Polyketides are an important class of bioactive small molecules valued not only for their diverse therapeutic applications, but also for their role in controlling interesting biological phenotypes in their producing organisms. While numerous polyketides are known to be derived from aerobic organisms, only a single family of polyketides has been identified from anaerobic organisms. Here we uncover a family of polyketides native to the anaerobic bacterium Clostridium acetobutylicum, an organism well-known for its historical use as an industrial producer of the organic solvents acetone, butanol, and ethanol. Through mutational analysis and chemical complementation assays, we demonstrate that these polyketides act as chemical triggers of sporulation and granulose accumulation in this strain. This study represents a significant addition to the body of work demonstrating the existence and importance of polyketides in anaerobes, and showcases a strategy of manipulating the secondary metabolism of an organism to improve traits relevant for industrial applications.
Polyketides are one of the most important classes of natural products given their wide range of applications in medicine and agriculture. Encompassing several different chemical classes such as macrolides, polyenes, and aromatics, polyketides are employed for clinical use as antibiotics, anti-cancer agents, immunosuppressants, and even cholesterol-lowering drugs. In addition to their role as therapeutic agents, many of these compounds are used by their producing organisms to access information about both the intracellular physiological status and extracellular environment, and control complex cellular processes such as morphological differentiation, virulence, stress response, and additional secondary metabolite production. As virtually all known polyketides are derived from aerobic organisms (such as bacteria, fungi, and plants), there has been a long-standing assumption that anaerobic organisms are unable to produce these compounds. However, recent genomic analysis has challenged this view by revealing that polyketide biosynthetic genes are widespread among anaerobic bacteria, and in particular, members from the genus *Clostridium*. This diverse genus, comprised of anaerobic endospore-forming Gram-positive Firmicutes, includes several notorious human pathogens as well as non-pathogenic species useful for industrial biotechnology. To date, only one family of polyketides, the clostrubins, has been identified from *Clostridium*, representing the only known polyketides from the anaerobic world. Clostrubins are pentacyclic polyphenolic polyketides biosynthesized by type II polyketide synthases (PKSs) in *Clostridium beijerinckii* and *Clostridium puniceum*, and display potent antibiotic activity against various pathogenic bacteria. Additionally, these aromatic polyketides have been shown to enable the plant pathogen *C. puniceum* to survive in an oxygen-rich environment. The discovery of clostrubins provided the first experimental evidence that anaerobes are capable of producing bioactive polyketides, and motivated further studies on anaerobes to reveal additional polyketide metabolites with novel structures and interesting biological functions.

*Clostridium acetobutylicum* is an organism historically used for industrial-scale production of the organic solvents acetone, *n*-butanol, and ethanol (ABE) through a process known as ABE fermentation. Batch ABE fermentation by *C. acetobutylicum* ATCC 824 (the model ABE producer) is characterized by two distinctive phases, an acid production phase (acidogenesis) and a solvent production phase (solventogenesis). During exponential growth, short-chain fatty acids (acetate and butyrate) are produced and accumulate in the media, causing a drop in the culture pH. As the culture approaches stationary phase, the previously formed acids are re-assimilated, the culture pH rises, and solvent production is initiated. The metabolic switch from acidogenesis to solventogenesis coincides with the initiation of the complex development program of sporulation. As part of these processes, a starch-like carbohydrate known as granulose is produced and accumulates in swollen, phase-bright clostridial forms, within which endospores develop. Further morphological development yields free spores, heat- and chemical-resistant cell types that do not contribute to solvent production.

Although ABE fermentation of *C. acetobutylicum* has been extensively studied, chemical signals responsible for triggering solventogenesis and/or sporulation have not been elucidated, and no secondary metabolite that plays a role in regulating these processes has been reported from this organism. Motivated by a recent transcriptomic analysis of *C. acetobutylicum*, which showed that the expression of a type I modular PKS gene was significantly upregulated during early stationary phase (~40-fold higher transcription level compared to mid-exponential phase), we postulated that the corresponding polyketide product might regulate one or more of the key fermentation phenotypes associated with solventogenesis and sporulation. Here we report the discovery of a family of polyketide metabolites using comparative, untargeted metabolomics followed by large-scale compound purification and molecular structure elucidation. Using a reverse genetics approach and chemical complementation, we further

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**Fig. 1** Identification of polyketides from wild-type extracts. **a** Results of XCMS analysis of wild-type *C. acetobutylicum* and ∆*pks* extracts taken from early stationary phase fermentation cultures (biological quadruplicates). Blue (upper) chromatograms depict MS traces of quadruplicate wild-type extracts, while orange/brown (lower) chromatograms depict MS traces of quadruplicate ∆*pks* extracts. Green circles represent MS peaks unique to wild-type extracts, with corresponding *m/z* values indicated by the *y*-axis. No significant MS peaks were identified, which were unique to ∆*pks*. The three peaks unique to wild type are identified as 1, 2, and 3. **b** UV–Vis traces (240 nm) of extracts from wild type, ∆*pks*, and ∆*pks* genetic complementation strain (∆*pks* (pCKO_pks)). **c** Elucidated structures of 1 and 3 based on NMR characterization.
show that deletion of the pks gene results in increased batch butanol production, and that the newly discovered polyketides are important in stimulating sporulation and granule accumulation in C. acetobutylicum.

Results

Analysis and inactivation of pks locus in C. acetobutylicum. All sequenced C. acetobutylicum strains possess one pks gene (ca_c3355 in C. acetobutylicum ATCC 834) that encodes a type I single-module PKS with a predicted catalytic domain organization of ketosynthase, acyltransferase, dehydratase, ketoreductase, and acyl carrier protein (KS-AT-DH-KR-ACP) (Supplementary Fig. 1a). Homologous PKSs with the same domain structure could be identified, but the genes for these homologous PKSs are typically part of much larger biosynthetic gene clusters that encode additional PKSs and/or modification enzymes. ca_c3355 appears to be an orphan gene with no other biosynthetic enzyme encoded either upstream or downstream, although several transcriptional regulators and efflux pumps are encoded in the vicinity (Supplementary Fig. 1b). While we were able to predict that malonyl-CoA was the likely substrate recognized by the AT domain (Supplementary Fig. 1a), it was difficult to predict the identity of polyketides, which might be synthesized by this PKS, or if any chemical modifications of the nascent polyketide intermediate would be made by proteins encoded elsewhere on the genome. To determine the identity of any PKS-associated metabolites and probe the broader function of the pks locus, we performed a targeted in-frame deletion of the pks gene (ca_c3355) using an allelic exchange method developed for C. acetobutylicum15. The resulting mutant (Δpks) was confirmed by PCR analysis (Supplementary Fig. 2).

Discovery of polyketides through comparative metabolomics. Quadruplicate batch fermentations with wild-type C. acetobutylicum and the mutant Δpks were performed and harvested at early stationary phase. Organic extracts from combined supernatants and cell pellets were obtained and analyzed via liquid chromatography-high-resolution mass spectroscopy (LC-HRMS). Following untargeted metabolomic comparisons of the two strains using XCMS16, three major species with molecular formulas C_{14}H_{22}O_{3} (1, calculated for C_{14}H_{22}O_{3}: 237.1496; found: 237.1496), C_{20}H_{32}O_{2} (2, calculated for C_{20}H_{32}O_{2}: 383.2075; found: 383.2080), and C_{26}H_{42}O_{12} (3, calculated for C_{26}H_{42}O_{12}: 545.2604; found: 545.2600) were found to be present in wild-type culture extracts and completely absent in extracts of Δpks (Fig. 1a, b; Supplementary Figs. 3–5). A majority of 1–3 was found in the culture medium rather than cell pellets, suggesting that they were secreted into the culture medium upon production. 1–3 were UV active and featured identical UV absorption spectra (Supplementary Figs. 3–5), indicating that they share the same chromophore and likely the same biosynthetic origin. Furthermore, when the pks gene was introduced back into the Δpks mutant, the genetic complementation strain was found to have resumed the production of 1–3, demonstrating a direct relationship between the pks gene and the production of 1–3 (Fig. 1b).

Isolation and structure elucidation of polyketides. To isolate compounds 1–3, we prepared 34 L of wild-type C. acetobutylicum culture broth. The culture was extracted with ethyl acetate and chromatographed on a silica gel column, followed by further purification via multiple rounds of HPLC using reverse-phase C18 columns (Supplementary Methods). These purification steps yielded pure compound 1 (1.1 mg) and 3 (0.9 mg). NMR spectra, including ^{1}H, ^{13}C, DQF-COSY, TOCSY, HSQC, and HMBC spectra, were obtained for compound 1 (Supplementary Table 1; Supplementary Fig. 6; Supplementary Methods). The proton signals at δ 5.43, 5.56, and 6.00 indicated a considerable overlap in the aliphatic double bond region, and their configuration (all E stereochemistry) was further resolved using the high-resolution HSQC spectrum without ^{13}C decoupling. These assignments of geometric stereochemistry were also supported from ^{13}C chemical shift values of allylic methylenes17. The carbon signals at δ 172.97 and 67.25, together with related HMBC and TOCSY correlations, indicated the presence of a carboxylic acid and a secondary alcohol. The absolute stereochemistry of 1 was determined to be in the R configuration by measuring the specific rotation of its fully reduced product, 3-hydroxytetradecanoic acid (3-HTA). In particular, 1 was reduced to 3-HTA through hydrogenation over a palladium catalyst, and the specific rotation of the product ([α]_{D}^{20}−15.7° (c 1, CHCl_{3})) was consistent with the reported value of optically pure (R)-3-HTA ([α]_{D}^{20}−16.2° (c 1, CHCl_{3})) (Supplementary Methods). From these data, we elucidated the molecular structure of 1 to be a modified tetradecenoic acid, which we termed clostrienoic acid (Fig. 1c).

In vitro biochemical analysis of PKS. Based on the structures of 1 and 3, we hypothesized that the single-module PKS could use malonyl-CoA substrates and function iteratively to yield a heptaketide intermediate. Further modifications by putative auxiliary enzymes such as enoyl reductase, isomerase/desaturase, and thioesterase would then form the clostrienoic core (1). To confirm that ca_c3355 encodes a functional iterative type I PKS, the 203 kDa megasynthase was overexpressed and purified from Streptomyces sp. RM-5-423, a microbial modification product of A58365A from Streptomyces chromofuscus NRRL 1509824, and a glycosyl ester of 3,4-seco-triterpene25. The minor compound 2 was indicated to contain one monosaccharide substituent (α-L-rhamnopyranoside) based on HRMS/MS analysis, but the titer was too low to be further confirmed by NMR spectroscopic analysis (Supplementary Fig. 4).

Further in vitro reconstitution of the activity of PKS with malonyl-CoA showed a dominant product, the triketide lactone 4, which is a typical shunt product after spontaneous cyclic off-loading of the unreduced triketide. Addition of NADPH to this reaction enabled function of the KR domain (and the subsequent DH domain), leading to the production of the known shunt products tetraakete pyrone 5 and pentaketide pyrone 6 (Supplementary Figs. 8,
obtained production time-course profiles of the three polyketides and the ABE fermentation profile associated with deletion of the corresponding gene. These results demonstrated that ca_c3355 encodes a highly reducing type I PKS that functions iteratively to condense malonyl-CoA monomers. Furthermore, these results indicated that additional biosynthetic enzymes are needed for generating 1 and 3, and their encoding genes are located elsewhere on the genome of C. acetobutylicum.

**Impact of pks gene on ABE fermentation.** To determine whether polyketide production influenced ABE fermentation, we compared the batch fermentation performance of wild-type C. acetobutylicum and Δpks (Fig. 2a–c; Supplementary Fig. 10). While both strains displayed similar growth curves and the expected acidogenic and solventogenic phases, Δpks showed stronger butanol production with ~10% increases in both butanol titer and productivity relative to wild type (Fig. 2a, b). Perhaps related to the difference in butanol production, the butyrate concentration profiles also differed for Δpks and wild type; in the Δpks culture, butyrate was produced more rapidly and reached a higher concentration during acidogenesis (0–20 h), and was re-assimilated earlier during the transition to solventogenesis (20–23 h) (Fig. 2c). This was also reflected in the pH profiles of the two strains, with the fall and rise of the culture pH (corresponding to the changes in metabolism) occurring earlier for Δpks and wild type, with 282 genes downregulated and 110 genes upregulated in Δpks. STRING network analysis showed that the expression of genes related to four major cellular processes was downregulated in Δpks, including sporulation (33 genes), carbohydrate transport (21 genes), carbohydrate metabolism (33 genes), amino-acid transport (8 genes) (Fig. 3; Supplementary Data 1). It is notable that no significant difference in the expression of the key solventogenic genes (including the crucial sol operon) was observed during the same period as maximum butyrate/acetate concentrations (20–23 h), while the production of Δpks and wild type acetone initiated at approximately the same time (~16 h) and increased for the remainder of the fermentation. These results suggest a direct, although currently unclear, link between polyketide production and the ABE fermentation phases, with the production of 1 and 2 associated with acidogenesis, and the production of 3 associated with solventogenesis. We propose that 1 and 2 are biosynthetic intermediates of the end product 3, and feeding studies with 1 and 2 showed that these compounds were readily converted to 3 in cultures of C. acetobutylicum Δpks.

**Polyketides affect sporulation and granulose accumulation.** To better understand the broader biological impacts of polyketide production on C. acetobutylicum metabolism and physiology, we performed a transcriptome comparison of Δpks and wild-type strains using RNA-Seq. Analysis of samples taken at early stationary phase revealed a total of 392 genes that were differentially expressed (expression fold change $> 2.0$, p-value $< 0.003$) between Δpks and wild type, with 282 genes downregulated and 110 genes upregulated in Δpks. STRING network analysis showed that the expression of genes related to four major cellular processes was downregulated in Δpks, including sporulation (33 genes), carbohydrate transport (21 genes), carbohydrate metabolism (33 genes), amino-acid transport (8 genes) (Fig. 3; Supplementary Data 1). It is notable that no significant difference in the expression of the key solventogenic genes (including the crucial sol operon) was observed during the same period as maximum butyrate/acetate concentrations (20–23 h), while the production of Δpks and wild type acetone initiated at approximately the same time (~16 h) and increased for the remainder of the fermentation. These results suggest a direct, although currently unclear, link between polyketide production and the ABE fermentation phases, with the production of 1 and 2 associated with acidogenesis, and the production of 3 associated with solventogenesis. We propose that 1 and 2 are biosynthetic intermediates of the end product 3, and feeding studies with 1 and 2 showed that these compounds were readily converted to 3 in cultures of C. acetobutylicum Δpks.

**Fig. 2** Time-course batch fermentation evaluation of wild-type C. acetobutylicum and Δpks. a Culture density (measured as optical density at 600 nm, OD$_{600}$) (left axis) and pH (right axis) from batch fermentations of wild type and Δpks. b Production of acetone, butanol, and ethanol from batch fermentations of wild type and Δpks. c Production of acetate and butyrate from batch fermentations of wild type and Δpks. d Production of compounds 1–3 from batch fermentation of wild-type C. acetobutylicum ATCC 824. As indicated, the green region represents the acid production phase of the fermentation (acidogenesis), while the blue region represents the solvent production phase (solventogenesis). Error bars represent the standard deviation of values from duplicate fermentations. Experiments were repeated at least three times independently.
Fig. 3 RNA-Seq comparison of wild-type *C. acetobutylicum* and Δpks. STRING network analysis of genes predicted to be transcriptionally downregulated (left) and upregulated (right) by RNA-Seq in Δpks fermentation culture relative to wild type. Nodes represent differentially expressed genes, and lines represent predicted connections between genes including shared functional pathways of encoded proteins, chromosomal proximity, co-occurrence of genes in other organisms, co-expression of genes, and protein homology. For clarity, only genes with at least one predicted connection are shown. Four major concentrations of connected nodes were observed for each group (indicated by a–h), signifying the cellular pathways most affected by deletion of the pks gene. Detailed results from the RNA-Seq analysis are presented in Supplementary Data 1.

was observed, suggesting that the difference in butanol production between Δpks and wild type was not due to the direct transcriptional regulation of solventogenic genes by the polyketides. Consistent with the higher solvent production observed for Δpks, the major pathways upregulated in Δpks (particularly class I, III, and IV heat shock response machinery) have previously been associated with improved solvent tolerance in *C. acetobutylicum*34,36.

The sporulation genes downregulated in Δpks include those encoding late-stage sporulation proteins such as spore coat and germination proteins, and the sporulation-specific sigma factor K (σK), one of the core regulators of sporulation in *C. acetobutylicum*34. Notably, transcription of the gene encoding the well-known master regulator of sporulation and solvent production, Spo0A35, was not significantly affected in Δpks. To determine whether reduced sporulation was an observable property of Δpks as suggested by the RNA-Seq analysis, sporulation assays in both liquid and solid media were performed for wild-type *C. acetobutylicum* and Δpks. Indeed, a significant decrease in sporulation was observed for Δpks, with spore formation decreasing by 3–4 orders of magnitude in liquid culture (Fig. 4a), and by ~2 orders of magnitude on solid media (Supplementary Fig. 11). Furthermore, the level of sporulation for Δpks was partially restored when Δpks culture broth was supplemented with clostrienose (3) (Fig. 4a). Since granulose biosynthesis and accumulation is related to the sporulation cycle, we then performed standard granulose accumulation assays using iodine staining. Colonies which have accumulated granule are expected to stain dark brown/purple following exposure to iodine vapor, with darker staining indicating higher granulose production. Following the trend observed for sporulation, a significant decrease in granulose accumulation was observed for Δpks (little to no staining), and the addition of 3 to Δpks culture completely restored granulose accumulation (even appearing to exceed levels observed for wild type) (Fig. 4b). These assays revealed that the polyketides are important, although not essential, for triggering both sporulation and granulose accumulation in wild-type *C. acetobutylicum*.

Interestingly, pks inactivation also affected colony morphology of *C. acetobutylicum*. While wild-type colonies were relatively flat in elevation and featured a distinctive “spore center” in the middle of the colony, Δpks colonies were distinctively raised in elevation and featured a highly textured surface with no distinguishable spore center (Fig. 4c). This behavior suggested that one or more of the polyketides might act as surfactants to permit colony spreading over the agar surface, as self-produced surfactants are known to permit surface motility in some microbes36. To test this, we performed standard assays for surfactant activity (oil spreading and drop collapse assays) using pure clostrienoic (3), given that this compound displays some structural similarities to known non-ionic surfactants such as *n*-dodecyl-β-D-maltosides37 and rhamnolipids38. As shown in Supplementary Tables 3 and 4, clostrienose displayed weak surfactant activity at relatively high concentrations (100 μM), but little to no surfactant activity near physiological concentrations (10 μM) according to the oil spreading assay. Surfactant activity was not observed using the drop collapse assay at either concentration of clostrienose (10 or 100 μM).

**Discussion**

*Clostridium* is one of the largest bacterial genera, ranking second in size only to *Streptomyces*39. While members of *Streptomyces* are known to be prolific producers of secondary metabolites40, only a handful of secondary metabolites have been discovered from *Clostridium*. However, recent genomic analysis has indicated that secondary metabolite gene clusters can be found among diverse members of this genus, prompting efforts to identify and characterize these “cryptic” secondary metabolites6. In this study, we identified a suite of polyketides (clostrienoic acid and clostrienose) from *C. acetobutylicum*, a well-studied solvent-producing anaerobe with no previously associated natural products.

Clostrienoic acid and clostrienose are biosynthesized by a predicted type I single-module PKS. Through in vitro reconstitution of the activity of purified PKS, we demonstrated that this megasynthase functions as a highly reducing iterative type I PKS. We propose that the PKS functions iteratively to generate a heptaketide intermediate, which is then modified by tailoring enzymes encoded elsewhere on the genome to yield clostrienoic acid. Two subsequent glycosylation events are proposed to install the rhamnopyranoside group, followed by the galactofuranoside group (generating clostrienose) (Supplementary Fig. 12). The proposed sequential biosynthesis of the three polyketides is consistent with the production timing of 1–3 observed in batch fermentation of *C. acetobutylicum*. Although galactofuranose has
studies identified with well-known examples such as 4-quinolones, in several Clostridium species, although none of these studies identified the molecular structure of the presumed bioactive small molecules. This work provides solid evidence that cellular differentiation can be regulated by secondary metabolites in Clostridium, and has revealed the molecular identity of the signal responsible for this behavior.

Although clostrienose displayed only weak surfactant activity near physiological concentrations observed under batch fermentation conditions (<6 µM), the possible role of clostrienose as both a biosurfactant as well as a signaling molecule draws interesting parallels to previously studied biosurfactants, which are known to act as signaling molecules to regulate gene expression. In the well-studied case of surfactin (widely classified as a quorum sensing signaling molecule), this self-generated biosurfactant is known to regulate gene expression necessary for triggering extracellular matrix (ECM) production and efficient sporulation in Bacillus subtilis. Interestingly, surfactin is able to alter gene expression by forming membrane pores, which selectively leak potassium ions. The decrease in intracellular potassium is sensed by the membrane histidine kinase KinC, which then activates the regulatory circuit leading to ECM production. In another case, N-acyl-homoserine lactones (AHLs), a well-known class of signaling molecules, are produced by the bacterium Rhizobium etli to act as both biosurfactants and quorum sensing molecules to promote surface colonization. These examples illustrate that self-produced biosurfactants are not restricted to their physiochemical role in decreasing surface tension, and can also serve to regulate gene expression by acting as signaling molecules for some microbes.

Although extensive future work is needed to characterize the full regulatory network through which clostrienose controls cellular differentiation, results from our RNA-Seq analysis suggest
that transcriptional regulation of the sporulation-specific sigma factor, σ^6, may play an important role in the mode of action. Based on our RNA-Seq results, we observed the downregulation of 33 genes related to sporulation, in particular, genes related to late endospore development (stages IV and V of the sporulation cycle^{[2]}) and spore germination in Δpks relative to wild type (Supplementary Data 1). Of the well-characterized regulators of sporulation in C. acetobutylicum ATCC 824 (including Spo0A, σ^D, σ^E, σ^F, and σ^K)^{[3], [63]}, only the gene encoding σ^K (ca_c1689) was significantly downregulated in Δpks at the time of analysis (26 h post inoculation). A putative sigma factor encoded by ca_p0157 (believed to be involved in regulating very late stage sporulation and granulose accumulation) was also significantly downregulated in Δpks, suggesting that transcription of this regulator may be under the control of σ^K. It was reported recently that σ^K performs two developmentally separated roles in C. acetobutylicum ATCC 824, one in early sporulation and one in late sporulation^{[54]}. In early sporulation, σ^K is important for upregulation of Spo0A, the master regulator of sporulation and solvent production. For this early role, σ^K likely requires transcriptional activation by σ^D. Since the latter is important for stimulating both solventogenesis and sporulation, σ^K deletion results in low solvent, non-sporulating cultures. In late sporulation, σ^K is important for stage IV spore development, including assembly of the spore coat. For this late role, σ^K activation is σ^G dependent, likely through contributions of the σ^G dependent genes spoIVFB (ca_c1253) and spoIVB (ca_c2072), which are proposed to be required for post-translational processing of pre-σ^K to the mature σ^K form. Considering our results, we hypothesize that transcriptional downregulation of σ^K in Δpks at this stage of the fermentation is relevant to the role of σ^K in late-stage sporulation. As Δpks displayed severe reductions in observed sporulation rates, but ABE production was not decreased as would be expected for weakened early-stage σ^K activity. As we expect that some or all of sporulation genes downregulated in Δpks are members of the σ^K regulon, this suggests that closotrienose may have some role in stimulating late-stage σ^K activity, and thus, late-stage sporulation. Given what is known about the regulatory relationships between well-characterized sporulation sigma factors in C. acetobutylicum,^{[12]} we hypothesize that closotrienose may act to regulate late-stage σ^K activity through an unknown pathway between σ^D and σ^K, possibly involving contributions in post-translational regulation of σ^K from the σ^G-dependent enzymes SpoIVFB and SpoIVB (the encoding genes of which were also downregulated in Δpks) (Supplementary Data 1). While extensive follow-up work is required to validate this hypothesis, this proposed mechanism could help to explain the means by which σ^K activates late σ^K activity in C. acetobutylicum, which is currently unknown^{[12]}. Solventogenic strains of Clostridium (such as C. acetobutylicum) were employed for industrial solvent production as early as 1916, and were eventually the source of 66% of US butanol production in 1945^{[10]}. Although industrial operations of this process largely ceased during the 1950s due to the growth of the petrochemical industry, ABE fermentation has recently gained renewed interest given the wide range of agricultural feedstocks, which can be converted to various commodity chemicals and potential biofuels using this process^{[35]}. However, some drawbacks still exist that prevent the widespread use of ABE fermentation, such as low solvent titer/productivity due to solvent toxicity, and unfavorable cellular differentiation^{[36]}. Rather than pursuing a traditional metabolic engineering strategy that focuses on the core metabolic pathway for solvent production, our work showcases an alternative approach by manipulating the secondary metabolism of the organism to improve traits significant for industrial ABE fermentation performance. In particular, given that both granulose biosynthesis and sporulation are undesirable traits for industrial fermentation (as granulose accumulation results in reduced solvent yields, and metabolically inactive spores do not contribute to solvent production)^{[39]}, the reduced granulose accumulation and sporulation associated with Δpks represent improved industrial traits. Furthermore, although none of the solvent producing genes were upregulated in Δpks relative to wild type, both butanol titer and productivity were increased in Δpks. This may be explained by the decreased commitment of cells to sporulation in Δpks (yielding a higher proportion of cells capable of solvent production), as well as the upregulation of cellular machinery related to butanol stress and adaptation as indicated by transcriptomic analysis.

In summary, we have discovered a family of novel polyketides that are biosynthesized by a highly reducing iterative type I PKS in C. acetobutylicum ATCC 824. In addition to the type II PKS-derived clostrubin, our work provides the second example of polyketide metabolites from a strictly anaerobic bacterium, and encourages continued efforts in exploring the unexploited terrain of secondary metabolites of this industrial strain. We have further hypothesized that the newly identified polyketides are important for stimulating sporulation and granulose accumulation in C. acetobutylicum, adding to the extremely limited inventory of known signaling molecules used by Clostridium to control cellular physiology and metabolism. Furthermore, this work has yielded an engineered strain of C. acetobutylicum with improved traits for industrial ABE fermentation (reduced sporulation, reduced granulose accumulation, and increased butanol titer and productivity), demonstrating a novel strategy of manipulating secondary metabolism as a means of improving this important renewable bioprocess.

**Methods**

**Bacterial strains and media.** C. acetobutylicum ATCC 824 was cultured in an anaerobic chamber (Coy Laboratory Products) containing an atmosphere of 97% nitrogen and 3% hydrogen. 2xTYG medium^{[67]} contained 16 g/L tryptone, 10 g/L yeast extract, 5 g/L NaCl, and 10 g/L glucose (unless noted otherwise) with the pH adjusted to 5.2 for liquid media, and 5.8 for solid media (also containing 15 g/L agar). Clostridial growth medium (CGM)^{[68]} contained 30 g/L glucose (unless noted otherwise), 6.25 g/L yeast extract, 2.5 g/L ammonium sulfate, 1.25 g/L NaCl, 2.5 g/L asparagine, 0.95 g/L monobasic potassium phosphate, 0.95 g/L dibasic potassium phosphate, 0.5 g/L magnesium sulfate heptahydrate, 13 mg/L manganese sulfate heptahydrate, and 13 mg/L iron sulfate heptahydrate with the pH adjusted to 6.4. P2 medium^{[66]} contained 80 g/L glucose (unless noted otherwise), 1.6 g/L yeast extract, 2.2 g/L ammonium acetate, 0.5 g/L potassium phosphate monobasic, 0.5 g/L potassium phosphate dibasic, 0.2 g/L magnesium sulfate heptahydrate, 1 mg/L paraaminobenzoic acid, 1 mg/L thiamine hydrochloride, 10 µg/L biotin, 10 mg/L manganese sulfate heptahydrate, 10 mg/L ferrous sulfate heptahydrate, and 10 mg/L NaCl with the pH adjusted to 6.4. Clostridial basal medium (CBM)^{[70]} contained 10 g/L glucose, 0.5 g/L monobasic potassium phosphate, 0.5 g/L dibasic potassium phosphate, 4 g/L tryptone, 0.2 g/L magnesium sulfate heptahydrate, 10 mg/L manganese sulfate heptahydrate, 10 mg/L ferrous sulfate heptahydrate, 1 mg/L paraaminobenzoic acid, 1 mg/L thiamine hydrochloride, and 2 µg/L biotin with the pH adjusted to 6.9. For solid CGM plates, 15 g/L agar was added. CBM-S (used for liquid sporulation assays) was identical to CBM except 50 g/L glucose was used, and 5 g/L CaCO3 was added just prior to inoculation of cultures. E. coli TOP10 (Thermo Fischer Scientific) was grown in Luria-Bertani (LB) medium at 37 °C. For the appropriate Clostridium strains, culture media was supplemented with erythromycin (Ery: 40 µg/mL for solid media, 80 µg/mL for liquid media) and/or tiamphenicol (Th: 5 µg/mL for solid and liquid media). Kanamycin (Kan; 60 µg/ mL) or chloramphenicol (Cm; 25 µg/mL) were added to E. coli culture media as indicated. Clostridium and E. coli strains were maintained as 20% v/v glycerol stocks stored at −80 °C.

**Plasmid construction.** Oligonucleotides were provided by Integrated DNA Technologies (Supplementary Table 3). Phusion polymerase (NEB) was used for all PCR reactions. For isolation of genomic DNA from C. acetobutylicum ATCC 824, an alkaline lysis method was used^{[71]}. C. acetobutylicum was cultured overnight in 2xTYG medium to stationary phase (OD600 > 2.0), at which point 10 mL of culture was centrifuged at 3500xg for 15 min (room temperature). The supernatant was removed and the cell pellet was resuspended in 5 mL SET buffer (25 mM NaCl, 25 mM EDTA pH 8.0, 20 mM Tris-HCl pH 7.5). Lysozyme was added to a final concentration of 2 mg/mL, and the solution was gently mixed. The mixture was
incubated at 37 °C for 60 min with gentle mixing performed every 15 min. Following the incubation period, 660 µL of lysis buffer (1 M NaOH, 10% w/v SDS) was added, and the sample was thoroughly mixed to a final concentration of 0.5 mg/mL, and the solution was incubated at 55 °C for 1 h. Following this incubation period, an equal volume of phenol-chloroform (1:1) was added, and the solution was mixed by inversion for 5 min. The solution was then centrifuged at 3500×g for 15 min (room temperature), and the upper aqueous phase was discarded. The DNA was precipitated with 2 volumes of 70% ethanol, and the DNA was resuspended in 20 µL TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0), and dissolved by incubation at 50 °C.

For constructing plasmid pKO_mazF_mod (which would later serve as a template for pKO_pks), primers pK0F and pK0R were used to PCR amplify a 5.0 kb region from the pKO_mazF template. This step was necessary to remove the sequence of the mazF gene under the constitutive crotonase promoter (P_cro) from the plasmid backbone. The chloramphenicol/thiamenol resistance gene and constitutive promoter (pP_cro from C. acetobutylicum) were PCR amplified from pKO_mazF_mod using primers CMR_F and CMR_R. The gel extracted PCR products were ligated via Gibson assembly and transformed into E. coli TOP10. Transformant clones were screened by plasmid test digestion, and Sanger sequencing was used to confirm the sequence of the final pKO_mazF_mod clone.

For constructing plasmid pICE_pks (for deletion of the pks gene from C. acetobutylicum), a 3.8 kb region from plasmid pICE was amplified using primers ICEE_F and ICEE_R. The PCR amplified plasmid pICE_pks_mod was further amplified using primers pK0_F and pK0_R. Additionally, primers UHR_F and UHR_R, and DHR_F and DHR_R were used to PCR amplify 1 kb regions representing the upstream and downstream homologous regions. The gel amplified 1 kb regions were then digested with DpnI and ligated to form pKO_mazF_mod, and transformed into E. coli TOP10. Transformant clones were screened by plasmid test digestion, and Sanger sequencing was used to confirm the sequence of the final pKO_pks clone. For constructing plasmid pAN315 (necessary for methylation of pKO_pks and pCKO_pks prior to transformation into C. acetobutylicum), a 6.4 kb region from plasmid pAN152 was amplified using primers pAN1_F and pAN1_R, and a 1.0 kb region containing the kanamycin resistance gene from vector pCOLA_Duet was amplified using primers KAN_F and KAN_R. The gel extracted PCR products were ligated via Gibson assembly and transformed in to E. coli TOP10. Transformant clones were screened by purified plasmid test digestion, and Sanger sequencing was used to confirm the sequence of the final pAN315 clone. For constructing plasmid pCKO_pks (for expression of the pks gene under the constitutive crotonase [P_cro] promoter from C. acetobutylicum), primers CPKS_Fo and CPKS_Ro were used to PCR amplify the 5.4 kb C. acetobutylicum ATCC 824 pks gene (C. acetobutylicum) with appropriate overhangs for Gibson assembly. The pWS_empty vector backbone (containing the constitutive crt promoter upstream of the multiple cloning site) was PCR amplified using primers pWS_F and pWS_R yielding a 5 kb product. The two gel purified PCR products were ligated via Gibson assembly and transformed into E. coli TOP10. Transformant clones were screened by purified plasmid test digestion, and Sanger sequencing was used to confirm the sequence of the final pCKO_pks clone.

For constructing plasmid pET24b_pks, the 5.4 kb C. acetobutylicum ATCC 824 pks gene was amplified from C. acetobutylicum genomic DNA. The gel extracted PCR products were ligated via Gibson assembly and transformed into E. coli TOP10. Transformant clones were screened by purified plasmid test digestion, and Sanger sequencing was used to confirm the sequence of the final pET24b_pks clone.

Electro-transfer of C. acetobutylicum. Prior to transformation into C. acetobutylicum, vector pKO_pks was co-transformed with pAN3 into E. coli TOP10 via electroperoration. This procedure permitted methylation of pKO_pks necessary to overcome the native resistance-modification system of C. acetobutylicum72. Plasmid purification of E. coli pKO_pks/pAN3 liquid culture was performed, and the resulting plasmid mixture was used for electroperoration of C. acetobutylicum using the previously published method72 which we detail here. To prepare electrocompetent cells, a single colony of C. acetobutylicum from a 2xYTG plate (grown for 1 week) was resuspended in 20 mL of liquid CGM (30 g/L glucose). Following overnight incubation (stagnant) until reaching OD600 ~1.0, these cultures were used to inoculate 10 mL of liquid CGM (80 g/L glucose) with a 10% inoculum for subculturing. After ~5 h (OD600 ~1.0) of stagnant growth, the subcultures were used to inoculate quadruplicate flask眼神 (70 mL CGM, 80 g/L glucose, 0.5 mL Antifoam 204, 3 mL Ery) aerated at 200 RPM and ~35 °C. Calcium carbonate (6 g/L) was supplemented to the fermentation media for pH buffering. All culturing was performed at 37 °C. About 5 g/L Th was included in all cultures of pKSs with the exception of the final fermentation culture, as certain antibiotics are known to perturb the ABE fermentation phases. Samples of fermentation broths (1 mL) from each replicate were taken during early stationary phase and extracted with 3 mL of 2:1 chloroform–methanol. The mixtures were vortexed, separated via centrifugation (2700g, 10 min), and the bottom chloroform-rich layer was transferred to a glass vial. These organic extracts were then dried with nitrogen gas, resuspended in 100 µL methanol, and 10 µL was injected into 4.6 µL LC-HRMS metabolomic analysis. Ion chromatograms were fit with an Agilent Eclipse Plus C18 column (4.6 × 100 mm). A linear gradient of 2–98% CH3CN (vol/vol) over 40 min in H2O with 0.1% formic acid (vol/vol) at a flow rate of 0.3 mL/min was used. The metabolomic analysis platform XCMS (The Scripps Research Institute) was used to compare the metabolomes of wild-type and Δpks C. acetobutylicum, single colonies of each strain were heat shocked at 42 °C for 10 min and placed on ice to induce C. acetobutylicum. To use the method described above, following electroperoration and colony PCR verification. Four sets of primers were used as the basis of colony PCR verification, as detailed in Supplementary Fig. 2.

Deletion of pks gene and genetic complementation. Target KO of the pks gene (CA_3355) in C. acetobutylicum ATCC 824 was achieved using the previously published method73. In detail, 5 µg of methylated pKO_pks/pAN3 plasmid mixture was transformed into E. coli TOP10. Following recovery in liquid 2xYTG medium for 4 h, the cells were centrifuged (3500×g, 15 min, room temperature), resuspended in 0.5 mL of fresh liquid 2xYTG, and 100 µL of the resuspended cell culture was plated on solid 2xYTG + 5 µg/mL Th + 40 mM β-lactose plates. Under these plating conditions, Th resistant mutants were enriched in low TE broth (10 mM Tris, 0.1 mM EDTA, pH 8.0), and dissolved by incubation at 50 °C.

LC-HRMS metabolic analysis. For untargeted metabolomic comparisons of wild-type and Δpks C. acetobutylicum, single colonies of each strain were heat shocked at 42 °C for 10 min and placed on ice to induce C. acetobutylicum. To use the method described above, following electroperoration and colony PCR verification, cultures were plated on solid 2xYTG + 5 µg/mL Th + 40 µg/mL Ery media. After twice restreaking on solid 2xYTG + 5 µg/mL Th + 40 µg/mL Ery media, potential colonies harboring pKO_pks were screened via colony PCR using primers pKO_F and pKO_R.

Bioreactor fermentations. To compare ABE fermentation profiles of wild-type and Δpks C. acetobutylicum, bioreactor fermentations were carried out in DASGIP Bioreactors (4 x GPI 100 Vessels, DASGIP Bioblock System) with 500 mL working volumes. Overnight cultures (10 mL CGM, 30 g/L glucose, 30 °C) inoculated with ATCC 824 (1:100) were used to inoculate 10 L of liquid CGM medium, 80 g/L glucose. The fermentations were cultured until reaching OD600 ~1. A 10% inoculum was then used to start a subculture (30 mL P2 media, 80 g/L glucose, 30 °C), and the subculture was incubated until reaching OD600 ~1. The 30 mL subcultures were then aseptically transferred into individual DASGIP Bioreactors pre-loaded with 500 mL P2 medium (80 g/L glucose, 100 µL Antifoam 204, 34 °C). The fermentations were allowed to proceed for 54 h with periodic sampling for optical density measurements, fermentation product analysis, and quantification of compounds 1, 2, and 3. The temperature was maintained at 34 °C throughout the fermentation, agitation was provided by stirring at 200 rpm, and the pH was maintained above 5.0 using automated addition of 5 M NaOH. Following fermentation, 1 L of spent-cell broth and 700 mL of spent-nitrogen gas was sparged at a rate of 2 L/h for the duration of the fermentation. For quantification of compounds 1, 2, and 3, 1 mL of fermentation broth was
mixed with 3 mL ethyl acetate, vortexed, separated via centrifugation (2700g, 10 min, room temperature), and the upper organic layer isolated. The organic layer was dried by rotary evaporation, resuspended in 200 µL methanol, and 20 µL was injected onto an Agilent Technologies 6120 Quadrapole LC-MS (with DAD) instrument fitted with an Agilent Eclipse Plus C18 column (4.6 x 100 mm). A linear gradient of 2–98% CH3CN (vol/vol) over 40 min in H2O with 0.1% formic acid (vol/vol) at a flow rate of 0.5 mL/min was used. Compounds 1, 2, and 3 were identified by UV absorption (240 nm) as demonstrated in Fig. 1b, and were quantified by the integrated peak area (absorbance at 240 nm).

Fermentation analytical procedures. A spectrophotometer was used to determine cell densities by measuring the optical density at 600 nm (OD600). A Shimadzu Prominence UFLC system fitted with a Biorad Aminex HPX-87H column (300 mm x 7.8 mm) was used to analyze C. acetobutylicum fermentation broth for the concentration of glucose and fermentation products (acetate, butyrate, lactate, acetone, butanol, and ethanol). Samples of fermentation broth were first pelleted by centrifugation at 10,000g for 3 min, followed by filtration of the supernatant using a 0.22 micron PVDF syringe filter. Samples of filtered supernatant (20 µL) were injected onto the UFLC system with 0.01 N sulfuric acid mobile phase flowing at 0.7 mL/min, column temperature of 35 °C, and a detection wavelength of 210 nm. Samples were resuspended and stored in 20 IU/ml RNase-free DNase I (NEB). RNA quality control, RNA quality, control, library construction, and library sequencing were performed by the University of California Genetics Functional Genomics Laboratory and Genomic Sequencing Laboratory. RNA quality and concentration was assessed according to the manufacturer’s instructions. An on-column DNase treatment was performed using DNase 1 (RNase-free) (NEB). RNA quality control, library construction, and library sequencing were performed by the University of California Genetics Functional Genomics Laboratory and Genomic Sequencing Laboratory. RNA quality and concentration was assessed using a nanochip on an Agilent 2100 Bioanalyzer. Bacterial 16S and 23S rRNA was quantified using a Leica MZ16 F dissecting microscope fitted with a Leica DFC300 FX camera.

RNA isolation and RNA-Seq analysis. Samples (10 µL) of fermentation broth were taken in biological triplicate from bioreactor fermentations of wild-type and Δpks C. acetobutylicum 26 h post inoculation. The samples were centrifuged (4000 x g, 10 min), the pellets were resuspended in Nucleic Acid Binding Reagent (Qiagen) according to the manufacturer’s instructions. An on-column DNAse treatment was performed using DNase 1 (RNase-free) (NEB). RNA quality control, library construction, and library sequencing were performed by the University of California Genetics Functional Genomics Laboratory and Genomic Sequencing Laboratory. RNA quality and concentration was assessed using a nanochip on an Agilent 2100 Bioanalyzer. Bacterial 16S and 23S rRNA was quantified using a Leica MZ16 F dissecting microscope fitted with a Leica DFC300 FX camera.

Phenotype comparison assays. Liquid sporulation assays were performed with minor modifications24. Samples were taken from biological triplicate liquid cultures after 5 days of incubation (30 mL CMB-S, 37 °C). The 20 mL samples were heated shocked (80 °C, 10 min), diluted (10–10) were spotted on 2XYTG plates, and colonies were estimated after 30 h of incubation (37 °C) to calculate the number of heat-resistant colony forming units (cfu/mL). For chemical complementation of Δpks in the liquid sporulation assay, purified clostrienose (final concentration 3.5 µM) was supplemented in CMB-S cultures of Δpks C. acetobutylicum at the time of inoculation. Since compound 3 was added as a concentrated solution in methanol, the equivalent volume of methanol (60 µL) was added to all other liquid sporulation assay cultures (wild-type and non-complemented Δpks) to control for this effect.

For solid sporulation assays, a previously described method19 was employed with some modifications. In detail, heat shocked (80 °C, 10 min) individual colonies were cultured in liquid media (10 mL CMB-S, 37 °C) for 24 h. Cultures were diluted by a factor of 104 and plated on solid CMB. Sampling was conducted 1, 2, 3, 4, and 6 days following initial plating. For sampling, three individual colonies were combined and thoroughly resuspended in 60 µL of liquid CGM. 20 µL of the resuspended colony mixture was then heat shocked (80 °C, 10 min), diluted (10–10) were spotted on 2XYTG plates, and colonies were estimated after 30 h of incubation (37 °C) to calculate the number of heat resistant colony forming units (cfu/colony). All samples were performed as biological triplicates.

Granulose accumulation assays were performed via iodine staining23. Mid-log phase (OD600 ~0.6) liquid cultures (P2, 37 °C) were plated on solid 2XYTG medium with elevated glucose levels (50 g/L) to enable granulose production. A droplet (150 µL) of sample was injected onto an Agilent Technologies 6120 Quadrapole LC-MS (with DAD) instrument fitted with an Agilent Eclipse Plus C18 column (4.6 x 100 mm). A linear gradient of 2–98% CH3CN (vol/vol) over 40 min in H2O with 0.1% formic acid (vol/vol) at a flow rate of 0.5 mL/min was used. LC-HRMS analysis of the extract was performed on an Agilent Technologies 6520 Accurate-Mass QTOF LC-MS instrument fitted with an Agilent Eclipse Plus C18 column (4.6 x 100 mm) using the same solvent gradient and flow rate described above.

Data availability. RNA-seq data generated in this study have been deposited in ArrayExpress (https://www.ebi.ac.uk/arrayexpress/) under accession number E-MTAB-6019. All other relevant data are available from the authors upon request.
References
1. Fischbach, M. A. & Walsh, C. T. Assembly-line enzymology for polyketide and nonribosomal peptide antibiotic logic, machinery, and mechanisms. Chem. Rev. 106, 4368–4396 (2006).
2. Hertweck, C. The biosynthetic logic of polyketide diversity. Angew. Chem. Int. Ed. 48, 4688–4716 (2009).
3. Newman, D. J. & Cragg, G. M. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J. Nat. Prod. 75, 311–335 (2012).
4. Raaijmakers, J. M. & Mazzola, M. Diversity and natural functions of antibiotics produced by beneficial and plant pathogenic bacteria. Annu. Rev. Phytopathol. 50, 403–424 (2012).
5. Behnken, S. & Hertweck, C. Anaerobic bacteria as producers of antibiotics. Appl. Microbiol. Biotechnol. 96, 61–67 (2012).
6. Letzel, A.-C., Pidot, S. J. & Hertweck, C. A genomic approach to the cryptic biosynthetic logic of polyketide diversity. Science 326, 589–592 (2009).
7. Xu, W., Cai, X., Jung, M. E. & Tang, Y. Analysis of intact and dissected fungal polyketide synthase-nonribosomal peptide synthetase in vitro and in Saccharomyces cerevisiae. J. Am. Chem. Soc. 132, 13604–13607 (2010).
8. Ma, S. M. et al. Complete reconstitution of a highly reducing iterative polyketide synthase. Science 336, 258–262 (2012).
9. Shabuer, G. et al. Plant pathogenic anaerobic bacteria use aromatic polyketides as metabolites. Appl. Microbiol. Biotechnol. 88, 665–674 (2011).
10. Al-Hinai, M. A., Jones, S. W. & Papoutsakis, E. T. The clostridium sporulation developmentally separated roles, one early and one late in sporulation. J. Bacteriol. 196, 287–294 (2014).
11. Steinert, E. et al. Multiple orphan histidine kinases interact directly with Spo0A – control of the initiation of endospore formation in Clostridium acetobutylicum. Mol. Microbiol. 80, 641–654 (2011).
12. Shabuer, G. et al. A field guide to bacterial swarming motility. Nat. Rev. Microbiol. 8, 634–644 (2010).
13. Ali, M. S. & Al-Lohedan, H. A. Interaction of biocompatible sugar based surfactant n-dodecyl β-D-maltoside with lysozyme. J. Mol. Liq. 209, 662–668 (2015).
14. Abdel-Mawgoud, A. M., Lépine, F. & Déziel, E. Rhamnolipids: diversity of structures, microbial origins and roles. Appl. Microbiol. Biotechnol. 86, 1323–1330 (2013).
15. Duerre, P. Handbook on Clostridia (CRC Press, Boca Raton, 2005).
16. Ikeda, H. Natural products discovery from micro-organisms in the post-genome era. Biosci. Biotechnol. Biochem. 81, 13–22 (2017).
17. Platt, T. G. & Fuqua, C. What’s in a name? The semantics of quorum sensing. Trends Microbiol. 18, 383–387 (2010).
18. Winzer, K., Hardie, K. R. & Williams, P. Bacterial cell-to-cell communication: so close, yet so far away! Curr. Opin. Microbiol. 5, 216–222 (2002).
19. Schuster, M., Sexton, D. J., Diggle, S. P. & Greenberg, E. P. Acyl-homoserine lactone quorum sensing: from evolution to application. Annu. Rev. Microbiol. 67, 63–63 (2013).
20. Diggle, S. P., Cornelis, P., Williams, P. & Cámara, M. 4-Quinolone signaling in Pseudomonas aeruginosa: old molecules, new perspectives. Int. J. Med. Microbiol. 296, 83–91 (2006).
21. Takano, E. y Butyrolactones: Streptomyces signaling molecules regulating antibiotic production and differentiation. Curr. Opin. Microbiol. 9, 287–294 (2006).
22. Ren, D., Bedzyk, L. A., Ye, R. W., Thomas, S. M. & Wood, T. K. Differential gene expression shows natural brominated furanoside furanosides interfere with the autoinducer-2 bacterial signaling system of Erwinia chrysanthemi. J. Bacteriol. 189, 630–642 (2004).
23. Li, J., Chen, J., Vidal, J. E. & McClane, B. A. The agr-like quorum-sensing system regulates sporulation and production of enterotoxin and Beta toxin by Clostridium perfringens type A non-food-borne human gastrointestinal disease strain F603. Infect. Immun. 79, 2451–2459 (2011).
24. Martin, M. J. et al. The agr locus regulates virulence and colonization genes in Clostridium difficile 027. J. Bacteriol. 195, 3672–3681 (2013).
25. Cookley, C. M. et al. Regulation of neurotoxin production and sporulation by a putative agrB/D signaling system in proteolytic Clostridium botulinum. Appl. Environ. Microbiol. 76, 4448–4460 (2010).
26. Platt, T. G. & Fuqua, C. What’s in a name? The semantics of quorum sensing. Trends Microbiol. 18, 383–387 (2010).
27. Winzer, K., Hardie, K. R. & Williams, P. Bacterial cell-to-cell communication: so close, yet so far away! Curr. Opin. Microbiol. 5, 216–222 (2002).
28. Platt, T. G. & Fuqua, C. What’s in a name? The semantics of quorum sensing. Trends Microbiol. 18, 383–387 (2010).
29. Martin, M. J. et al. The agr locus regulates virulence and colonization genes in Clostridium difficile 027. J. Bacteriol. 195, 3672–3681 (2013).
30. Cookley, C. M. et al. Regulation of neurotoxin production and sporulation by a putative agrB/D signaling system in proteolytic Clostridium botulinum. Appl. Environ. Microbiol. 76, 4448–4460 (2010).
31. Platt, T. G. & Fuqua, C. What’s in a name? The semantics of quorum sensing. Trends Microbiol. 18, 383–387 (2010).
54. López, D. & Kolter, R. Extracellular signals that define distinct and coexisting cell fates in Bacillus subtilis. FEBS Microbiol. Rev. 34, 134–149 (2010).
55. López, D., Fischbach, M. A., Chu, F., Loisick, R. & Kolter, R. Structurally diverse natural products that cause potassium leakage trigger multicellularity in Bacillus subtilis. Proc. Natl Acad. Sci. USA 106, 280–285 (2009).
56. Shank, E. A. & Kolter, R. Extracellular signaling and multicellularity in Bacillus subtilis. Curr. Opin. Microbiol. 14, 741–747 (2011).
57. Nakagawa, M. An islet enzyme required for surfacein production, competence development, and efficient sporation in Bacillus subtilis. J. Bacteriol. 173, 1770–1778 (1991).
58. Camilli, A. & Bassler, B. L. Bacterial small-molecule signaling pathways. Science 311, 1113–1116 (2006).
59. Williams, P., Winzer, K., Chan, W. C. & Cámara, M. Look who’s talking: communication and quorum sensing in the bacterial world. Philos. Trans. R. Soc. B Biol. Sci. 362, 1119–1134 (2007).
60. Daniels, R. et al. Quorum signal molecules as biosurfactants affecting swimming in B. subtilis. Curr. Opin. Biotechnol. 18, 280–287 (2007).
61. Jones, S. W., Tracy, B. P., Fast, A. G., Indurthi, D. C. & Papoutsakis, E. T. Inactivation of epsF and epsG in Clostridium acetobutylicum ATCC 824 blocks sporulation prior to asymmetric division and abolishes epsE and epsG protein expression but does not block solvent formation. J. Bacteriol. 193, 2429–2440 (2011).
62. Tracy, B. P., Jones, S. W. & Papoutsakis, E. T. Inactivation of epsF and epsG in Clostridium acetobutylicum illuminates their roles in clastrodial-cell-form biogenesis, granule synthesis, solventogenesis, and spore morphogenesis. J. Bacteriol. 193, 1414–1426 (2011).
63. Bi, C., Jones, S. W., Hess, D. R., Tracy, B. P. & Papoutsakis, E. T. SpoIE is necessary for asymmetric division, sporulation, and expression of epsF, epsE, and epsG but does not control solvent production in Clostridium acetobutylicum ATCC 824. J. Bacteriol. 193, 5130–5137 (2011).
64. Jones, S. W. et al. The transcriptional program underlying the physiology of C. acetobutylicum ATCC 824. J. Bacteriol. 193, 2854–2861 (2011).
65. Tracy, B. P., Jones, S. W., Fast, A. G., Indurthi, D. C. & Papoutsakis, E. T. Clostridia: the importance of their exceptional substrate and metabolite diversity for biofuel and biorefinery applications. Curr. Opin. Biotechnol. 23, 364–381 (2012).
66. Papoutsakis, E. T. Engineering solventogenic clostridia. Curr. Opin. Biotechnol. 19, 420–429 (2008).
67. J. D. Oultram et al. Introduction of plasmids into whole cells of Bacillus subtilis through high-efficiency transformation. FEMS Microbiol. Lett. 177, 38–88 (1998).
68. Dusseau, S., Croux, C., Soucaille, P. & Meynial-Salles, I. Metabolic engineering of Clostridium acetobutylicum ATCC 824 for the high-yield production of a biofuel composed of an isopropanol/butanol/ethanol mixture. Metab. Eng. 18, 1–8 (2013).
69. Baer, S. H., Blaschek, H. P. & Smith, T. L. Effect of butanol challenge and implementation of rational metabolic engineering for the industrial production of Clostridium acetobutylicum ATCC 824. Biotechnol. Biofuels 5, 280–288 (2012).
70. O’Brien, R. W. & Morris, J. G. Oxygen and the growth and metabolism of Clostridium acetobutylicum. Microbiology 68, 307–318 (1971).
71. Herman, N. A. et al. Development of a high-efficiency transformation method and implementation of rational metabolic engineering for the industrial butanol hyperproducer Clostridium saccharoperbutylacetonicum strain N1-4. Appl. Environ. Microbiol. 83, e02942–16 (2017).
72. Mermelstein, I. D. & Papoutsakis, E. T. In vivo methylation in Escherichia coli by the Bacillus subtilis phage phi T1 methyltransferase to protect plasmids from restriction upon transformation of Clostridium acetobutylicum ATCC 824. Appl. Environ. Microbiol. 59, 1077–1081 (1993).
73. Benjamini, Y. & Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. R. Stat. Soc. Ser. B Methodol. 57, 289–300 (1995).
74. Ehsaan, M. et al. Mutant generation by allelic exchange and genome resequencing of the biobutanol organism Clostridium acetobutylicum ATCC 824. Biotechnol. Biofuels 9, 4 (2016).
75. Ren, C. et al. Pleiotropic functions of catalase control protein CcpA in butanol-producing Clostridium acetobutylicum. BMC Genomics 13, 349 (2012).
76. Morikawa, M., Hirata, Y. & Imanaka, T. A study on the structure-function relationship of lipopeptide biosurfactants. Biochim. Biophys. Acta 1488, 211–218 (2000).
77. Plaza, G. A., Zjawiony, I. & Banat, I. M. Use of different methods for detection of thermophilic biosurfactant-producing bacteria from hydrocarbon-contaminated and bioremediated soils. J. Pet. Sci. Eng. 50, 71–77 (2006).
78. Youssef, N. H. et al. Comparison of methods to detect biosurfactant production by diverse microorganisms. J. Microbiol. Methods 56, 339–347 (2004).
79. Boudour, A. A. & Miller-Maier, R. M. Application of a modified drop-collapse technique for surfactant quantitation and screening of biosurfactant-producing microorganisms. J. Microbiol. Methods 32, 273–280 (1998).
80. Jain, D. K., Collins-Thompson, D. L., Lee, H. & Trevors, J. T. A drop-collapsing test for screening surfactant-producing microorganisms. J. Microbiol. Methods 13, 271–279 (1991).

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Author contributions
N.A.H. and W.Z. designed the experiments, analyzed the data, and wrote the manuscript. S.J.K. and W.C. performed purification and structure characterization of the polyketides. H.K. assisted with interpretation of NMR spectra of the polyketides. I.S.L. assisted with fermentation and phenotype characterization of C. acetobutylicum strains. N.A.H. performed all other experiments.

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