encoded by CPZ25_RS02235), the product of which was not detected in our current proteomic analysis, since it is a likely transmembrane protein. The organization of the DL-lactate utilization gene cluster of lctABCDEFG in E. maltosivorans is depicted in Fig. 4B.
Identification of the WLP in the proteome of E. maltosivorans and induction of two highly similar TMA methyltransferases

Hydrogen is a common gaseous microbial metabolite produced in the intestine through the fermentation of carbohydrates. CO₂ is also produced through a variety of decarboxylation reactions, notably from amino acid fermentations. Homoacetogenic bacteria can convert hydrogen together with CO₂ to acetate through WLP. Therefore, we sought to reconstruct the WLP in E. maltosivorans leading to the conversion of H₂/CO₂ to acetate.

The genes encoding proteins of the WLP in E. maltosivorans are mainly grouped in three clusters on the chromosome, consisting of a formate dehydrogenase (Fdh) cluster (CP225_RS17665-17675), a methyl (CP225_RS11820-11850) and a carbonyl branch (CP225_RS18530-18570) to form the central intermediate, acetyl-CoA (Supplementary Fig. 3). All of the predicted genes involved in the WLP were detected in the current proteome study, except for MoeA (molybdenum cofactor guanylyltransferase, encoded by CP225_RS17670) and a Fdh accessory protein (encoded by CP225_RS17675), indicating the activity of WLP in E. maltosivorans. However, most of the detected proteins were not higher produced in homoacetogenic growth, when compared with butyrogenic growth cells, suggesting constitutive expression of their genes (Tables S1 and S3). Remarkably, during growth on H₂/CO₂ two highly similar TMA methyltransferases (encoded by CP225_RS14065 and CP225_RS14915) were highly overproduced (over 150-fold change) compared to growth on glucose or lactate and acetate (Fig. 4A). These two TMA methyltransferases (considered as the MttB prototype) have an identical sequence and hence could not be distinguished from each other. Presently, it is not known whether these two TMA methyltransferases have a specific function in the homoacetogenic metabolism of E. maltosivorans.

Growth on maltose induces maltose-specific phosphotransferase and a set of internalin B-like proteins

Starch is a major source of energy in the human diet and is consumed in diverse forms. Resistant starch escapes digestion by glucoamylases in the upper GI tract and is degraded by the combined action of many gut bacteria in the colon, leading to beneficial impacts on colonic function and host health. Maltose is one of the by-products
from the degradation of resistant starch that in the colon is mainly converted by *Ruminococcus bromii* (Ze et al., 2012). The ability to utilize maltose is one of the differentiating features for *E. maltosivorans* in respect to *E. limosum*. Therefore, we sought to identify the proteins involved in the metabolic pathway that converts maltose to acetate and butyrate by comparing the proteomes of *E. maltosivorans* grown on maltose and 1,2 PDO.

Fig. 4. Differentially abundant proteins in *E. maltosivorans* cells grown on lactate/acetate and hydrogen (A) and organization of gene clusters involved in lactate utilization in the genome of *E. maltosivorans* (B). Proteins that are significantly different between the two conditions in the proteome are coloured in orange, with the locus tags of the genes encoding for these proteins indicated. The locus tag for the genes encoded in *E. maltosivorans* starts with CPZ25_RS, which is omitted to avoid repetition in the figures. For clarity, proteins related to lactate utilization and the MttB are labelled in bold, and a complete list with product names for locus tags is given in Table S1. EtfA: electron transfer flavoprotein alpha; EtfB: electron transfer flavoprotein beta; Ldh: lactate dehydrogenase; LctF: lactate racemase; Lud: lactate utilizing domain; MttB: trimethylamine methyltransferase.

Genome analysis predicted that maltose uptake in *E. maltosivorans* depends on a maltose phosphoenolpyruvate phosphotransferase (Mal-PTS) system encoded by *malP* (CPZ25_RS08305). The phosphorylated maltose-6-phosphate is then degraded by a maltose-6-phosphate hydrolase (encoded by *malA*, CPZ25_RS08300) into glucose and glucose-6-phosphate, which both enter the glycolysis pathway and lead to production of acetate and butyrate. Lastly, there are two potential maltose operon regulators encoded by *malQ* and *malR* (CPZ25_RS08290 and CPZ25_RS08295 respectively) that may control the expression of the PTS genes and are located...
in the proximity of the genes for the maltose utilization cluster (Fig. 5B).

The proteins encoded by the malPQR cluster of genes had significantly higher abundance in the proteome of cells grown on maltose compared to propionogenic growth (Fig. 5A). Among these proteins, the products of malR and malP were uniquely detected in the cells grown with maltose, indicating that the Mal-PTS is specific for transporting maltose. Remarkably, we also noticed that the products of genes closely located around the maltose utilization cluster were coordinately expressed and highly induced in the presence of maltose (Fig. 5A). Among these genes, CPZ25_RS08280, CPZ25_RS08315, CPZ25_RS08325 and CPZ25_RS08330 encode large, over 1000 amino acid residue proteins. The protein products of CPZ25_RS08280, CPZ25_RS08325 and CPZ25_RS08330 share ~40%–72% similarities to each other and their functions remain unknown although their genes were found to be conserved in all related E. limosum genomes. All proteins have signalP-predicted N-terminal signal sequences and those encoded by locus tags CPZ25_RS08315 and CPZ25_RS08320 have considerable similarity to internalin B, a well-studied Listeria monocytogenes surface protein involved in human cell binding (Parida et al., 1998). Moreover, these both contain remnants of sortase recognition sequences in their C-terminus. Of note, the E. maltosivorans genome is predicted to encode two class B sortases (CPZ25_RS01590 and CPZ25_RS01785), a protein that is known to covalently couple this kind of proteins to the cell envelope (Ilangovan et al., 2001; Marraffini et al., 2006). Whether this is also the case with these internalin B-like proteins remains to be confirmed since cell-envelope binding usually precludes detection in the cytoplasmic proteome as we report here.

Growth on 1,2-PDO induces proteins involved in BMCs

To further study the versatility of E. maltosivorans, we grew it on 1,2-PDO resulting in the production of equal amounts of propionate and 1-propanol (Fig. S4). Interestingly, we did not observe accumulation of intermediate propionaldehyde, which is known as a toxic compound to the cell. Therefore, we hypothesized that propionaldehyde could be sequestered within an organ-elle so-called a BMC which is a distinct mechanism to avoid lethal effects while growing on 1,2-PDO or other substrates with toxic intermediates ( Chowdhury et al., 2014). This is in line with the detection of a total 27 pdu genes encoding BMC production and degradative enzymes in genome of E. maltosivorans, with 17 of which are closely located in a cluster. We found homologues of major (PduABB) and minor (PduKNTU) BMC shell proteins (Fig. 5D1). This confirms earlier predictions for the presence of a PDU1 type BMC encoded by multiple loci in the genome of E. limosum KIST612 (Axen et al., 2014).

Of note, an ethanolamine utilization (eutU) homologue is located among the pdu cluster, which is a part of ethanolaminse BMC and encodes a chaperone whose specific function is currently unknown (Kofoid et al., 1999). When the proteome of 1,2-PDO-grown cells was compared with those grown on maltose, all these BMC shell proteins were detected at a significantly higher abundance and PduABKNU, as well as EutU, were significantly over-produced (Fig. 5A). The formation of BMCs during growth with 1,2-PDO was confirmed by analyzing cells grown on this substrate by transmission electron microscopy (TEM) and comparing these with cells grown on glucose (Fig. 5D).

Growth on quaternary amines is associated with induction of BMCs in E. maltosivorans

In our previous analysis we found that E. maltosivorans can grow on betaine ( Feng et al., 2018). To further test other quaternary amines, we grew E. maltosivorans on betaine, carnitine, or choline (each 20 mM) on a medium with yeast extract and peptone (each 0.25%). Compared to the negative control (without substrate), E. maltosivorans produced 5 and 8 mM acetate when grown on carnitine and betaine respectively, and small amounts of butyrate productions (1–2 mM) were observed from both substrates (Fig. S5). However, choline did not generate these SCFA above background level but resting cells appeared to adapt to this substrate as evident from the determined proteome (see below).

Proteome analysis of E. maltosivorans grown on the quaternary amines and glucose detected 812 proteins with 420 as core proteins detected in all conditions. Importantly, 11 out of the 27 proteins encoded by the pdu cluster were detected to be higher expressed when E. maltosivorans was grown on quaternary amines than on glucose, especially the genes involved in cobalamin assimilation (encoded by pduS) and the formation of the BMC shells (encoded by pduABN, Table 3). Functional identification of BMCs was provided by TEM image analysis of E. maltosivorans cells grown on betaine that clearly revealed BMC structures that were similar in number and structure as those visible in cells grown on 1,2-PDO but absent in glucose-grown cells (Fig. 5D2). The involvement of BMCs in the degradation of quaternary amines such as choline has been earlier established for Desulfovibrio alaskensis, Proteus mirabilis and an uropathogenic Escherichia coli (Kuehl et al., 2014; Jameson et al., 2016; Herr et al., 2018). However, these bacteria all contain choline TMA lyase and its activating enzyme (CuTCD) generates TMA and acetaldehyde. The
Comparative analysis of proteomics (A), prediction of gene clusters (B & C) and presence of BMCs (D) in *E. maltosivorans* grown on 1,2-PDO and maltose.

A. Differentially abundant *E. maltosivorans* proteins in cells grown on 1,2-PDO versus on maltose. Proteins that are significantly different in abundance between the two conditions in the proteome are coloured in orange, with the locus tags of the genes encoding for these proteins indicated. The locus tag for the genes encoded in *E. maltosivorans* starts with CPZ25_RS, which is omitted to avoid repetition in the figures. For clarity, locus tags related with maltose utilization and microcompartments formation are labelled in bold, and a complete list with product names for locus tags is given in Table S4. B, C. Operon of the genes involved in maltose transportation and 1,2-PDO degradation in the genome of *E. maltosivorans* with colour coded predicted functions. D. TEM visualization of microcompartments in *E. maltosivorans* when grown with 1,2-PDO (1), betaine (2) and negative control glucose (3). Green arrowheads point to microcompartments structures, with the black scale bars.

**Fig. 5.** Comparative analysis of proteomics (A), prediction of gene clusters (B & C) and presence of BMCs (D) in *E. maltosivorans* grown on 1,2-PDO and maltose.

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Table 3. Proteins involved in bacterial microcompartments in E. maltosivorans were higher expressed when grown with betaine (BE), carnitine (CA) and choline (CH) than with glucose (Glu).

| Locus tag | Protein                              | BE vs. Glu | CA vs. Glu | CH vs. Glu |
|-----------|--------------------------------------|------------|------------|------------|
| 10565     | Polyhedral organelle shell protein PduT | 2.7        | 3.4        | 1.9        |
| 10575     | Cobalamin reductase, PduS             | 14.8       | 13.5       | 10.7       |
| 10580     | Propionaldehyde dehydrogenase, PduP   | 2.4        | 3.2        | 2.5        |
| 10590     | Shell protein, PduN                   | 27.5       | 1.0        | 1.0        |
| 10620     | Glycerol dehydratase reactivase subunit alpha | 1.0        | 1.8        | 1.5        |
| 10625     | PD dehydratase small subunit PduE     | 4.7        | 12.3       | 6.6        |
| 10630     | PD dehydratase medium subunit PduD    | 5.4        | 12.3       | 4.3        |
| 10635     | PD dehydratase large subunit PduC     | 9.1        | 13.2       | 9.1        |
| 10640     | Microcompartment protein PduB         | 12.6       | 18.2       | 13.2       |
| 10645     | Microcompartment protein PduA         | 6.2        | 6.5        | 2.3        |
| 19865     | Phosphate propanoyl transferase, PduL  | 1.8        | 2.3        | 1.1        |

Fold change induction was calculated by dividing the protein abundances in the different growth conditions.

BMCs are involved to sequester acetaldehyde to prevent toxicity, and subsequently metabolize acetaldehyde to ethanol and acetate (Herring et al., 2018). On the other hand, the resulting TMA is excreted in the gut lumen and cannot be used as a nitrogen or carbon source by most of the gut microbes. TMA then can enter the bloodstream, and once transported to the liver it is converted to TMAO predominantly by flavin monooxygenase 3. High serum levels of TMAO have been shown to promote formation of atherosclerotic plaques in a mouse model (Koay et al., 2021). Furthermore, serum levels of TMAO were significantly correlated with the incidence of heart attack and stroke (Wang et al., 2011). Moreover, it is a common uremic toxin (Velasquez et al., 2016). In contrast to the TMA-producing species, E. maltosivorans deaminates betaine and other quaternary amines in a process that likely involves a BMC. Quaternary amines are thought to be actively transported into the cell by BCCT transporter family proteins that also likely export the generated deaminated products (Ziegler et al., 2010). In addition, based on earlier discoveries in another strain (Müller et al., 1981), recent studies have elucidated the biochemical pathway of quaternary amines in E. limosum ATCC 8486T into acetate and butyrate during demethylation of betaine, choline and carnitine into dimethylglycine, dimethylethanolamine and norcarnitine respectively (Müller et al., 1981; Picking et al., 2019; Kountz et al., 2020). In this way, one of three methyl groups from quaternary amines is transferred to MtqC (a corrinoid protein), together with a cob(I)alamin by MtB homologues, forming methyl-Co(Ill)-MtqC. The N,N-dimethyl compound is thus produced and released. The methyl group is subsequently transferred to tetrahydrofolate (THF) by a methylocorrinoid:THF methyltransferase (MtqA). The resulting methyl-THF enters the WLP, leading to cell biomass, and acetate/butyrate and CO₂ production.

Co(I) MtqC can be adventitiously oxidized to catalytically inactive cobalt(II), which is reduced to active cobalt(I) by the ATP and reducing power-dependent activity of RamQ (a corrinoid activation enzyme) (Picking et al., 2019; Kountz et al., 2020). Based on genomic reconstructions a similar pathway is expected to operate in E. maltosivorans (Fig. 3). Moreover, the present proteomic and TEM data provide further support for the fact that BMCs play a role in this unique pathway, rationalize the observation that the WLP genes are rather constitutively expressed as needed for growth on quaternary amines, and explain the requirement for the high number of MtB homologues predicted from the genomes of E. maltosivorans and related species (Table 1; see below). In general, the BMCs are thought to protect the cell from toxic metabolic intermediates, prevent unwanted side reactions and may enhance flux through a multistep pathway (Kerfeld et al., 2018). In absence of further data, we hypothesize that the role of a BMC in the demethylation of quaternary amines by E. maltosivorans may be to encapsulate the vitamin B12-depending demethylation cascade and contain the appropriate TMA methyltransferase within the BMC.

Demethylation of quaternary amines is associated with induction of TMA methyltransferases in E. maltosivorans

Next, we focused on the expressed products of mtb genes in E. maltosivorans when induced by the growth with different quaternary amines and the other tested substrates (see Fig. 3). Among the total of 41 MtB homologues predicted by the genome of E. maltosivorans, 16 were detected to be present in the proteomes of cells grown on the quaternary amines. Of note, most of these detected MtB homologues were detected in the cells grown at all quaternary amines, except for protein...
were >300 times higher produced when grown on betaine homologues encoded by CPZ25_RS13805 and 18250 respectively (Table 4). For instance, MttB and carnitine as well as choline, though we only analyzed MttB proteins for each of the quaternary amines betaine, carnitine respectively (Table 4). Remarkably, although choline did not seem to support the growth of E. maltosivorans, the proteomes of the cells were affected, as one MttB homologue (encoded by CPZ25_RS12890) was uniquely produced when cells were incubated in the presence of choline. Based on our present proteome data, we hypothesize that candidate TMA methyltransferases specific for betaine and choline.

To fully picture the 41 MttB homologues encoded by the genome of E. maltosivorans, a Maximum-likelihood phylogenetic tree was constructed (Fig. 6). The amino acid sequence of these E. maltosivorans MttB proteins varied considerably, as when compared to the prototype MttB (CPZ25_RS14065 and 14915), these vary from 100% to approximately 27% in sequence identity. Through our current proteome and genome studies, we identified 16 active MttB homologues induced by growth or incubation with three different quaternary amines and proposed specific substrates for eight MttB homologues. The substrates for the other MttB homologues in E. maltosivorans remain unknown but we speculate that these may be involved in growth on other C1 compounds like methanol, CO or formate. Moreover, these may be involved in the ability to demethylate other substrates.

products of CPZ25_RS12890 and CPZ25_RS13475, and CPZ25_RS13805, which was only detected when E. maltosivorans was grown on choline, betaine and carnitine respectively (Table 4).

A recent proteome study of E. limosum grown on proline betaine showed high expression of only one of the MttB homologues, in line with earlier finding that this demethylating enzyme systems seem to be very specific (Müller et al., 1981). The MttB enzymes catalyzed THF methylation of proline betaine specifically, forming the key intermediate methyl-THF in the WLP (Picking et al., 2019). Later, Kountz et al. (2020) also revealed a carnitine-specific MttB protein, namely, MtcB, which catalyzes specifically l-carnitine demethylation. We found the homologues of MtpB and MtCB in the genome of E. maltosivorans, with 98.3% and 77.0% amino acid similarities (encoded by CPZ25_RS13805 and CPZ25_RS13245 respectively) to those in E. limosum. In addition, we identified MtqA and MtqC homologues in the genome of E. maltosivorans, sharing high similarity (>96% protein identity) to those of E. limosum.

In apparent contrast with the results obtained for E. limosum (Picking et al., 2019; Kountz et al., 2020), the E. maltosivorans proteome showed multiple overproduced MttB proteins for each of the quaternary amines betaine and carnitine as well as choline, though we only analyzed resting cells for the latter (Table 4). For instance, MttB homologues encoded by CPZ25_RS13805 and 18250 were >300 times higher produced when grown on betaine than when grown on carnitine, while MttB homologue encoded by CPZ25_RS13805 was >900 times overproduced when grown with carnitine than grown with betaine. Moreover, choline did not seem to support the growth of E. maltosivorans, the proteomes of the cells were affected, as one MttB homologue (encoded by CPZ25_RS12890) was uniquely produced when cells were incubated in the presence of choline. Based on our present proteome data, we hypothesize that candidate TMA methyltransferases specific for betaine are CPZ25_RS05865, 13475, 18250 and 13755, specific for carnitine are CPZ25_RS13805, 14065 and 14915, and CPZ25_RS12890 specific for choline.

Table 4. Differential expression of 16 MttB homologues and other components of a corrinoid-dependent methyltransferase in E. maltosivorans when grown with betaine (BE), carnitine (CA), choline (CH) or glucose (Glu).

| Locus tag   | Protein (potential function) | Fold change induction |
|-------------|------------------------------|------------------------|
| 04610       | MttB protein                 | BE vs. Glu: 15.8, CA vs. Glu: 16.8, CH vs. Glu: 16.8 |
| 05865       | Betaine-specific MttB protein| BE vs. Glu: 114.5, CA vs. Glu: 29.8, CH vs. Glu: 1.0 |
| 06255       | MttB protein                 | BE vs. Glu: 33.9, CA vs. Glu: 6.6, CH vs. Glu: 0.7 |
| 12920       | MttB protein                 | BE vs. Glu: 6.6, CA vs. Glu: 5.3, CH vs. Glu: 4.4 |
| 12935       | MttB protein                 | BE vs. Glu: 60.3, CA vs. Glu: 19.1, CH vs. Glu: 1.0 |
| 13475       | Betaine-specific MttB protein| BE vs. Glu: 25.7, CA vs. Glu: 1.0, CH vs. Glu: 1.0 |
| 13640       | MttB protein                 | BE vs. Glu: 5.9, CA vs. Glu: 2.6, CH vs. Glu: 3.0 |
| 13755; 18250| Betaine-specific MttB protein| BE vs. Glu: 1148.2, CA vs. Glu: 3.2, CH vs. Glu: 17.2 |
| 14265       | MttB protein                 | BE vs. Glu: 22.4, CA vs. Glu: 10.7, CH vs. Glu: 14.6 |
| 13805       | Carnitine-specific MttB protein| BE vs. Glu: 1.0, CA vs. Glu: 933.2, CH vs. Glu: 1.0 |
| 14255       | MttB protein                 | BE vs. Glu: 5.9, CA vs. Glu: 6.3, CH vs. Glu: 5.1 |
| 14915; 14065| Carnitine-specific MttB protein| BE vs. Glu: 3.1, CA vs. Glu: 575.4, CH vs. Glu: 9.6 |
| 17920       | MttB protein                 | BE vs. Glu: 1.3, CA vs. Glu: 5.4 |
| 12890       | Choline-specific MttB protein| BE vs. Glu: 1.0, CA vs. Glu: 61.5 |
| 11850       | MtqA                         | BE vs. Glu: 5.6, CA vs. Glu: 2.2, CH vs. Glu: 0.9 |
| 13765       | MFS transporter              | BE vs. Glu: 70.8, CA vs. Glu: 1.0 |
| 14190       | MtqC                         | BE vs. Glu: 0.5, CA vs. Glu: 0.04, CH vs. Glu: 0.0 |
| 18535       | RamQ                         | BE vs. Glu: 4.2, CA vs. Glu: 1.8, CH vs. Glu: 0.9 |

MFS: Major facilitator superfamily protein; RamQ: corrinoid activation enzyme; MtqA: methylcorrinoid:tetrahydrofolate methyltransferase; MtqC: a corrinoid protein. The locus tag for the genes encoded in E. maltosivorans starts with CPZ25_RS, which is omitted to avoid repetition in the table. Fold change induction was calculated by dividing the protein abundances in the different growth conditions. Bold entries reflect putative TMA methyltransferases involved in the utilization of betaine, carnitine or choline.
such as dietary lignans and (iso)flavonoids, which have been linked to possible health benefits and longevity (Jin et al., 2007; Possemiers et al., 2008; Struijs et al., 2009).

Conclusions

Our genomic and proteomic analyses revealed key metabolic features of *E. maltosivorans* (Fig. 2). *E. maltosivorans* has lactate utilization gene clusters, Ldh/Etf complex and butyryl-CoA dehydrogenase to convert low energy compounds D,L-lactate with acetate into butyrate. *E. maltosivorans* also catalyzes the conversion of 1,2-PDO to propionate and propanol and by proteome analysis and TEM, we confirmed that microcompartments were formed during the growth of *E. maltosivorans* with 1,2-PDO.

Genome analysis predicted *E. maltosivorans* to produce 41 trimethylamine methyltransferase (MttB). In our current proteome studies, 16 of these were detected when strain Y17 was grown with the quaternary amines betaine, carnitine and choline. In addition, two MttB proteins were produced when the cells were grown under

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**Fig. 6.** Maximum likelihood phylogenetic tree of 41 trimethylamine methyltransferase (MttB) homologues in *E. maltosivorans*. Label represents locus tag of the gene product, which in *E. maltosivorans* starts with CPZ25_RS. To avoid repetition of the prefix in the figure, the locus tags are represented only by the specific identifier. The numbers on branches represent the bootstrap values from 1000 replicates. The proteins detected in the proteomes described here are marked with arrows and the growth conditions are indicated.
The present study demonstrates that leading to a lower TMAO level in the blood serum. The deamination of betaine and other quaternary amines might also involve a microcompartment, possibly due to the vitamin B12-dependent nature of these MttB homologues. By this pathway, TMA production thus can be reduced in the human gut ecosystem and consequently resulting in a lower TMAO level in the blood serum. The present study demonstrates that E. maltosivorans is highly versatile in converting low-energy fermentation end-products in the human gut into health-promoting SCFA and may be a useful therapeutic strain to prevent the production of the undesired TMAO.

Experimental procedures

Growth medium and conditions

Cultivation of E. maltosivorans YIT (DSM 105863 T) was handled as previously described (Feng et al., 2018). Briefly, it was grown based on anoxic basal medium (Stans et al., 1993) supplemented with 1 g L^{-1} yeast extract and 20 mM of each substrate separately. Eight different substrates were selected to conduct the comparative proteomics study for E. maltosivorans: homoacetogenic growth with H_2/CO_2 (80:20 vol./vol.), butyrogenic growth with 20 mM lactate with 15 mM acetate, fermentation growth with 20 mM glucose or 20 mM maltose, propionogenic growth with 20 mM 1,2-PDO, and demethylate growth on either 20 mM carnitine, betaine, or choline. Each condition was run in triplicates. For growth on carnitine, betaine and choline (20 mM each), the growth medium was amended with 2.5 g L^{-1} peptone and 2.5 g L^{-1} yeast extract. Each condition was run in duplicates. Except otherwise indicated, cultures were incubated statically at 37°C in the dark without shaking.

Genomic DNA preparation, assembly and annotation

Exponentially growing cells on maltose (50 ml) were harvested by centrifuging at 4°C for genomic DNA isolation. MasterPure Gram-positive DNA purification kit (Epicentre) was used to extract the genomic DNA of E. maltosivorans YIT. The quality of the extracted DNA was measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). The genome was sequenced using both PacBio RSII instrument and Illumina. Both Illumina paired-end reads and PacBio sub-reads were used as input for the assembler spades (v.3.11.1) with a custom kmer setting of 21,31,51,81,91 and coverage cutoff set to auto. After removing scaffolds smaller than 500 bp, one scaffold was obtained of 43 375 050 bp (0 N’s). Illumina paired-end reads were mapped back to the assembly using bowtie2 (v2.3.3.1). The resulting sam files were converted with samtools (v1.3.1). The indexed bam file was used as input for Pilon (v1.2.2) which resulted in a 4 337 503 bp (N’s) polished assembly. The complete genome sequence of E. maltosivorans YIT (DSM 105863 T) was deposited at GenBank/EMBL-EBI under the accession number CP029487.1, and proteins were annotated by the NCBI Prokaryotic Genome Annotation Pipeline (Tatusova et al., 2016; Haft et al., 2018; Li et al., 2021). The complete genomes of the three known E. limosum strains, including the type strain ATCC8486 T, strain KIST612 and strain SA11, were obtained from NCBI with accession numbers CP019962.1, NC_014624.2 and CP011914.1 respectively.

Specific annotations

The core genome of the E. maltosivorans and E. limosum strains was identified using Spine and the pan-genome was identified using AGEnt (Ozer et al., 2014). CRISPR genes of the four strains were annotated using CRISP-RTfinder (Grissa et al., 2007). The CRISPRTarget tool identifies target phages using different databases and the search algorithm has its own scoring system with a score of 20 which was kept as default minimum as suggested by the authors (Biswas et al., 2013). Prophage sequences were detected using PHASTER (Amdt et al., 2016). In general, if a given phage region’s total score is <70, it is recognized as incomplete; between 70 and 90, it is recognized as questionable; if >90, it is intact. Genomic islands were identified using online IslandViewer version 4 (Bertelli et al., 2017).

Proteomics

For proteomics analysis, E. maltosivorans cells cultivated with different substrates as described above were harvested at middle or late exponential growth phase by centrifugation for 20 min at 4700 g at 4°C (Supplementary Fig. 2). The pellets were washed with 1 ml PBS and immediately stored at −80°C until protein extraction. In total, we conducted three sets of proteomic analyses, with the first set comparing homoacetogenic and butyrogenic growth to glucose fermentation in biological triplicates, the second comparing propionogenic growth to maltose fermentation in biological triplicates and the last comparing growth with quaternary amines to glucose fermentation in biological duplicates.

To prepare the protein samples for the first set proteomes, an in-stage-tip sample preparation method was used (Kulak et al., 2014). The harvested washed pellets were thawed on ice and 2 mg wet weight was
transferred and resuspended in 0.5 ml of 100 mM Tris/HCl at pH 8. The suspension was sonicated six times at 30% amplitude in cycles of 30 s pulse and 30 s rest on ice by a Branson sonifier SFX150 equipped with a 3 mm tip (Branson, Carouge, CH). Proteins in the supernatant were collected by centrifugation at 10 000g for 10 min at 4°C and transferred to low binding Eppendorf microtubes. Disulphide bonds of the proteins were reduced by incubating in 20 mM dithiothreitol (DDT) in a thermomixer at 500 rpm at 45°C for 15 min. When the mixture cooled down to room temperature, AcrylAmide was added up to 25 mM to alkylate the reduced cysteines in the dark at room temperature for 30 min. Followed by centrifugation at 14 000g for 2 min, 70–80 μl protein extract (ca. 40 μg protein) was collected and loaded onto a C18 stage-tip using a double membrane (Empore C18, 3M), as described by Sánchez-Andrea et al. (2020). After a clean-up with 150 μl 5% acetonitrile (AcNi) in 50 mM ammonium bicarbonate buffer (ABC), stage tips were moved to clean 0.5 ml low binding microcentrifuge tubes and 500 ng of trypsin in 50 mM ABC was added on top to perform the enzymatic digestion of the proteins at room temperature overnight. The enzymatic digestion was stopped by adding 75 μl 0.1% formic acid and all liquid was eluted into the low binding tubes. In the end, hydrophobic peptides were eluted from the membrane manually with 5 μl 50% AcNi/50% 0.1% formic acid. Finally, after drying most of the AcNi and water in a concentrator, the final volume was made up to 50 μl with 0.1% formic acid. The peptide samples were analyzed as described by Sánchez-Andrea et al. (2020) using the same machine and setup. LCMS runs with all MSMS spectra obtained in first set proteome comparisons were analyzed with MaxQuant 1.6.0.1 as previous as well (Sánchez-Andrea et al., 2020).

For the second and the third set proteomes, around 10 mg harvested pellets were thawed, resuspended into 100 mM Tris pH 8 and sonicated using the same procedure as described for the first set. After sonication, for the protein determination, samples were centrifuged at 10 000g for 10 min at 4°C and the protein concentration was measured with Qubi™ Protein Assay Kit (Thermo Scientific). 40 μg protein of each (not centrifuged) sample was transferred to a 2 ml low binding tube. Proteins were reduced with 15 mM DDT at 45°C for 30 min, unfolded with 6 M urea and alkylated with 20 mM AcrylAmide at 21°C for 30 min. The pH was adjusted to 7 with 10% trifluoroacetic acid (TFA) and proteins were cleaned up by using a PAC method (Bath et al., 2019) using speedbeads and washing the proteins with 1 ml 70% ethanol and then 1 ml 100% acetonitrile. Digestion was conducted with 100 μl 0.5 pg μl⁻¹ trypsin in 50 mM ABC at room temperature overnight. To stop the digestion, 10% TFA in H₂O was added to the samples until the pH dropped to 3. After filtering the peptide samples, volumes were adjusted to 50 μl with 0.1% formic acid.

5 μl of peptide samples were loaded directly onto a 0.10 × 250 mm ReproSil-Pur 120 C18-AQ 1.9 μm beads analytical column (prepared in-house) at a constant pressure of 825 bar (flow rate of circa 700 nl min⁻¹) with 1 ml L⁻¹ HCOOH in water and eluted at a flow of 0.5 μl min⁻¹ with a 50 min linear gradient from 9% to 34% acetonitrile in water with 1 ml L⁻¹ formic acid with a Thermo EASY nanoLC1000. An electrospray potential of 3 kV was applied directly to the eluent via a stainless-steel needle fitted into the waste line of the micro cross that was connected between the micro cross and the analytical column. Full scan positive mode FTMS spectra were measured between m/z 380 and 1400 on an Exploris 480 at resolution 60 000. MS and MSMS AGC targets were set to 300% and 100% respectively or maximum ion injection times of 50 ms (MS) and 30 ms (MSMS) were used. HCD fragmented (Isolation width 1.2 m/z, 28% normalized collision energy) MSMS scans in a cycle time of 1.1 s were recorded for the most abundant 2–5+ charged peaks in data-dependent mode (Resolution 15 000, threshold 2e4, 15 s exclusion duration for the selected m/z ± 10 ppm).

LCMS runs with all MSMS spectra obtained were analyzed with MaxQuant 1.6.3.4 (Cox and Mann, 2008). For all three sets of proteome analysis, an E. maltosivorans sequence database (NCBI accession number 72721) was used together with a contaminants database that contains sequences of common contaminants including Trypsins (P00760, bovin and P00761, porcin) and human keratins [Keratin K22E (P35908), Keratin K1C9 (P35527), Keratin K2C1 (P04264) and Keratin K1C1 (P35527)]. Propionamide (C) set as a fixed modification, while variable modifications were set for Protein N-terminal acetylation and M oxidation which were completed by non-default settings for de-amidation of N and Q, the maximum number of modifications per peptide was 5. The label-free quantification (LFQ), as well as the match between runs options, was enabled. De-amidated peptides were allowed to be used for protein quantification and all other quantification settings were kept default. Filtering was done in MaxQuant by using a False Discovery Rate of 1% on protein and peptide level and on the MaxQuant result table by only accepting proteins with at least two identified peptides of which at least one should be unique and at least one should be unmodified. Reversed hits were deleted from the MaxQuant output. No extra filtering was applied after grouping the samples. We carefully checked the data and saw that protein groups with just one measured value all have log-fold changes below 0.6 and log p-values lower than 1. These are therefore never considered to be significantly different. The normal logarithm was taken from protein LFQ values.
MS1 intensities as obtained from MaxQuant. Zero ‘Log LFQ’ values were replaced by a value of 4.4 (A value slightly lower than the lowest measured value) to make sensible ratio calculations possible. Relative protein quantitation of the first and second set proteomes was done with Perseus by applying a two-sample T-test using the ‘LFQ intensity’ columns obtained with FDR set to 0.05 and S0 set to 1. Total non-normalized protein intensities corrected for the number of measurable tryptic peptides (intensity-based absolute quantitation) intensity were, after taking the normal logarithm, used for plotting on the y-axis in a protein ratio versus abundance plot. All three mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (Vizcalino et al., 2016) partner repository with the dataset identifiers PXD028586, PXD028574 and PXD028575.

**Transmission electron microscopy**

Biomass of *E. maltosivorans* Y17 grown with 1,2-PDO, betaine, or glucose was obtained by centrifugation (5 min, 4°C at 14 000g) from late exponential cultures. Biomass were resuspended and fixed in 1 ml 2.5% (vol./ vol.) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 1 h at room temperature. After rinsing in the same buffer, biomass was resuspended and incubated in 100 μl of 3% gelatin in 0.1 M phosphate at room temperature for 20 min till gelatin solidified. The solidified specimens were incubated in 0.1 M sodium cacodylate buffer for 15 min at room temperature to become loose from tube wall. The specimens were infiltrated by ethanol and were then embedded in resin for 8 h at 70°C. Thin sections (<100 nm) of polymerized resin samples were obtained with microtomes. After staining with 2% (wt./ vol.) aqueous uranyl acetate, the samples were analyzed with a Jeol 1400 plus TEM.

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**Supporting Information**

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site:

**Appendix S1**: Supporting Information.