Production of Food and Feed Additives From Non-food-competing Feedstocks: Valorizing *N*-acetylmuramic Acid for Amino Acid and Carotenoid Fermentation With *Corynebacterium glutamicum*

Elvira Sgobba[†], Luisa Blöbaum[†] and Volker F. Wendisch*

Chair of Genetics of Prokaryotes, Faculty of Biology and CeBiTec, Bielefeld University, Bielefeld, Germany

Corynebacterium glutamicum is used for the million-ton-scale production of food and feed amino acids such as L-glutamate and L-lysine and has been engineered for production of carotenoids such as lycopene. These fermentation processes are based on sugars present in molasses and starch hydrolysates. Due to competing uses of starch and sugars in human nutrition, this bacterium has been engineered for utilization of alternative feedstocks, for example, pentose sugars present in lignocellulosic and hexosamines such as glucosamine (GlcN) and *N*-acetyl-D-glucosamine (GlcNAc). This study describes strain engineering and fermentation using *N*-acetyl-D-muramic acid (MurNAc) as non-food-competing feedstock. To this end, the genes encoding the MurNAc-specific PTS subunits MurP and Crr and the etherase MurQ from *Escherichia coli* K-12 were expressed in *C. glutamicum*Δ*nanR*. While MurP and MurQ were required to allow growth of *C. glutamicum*Δ*nanR* with MurNAc, heterologous Crr was not, but it increased the growth rate in MurNAc minimal medium from 0.15 h^{-1} to 0.20 h^{-1}. When in addition to *murP-murQ-crr* the GlcNAc-specific PTS gene *nagE* from *C. glycinophilum* was expressed in *C. glutamicum*Δ*nanR*, the resulting strain could utilize blends of GlcNAc and MurNAc. Fermentative production of the amino acids L-glutamate and L-lysine, the carotenoid lycopene, and the L-lysine derived chemicals 1,5-diaminopentane and L-pipecolic acid either from MurNAc alone or from MurNAc-GlcNAc blends was shown. MurNAc and GlcNAc are the major components of the bacterial cell wall and bacterial biomass is an underutilized side product of large-scale bacterial production of organic acids, amino acids or enzymes. The proof-of-concept for valorization of MurNAc reached here has potential for biorefinery applications to convert non-food-competing feedstocks or side-streams to valuable products such as food and feed additives.

Keywords: L-lysine, diamino pentane, lycopene, L-glutamate, biorefinery, food additives, peptidoglycan, *N*-acetyl-muraminic acid

INTRODUCTION

Corynebacterium glutamicum is a predominantly aerobic, rod-shaped, Gram-positive soil bacterium which is generally recognized as safe (GRAS). Since the 1960s, *C. glutamicum* was first used for the production of the flavor enhancer (Kinoshita et al., 1957) under biotin limiting conditions (Shiio et al., 1962). *C. glutamicum* was developed into an important organism for the biotechnological industry, producing amino acids on a million-ton scale (Wendisch, 2014). *C. glutamicum* has also been engineered to produce diamines, organic acids, carotenoids, proteins and biopolymers (Wendisch et al., 2016). Recently, metabolic engineering of *C. glutamicum* to expand its substrate scope allowed to use alternative carbon sources that do not have competing uses in the food industry (Zahoor et al., 2012). Access to the hexosamines GlcN (Uhde et al., 2013) and GlcNAc (Matano et al., 2014) has been reported, but utilization of the hexosamine MurNAc as alternative carbon source by *C. glutamicum* has not been described (Dominguez et al., 1997; Cramer and Eikmanns, 2007).

GlcN and GlcNAc can be gained by hydrolysis of chitin and chitosan that make up the arthropod exoskeleton and are present in fungal cell walls. Every year, circa 100 billion tons of chitin are produced in Nature and GlcNAc and GlcN can be obtained by acid hydrolysis (Chen et al., 2010; Zhang and Yan, 2017) and are available, e.g., from shrimp shell waste, an abundant side stream of the fishery industry. MurNAc and GlcNAc are the hexosamine constituents of peptidoglycan which makes up about 5% of the cell mass of Gram-negative bacteria and up to 20% of the cell mass of Gram-positive bacteria (Munoz et al., 1967; Reith and Mayer, 2011). The peptidoglycan constituents that can be found in all bacterial habitats have been used as indicators of bacterial biomass content in soils (Domsch, 1982). The Gram-positive *C. glutamicum* is the main producing organism for the annual production of 5 million tons of amino acids (Wendisch et al., 2016). Under the assumptions that (a) the same amount of cell dry weight is produced, (b) 20% of the cell dry weight is peptidoglycan and (c) about half of peptidoglycan is GlcNAc and MurNAc, about 500.000 tons of MurNAc and GlcNAc would be available from the amino acid fermentation industry. Biotechnological processes with bacterial hosts are used at the million-ton scale to produce secreted compounds such as organic acids, amino acids and enzymes. The spent biomass may be used in waste-to-energy applications either by thermal (e.g., incineration), thermo-chemical (e.g., torrefaction) or by biochemical treatments (e.g., anaerobic digestion). However, it is desirable to make use of the carbon and nitrogen containing hexosamine fraction of peptidoglycan in food and feed fermentation processes. The hexosamines may function both as carbon and nitrogen source for bacterial fermentations.

In chemical hydrolysis, the hexosamine fraction of peptidoglycan is accessible via enzymes of bacterial cell wall recycling. The degradation of the own cell wall by autolytic enzymes as a part of cell wall recycling is a common pathway in bacteria. When an *Escherichia coli lys dap* mutant was labeled with [3H]diaminopimelate for two generations and then chased, about 45% of its cell wall peptidoglycan was recycled per generation (Goodell and Schwarz, 1985; Uehara et al., 2006; Uehara and Park, 2008). The Gram-positive *Bacillus subtilis* can degrade, uptake and metabolize the cell wall component MurNAc in the stationary phase (Borisova et al., 2016). While all bacteria require cell wall peptidoglycan remodeling during growth and cell division, not all can utilize the monomeric components as carbon or nitrogen sources for growth. *C. glutamicum* may possess a minimal set of autolytic enzymes, however, many orthologs of the peptidoglycan degradation machinery from *E. coli* are absent (Dahl et al., 2004; Reith and Mayer, 2011). Catabolism of MurNAc in *E. coli* involves uptake and phosphorylation of MurNAc and GlcNAc via the phosphoenolpyruvate dependent phosphotransferase system (PTS). The MurNAc-specific PTS subunits are MurP and Crr (**Figure 1**). MurP, a two-domain protein that lacks a PTS-EIIA domain, is phosphorylated by EIIA^{Glc}, a kinase encoded by the *crr* gene (carbohydrate repression resistance), which interacts with several members of the glucose PTS family (Nuoffer et al., 1988; Tchieu et al., 2001; Dahl et al., 2004). MurNAc-6-phosphate is further catabolized by the etherase MurQ (**Figure 1**) that cleaves the lactyl ether bond yielding GlcNAc-6-phosphate and D-lactate (Jaeger et al., 2005; Hadi et al., 2008). GlcNAc and GlcN are also taken up via the PTS with the specific subunits NagE (Plumbridge, 2009) and PTS^{Man} (Curtis and Epstein, 1975). NagA deacetylates GlcNAc-6-phosphate to GlcN-6-phosphate which is deaminated by NagB to the glycolytic intermediate fructose-6-phosphate (**Figure 1**).
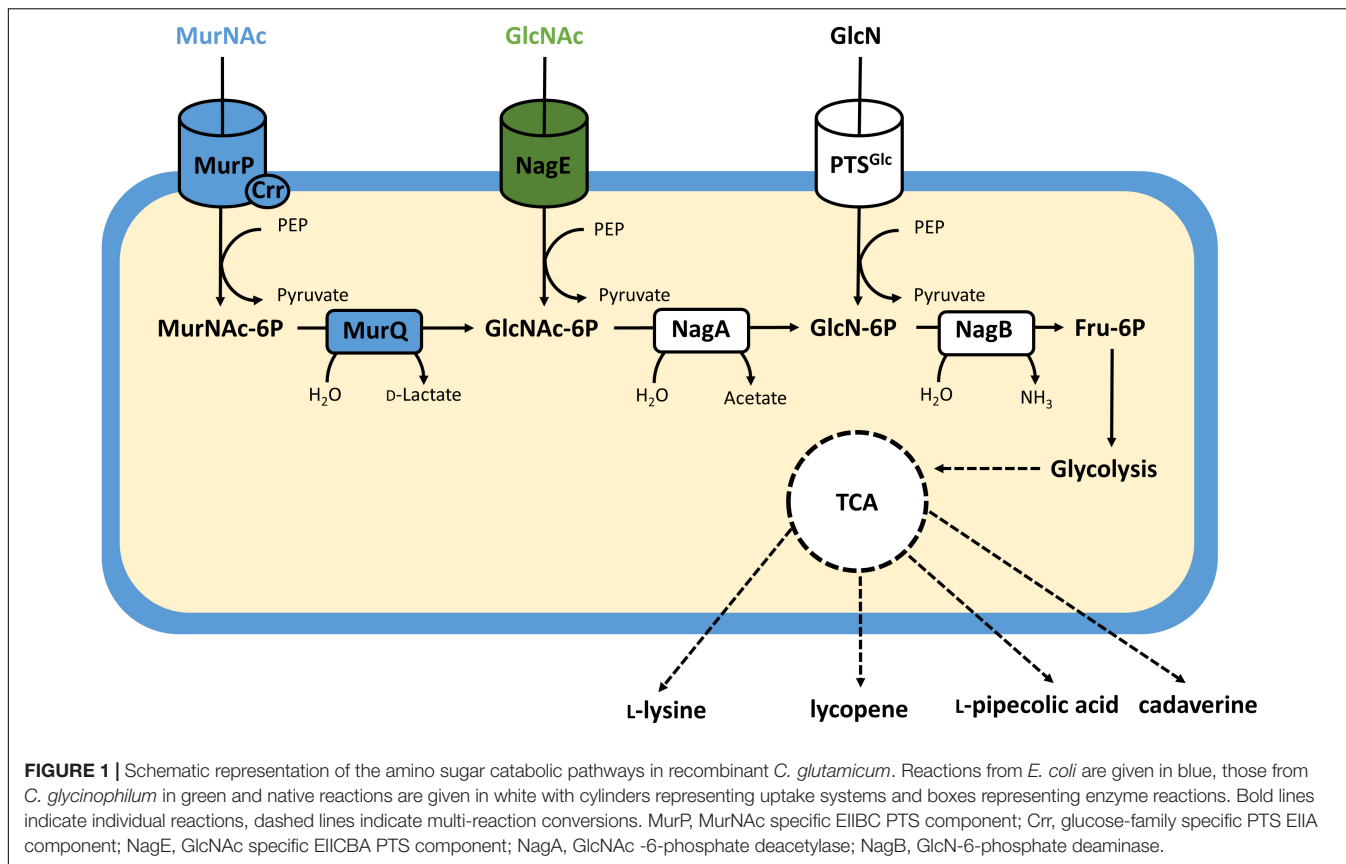
Corynebacterium glutamicum is able to take up GlcN (**Figure 1**) by using its glucose specific PTS PtsG (Arndt and Eikmanns, 2008; Uhde et al., 2013). Efficient growth with GlcN required high levels of the endogenous NagB, e.g., in the absence of the repressor protein NanR (Matano et al., 2016). By contrast, high levels of NagA and NagB were not sufficient to support growth with GlcNAc unless *nagE* from *C. glycinophilum* was expressed heterologously (Matano et al., 2014). Recombinant *C. glutamicum* strains carrying a *nanR* deletion and expressing *nagE* from *C. glycinophilum* produced several value-added products from GlcN or GlcNAc.

Here, *C. glutamicum* strains were developed for utilization of MurNAc as carbon, nitrogen and energy source and for MurNAc-based production of the food amino acid L-glutamate, the feed amino acid L-lysine, L-lysine-derived chemicals as well as the carotenoid lycopene.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

The strains and plasmids used in this work are listed in **Table 1**. Pre-cultivation of *C. glutamicum* strains was carried out at 30°C in baffled shake flasks using BHI supplemented with 45.5 g/L D-sorbitol. *E. coli* was grown at 37°C in LB (Lysogeny Broth) medium. Kanamycin (50 μg/mL), chloramphenicol (4.5 and 25 μg/mL for *C. glutamicum* and *E. coli*, respectively) or tetracycline (5 μg/mL) were added, if necessary. To adjust both cultures to growth conditions in the Biolector® system (m2pLabs,

FIGURE 1 | Schematic representation of the amino sugar catabolic pathways in recombinant *C. glutamicum*. Reactions from *E. coli* are given in blue, those from *C. glycinophilum* in green and native reactions are given in white with cylinders representing uptake systems and boxes representing enzyme reactions. Bold lines indicate individual reactions, dashed lines indicate multi-reaction conversions. MurP, MurNAc specific EIIBC PTS component; Crr, glucose-family specific PTS EIIA component; NagE, GlcNAc specific EIICBA PTS component; NagA, GlcNAc -6-phosphate deacetylase; NagB, GlcN-6-phosphate deaminase.

Baesweiler), precultures were washed after 24 h with TN-buffer and transferred to CGXII medium (Eggeling and Bott, 2005) with 100 mM GlcN and antibiotics, if necessary. After 24 h, these were transferred to 1 mL cultures in the Biolector® system (1100 rpm) with CGXII medium containing and, if not otherwise stated, 25 mM MurNAc (BACHEM, Bubendorf, Switzerland) as sole carbon source or a combination of 25 mM MurNAc and 25 mM GlcNAc. To trigger glutamate production, penicillin G (10 µM) was added in the main culture. The initial OD_{600} was 1 and gene expression from plasmids pVWEx1 and pEC-XT99A was induced by addition of 25 µM IPTG, if not otherwise stated. Correlation factors for light scattering in the Biolector system, OD_{600} and biomass concentrations were determined.

Construction of Expression Vectors

Escherichia coli DH5α was used for cloning. Codon usage of *murP*, *crr* and *murQ* from *E. coli* for *C. glutamicum* was examined using the graphical codon usage analyzer[1]. The analysis showed, that the codon ATA occurred twice in the sequence of *murP*. This codon is rarely used in *C. glutamicum* and was changed to the more frequently used codon of ATC via site directed mutagenesis (SDM). The mutated variation of *murP* was called *murP*[opt]. The genes of *murP*, *crr* and *murQ* were amplified via PCR from genomic DNA of *E. coli* K-12, while *nagE* from *C. glycinophilum*, was amplified from

pVWEx1_*nagE* (Matano et al., 2016). The primers used in this study (see **Supplementary Table S1**) were obtained from Metabion international AG, Planegg. Using Gibson assembly (Gibson, 2011), the vectors pVWEx1_*murP*, pVWEx1_*murP*[opt], pVWEx1_*murPcrr*, pVWEx1_*murP*[opt]*crr*, pCXE50_*murQ* and pEC-XT99A_*nagE* were constructed. The vectors pVWEx1 and pEC-XT99A are IPTG inducible while pCXE50 has a constitutive EF_{tu} promotor. *E. coli* was transformed by the $CaCl_2$ method while transformation through electroporation was applied for *C. glutamicum* at 2500 V, 25 µF and 200 Ω.

Carotenoid Extraction

Lycopene was extracted as described before (Heider et al., 2012). In short, 5 wells each containing 1 ml cell suspension were combined and pelleted in safe lock micro reaction tubes by centrifugation at 10,000 *g* for 15 min and resuspended in 800 µL of a 7:3 methanol/acetone mixture and incubated for 15 min at 60°C and 750 rpm in a thermomixer (Eppendorf). The cell debris was removed by centrifugation and the supernatant used for HPLC analysis. The procedure was repeated to ensure complete extraction until white pellets were obtained.

Quantitation of Fermentation Products

The quantification of MurNAc, GlcNAc, lycopene, L-glutamate, and L-lysine was conducted by HPLC analysis (1200 series

---

[1]http://gcua.schoedl.de/

TABLE 1 | Plasmids and strains used in this study.

Strain ID	Characteristics	Reference
E. coli		
DH5α	*supE*44 Δ*lacU*169(φ80*dlacZ*ΔM15) *hsdR*17 *recA*1 *endA*1 *gyrA*96 thi-1 *relA*1	Hanahan, 1983
JW2421-1	F-, Δ(*araD-araB*)567, Δ*lacZ*4787(:*rrnB*-3), λ-, Δ*murQ*757::*kan*, rph-1, Δ(*rhaD-rhaB*)568, *hsdR*514; Kan^R	Baba et al., 2006
JW2421-1 (pCXE50_*murQ*)	JW2421-1 carrying pCXE50_*murQ*	this work
C. glutamicum		
Δ*nanR*	ATCC 13032 Δ*nanR*	(Matano et al., 2016)
Δ*nanR* PQ	Δ*nanR* carrying pVWEx1_*murP* and pCXE50_*murQ*	This work
Δ*nanR* POQ	Δ*nanR* carrying pVWEx1_*murP*^opt and pCXE50_*murQ*	This work
Δ*nanR* PCQ	Δ*nanR* carrying pVWEx1_*murP_crr* and pCXE50_*murQ*	This work
Δ*nanR* POCQ	Δ*nanR* carrying pVWEx1_*murP*^opt_*crr* and pCXE50_*murQ*	This work
Δ*nanR* PCQnE	Δ*nanR* carrying pVWEx1_*murP_crr*, pCXE50_*murQ* and pEC-XT99A_*nagE*	This work
DM1729Δ*nanR* PCQ	DM1729Δ*nanR* carrying pVWEx1_*murP_crr* and pCXE50_*murQ*	This work
DM1729Δ*nanR*	ATCC 13032 *pycP*^458S *hom*^V59A *lysC*^T311I Δ*nanR,* L-lysine overproducing strain	Matano et al., 2014
DM1729Δ*nanR* PCQnE	DM1729Δ*nanR* carrying pVWEx1_*murP_crr*, pCXE50_*murQ* and pEC-XT99A_*nagE*	This work
DM1729Δ*nanR* PCQ *ldcC*	DM1729Δ*nanR* carrying pVWEx1_*murP_crr*, pCXE50_*murQ* and pEC-XT99A_*ldcC*	This work
DM1729Δ*nanR* PCQ LPA	DM1729Δ*nanR* carrying pVWEx1_*murP_crr*, pCXE50_*murQ* and pECXT99A_*lysDH*_S.prom-*proC*_C.g	This work
Δ*crtYEb* Δ*nanR*	lycopene producing derivative of WT carrying in-frame Δ*crtYEb* Δ*nanR*	Matano et al., 2014
Δ*crtYEb* Δ*nanR* PCQ	Deletion of *crtY* and *crtEb* carrying pVWEx1_*murP_crr*, pCXE50_*murQ*	This work
Δ*crtYEb* Δ*nanR* PCQnE	Δ*crtYEb* Δ*nanR* carrying pVWEx1_*murP_crr*, pCXE50_*murQ* and pEC-XT99A_*nagE*	This work
Plasmids		
pVWEx1	*E. coli/C. glutamicum* shuttle vector, Kan^R	Peters-Wendisch et al., 2001
pEC-XT99A	TetR, *C. glutamicum/E. coli* shuttle vector (Ptrc, *lacI*, pGA1 OriV_C.g.)	Kirchner and Tauch, 2003
pCXE50_*porB*	Constitutive pEf_tu promotor, Cm^R	Lee, 2014
pVWEx1_*murP*	pVWEx1-derivative for IPTG inducible expression of *murP* from *E. coli*, Kan^R	This work
pVWEx1_*murP*^opt	pVWEx1-derivative for IPTG inducible expression of the for *C. glutamicum* codon optimized *murP* from *E. coli*, Kan^R	This work
pVWEx1_*murP_crr*	pVWEx1-derivative for IPTG inducible expression of *murP* and *crr* from *E. coli*, Kan^R	This work
pVWEx1_*murP*^opt_*crr*	pVWEx1-derivative for IPTG inducible expression of the for *C. glutamicum* codon optimized *murP* and *crr* from *E. coli*, Kan^R	This work
pCXE50_*murQ*	Cm^R, expressing *murQ* from *E. coli K12*	This work
pEC-XT99A_*nagE*	Tet^R, expressing *nagE* from *Corynebacterium glycinophilum* DSM45794	This work
pEC-XT99A_*ldcC*	Tet^R, expressing *ldcC* from *E. coli K12*	This work
pECXT99A_*lysDH*_S.prom-*proC*_C.g	Tet^R, expressing *lysDH* from *Silicibacter pomeroyi* and *proC* from *C. glutamicum*	This work

Kan^R, kanamycin resistance; Tet^R, tetracycline resistance; Cm^R, chloramphenicol resistance.

HPLC system, Agilent Technologies Sales & Services GmbH & Co. KG, Waldbronn). The supernatant of 1 ml pelleted cell suspension was diluted and analyzed. For quantification of organic acids, the carbo column (300 × 8 mm, 10 μm particle size, 25 Å pore diameter, CS Chromatographie Service GmbH) and a refractive index detector (RID G1362A, 1200 series, Agilent Technologies) was used for quantification of MurNAc and GlcNAc with 5 mM H_2SO_4 as buffer.

Applying OPA derivatisation, L-lysine and L-glutamate were analyzed using an RP8 column with a sodium acetate (0.25 v/v %) buffer at pH 6 and a 1:50 dilution with an internal L-asparagine standard. Using the RP18 column with a Methanol-Milli-Q-water mixture (9:1), lycopene was quantified (Heider et al., 2012).

RESULTS

Metabolic Engineering of *C. glutamicum* for Growth With MurNAc as Carbon Source

Corynebacterium glutamicum, which has been engineered to utilize GlcN and GlcNAc (Matano et al., 2014, 2016), cannot

FIGURE 2 | (A) Growth parameters of various *C. glutamicum* strains in MurNAc minimal medium. Growth of the strains *C. glutamicum* ΔnanR EV (empty vector) and *C. glutamicum* ΔnanR PCQ was monitored for 25 h at 30°C and 1100 rpm in the Biolector with CGXII medium containing IPTG 25 μM and 20 mM MurNAc. **(B)** Biomass concentrations (gCDW/L; filled) and maximal growth rates (μmax, checkered) of the strains PQ, POQ, PCQ and POCQ grown for 48 h in the Biolector® system in CGXII medium with MurNAc 20 mM. Values and error bars represent means and standard deviations of triplicates.

utilize MurNAc since no growth was observed in minimal medium with 25 mM MurNAc and 25 ± 0.1 mM MurNAc remained in the growth medium after 25 h of incubation (**Figure 2A**). As expected, the *C. glutamicum* genome lacks genes encoding a MurNAc PTS and MurNAc-6-phosphate etherase for uptake and conversion of MurNAc to GlcNAc-6-phosphate, an endogenous intermediate of *C. glutamicum* metabolism. As described in Section "Materials and Methods," the genes for the MurNAc PTS *murP* from *E. coli* or codon optimized allele *murP*^opt (Nuoffer et al., 1988; Tchieu et al., 2001; Dahl et al., 2004) were cloned into the IPTG-inducible plasmid pVWEx1 alone or as operon with *crr*. The gene for the MurNAc-6-phosphate etherase *murQ* from *E. coli* (Jaeger et al., 2005) was cloned into the constitutive expression vector pCXE50 (Lee, 2014). Functional expression of *murQ* from pCXE50_*murQ* was tested by complementation of the *E. coli murQ* mutant *E. coli* JW2421-1. While *E. coli* JW2421-1(pCXE50_*murQ*) utilized MurNAc as sole carbon source (ΔOD$_{600}$ of 3.2 ± 0.1 and μ$_{max}$ of 0.07 ± 0.01 h^{-1}), *E. coli* JW2421-1ΔmurQ showed no growth (see **Supplementary Figure S1**). Only *murQ* was tested by complementation of a *E. coli* mutant. We neither tested *murP* nor *crr* because we expected perturbations due to overexpression since MurP is a membrane protein and Crr serves a regulatory function. *C. glutamicum* ΔnanR was transformed with the constructed pVWEx1 plasmids and with the pCXE50_*murQ*. The respective strains were named *C. glutamicum* ΔnanR PQ, POQ, PCQ, POCQ (**Table 1**).

Strains expressing *crr* from *E. coli* grew faster in minimal medium containing 25 mM MurNAc as sole source of carbon and energy than strains lacking *crr* (**Figure 2B**). Strains with native *murP* grew better than strains expressing codon optimized *murP*
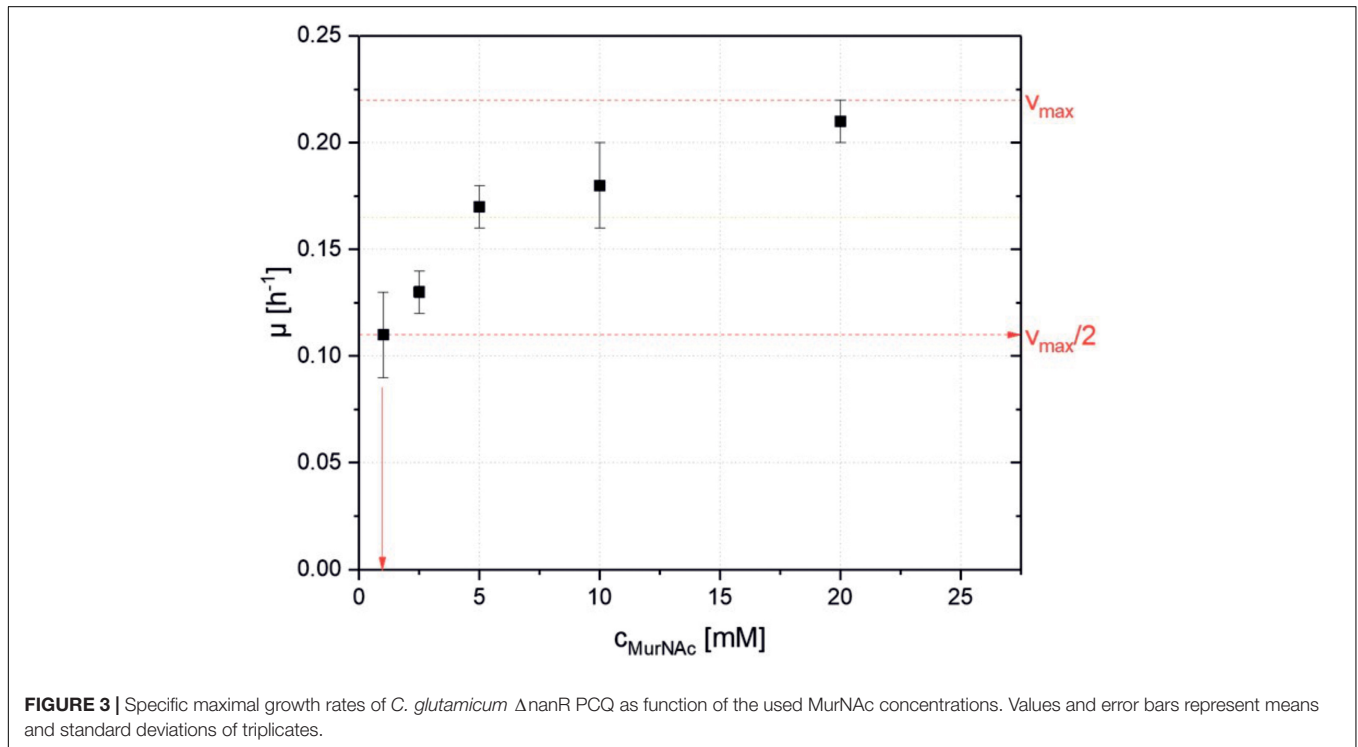
(**Figure 2B**). IPTG was used at a low concentration (25 μM) to induce heterologous gene expression, since higher concentrations slowed growth (**Table 2**). This is not unexpected and presumably due to too high expression of transport protein genes as seen previously for *dccT* (Youn et al., 2008) and *dctA* (Youn et al., 2009), coding for dicarboxylate transporters. With 25 μM IPTG, strain ΔnanR PCQ expressing native *murP*, *crr* and *murQ* grew in minimal medium containing 25 mM MurNAc to a biomass concentration of 1.2 ± 0.3 gCDW/L and with 50 mM MurNAc to a biomass concentration of 2.0 ± 0.2 gCDW/L (**Table 2**). Biphasic exponential growth was observed: faster growth between 0 and 6 h and slower growth between 6 and 27 h (**Figure 2A**). The curves appear linear as the Y axis has been logarithmized (**Figure 2**). During the transition from the first to the second growth phase the medium contained 3.10 ± 0.12 mM lactate. Thus, lactate released by MurQ from MurNAc-6-phosphate may not have been utilized as fast as GlcNAc-6-phosphate, the other product of the MurQ reaction. In consequence, lactate accumulated in the culture medium in the first exponential growth phase and presumably slowed growth in the second exponential growth phase. Transient accumulation of lactate to growth inhibitory concentrations has been observed during growth of *C. glutamicum* with various carbon sources (Engels et al., 2008).

As PTS systems typically support growth on their cognate substrates with high affinity, the dependence of the growth rate on the initial MurNAc concentration in the growth medium was determined using strain ΔnanR PCQ. Different concentrations of MurNAc (1, 2.5, 5, 10, and 20 mM) were used and the maximal growth rates were plotted against the MurNAc concentration to derive the maximal growth rate of 0.22 h^{-1} and the Monod constant of 0.9 ± 0.1 mM as shown in **Figure 3**.

TABLE 2 | Growth characteristics of *C. glutamicum* Δ*nanR PCQ* on different MurNAc and IPTG concentrations.

C_{MurNAc} [mM]	C_{IPTG} [µM]	gCDW/L	μ_{max1} [h^{-1}]	μ_{max2} [h^{-1}]	$Y_{x/S}$ [g·mol^{-1}]
20	25	1.2 ± 0.3	0.25 ± 0.01	0.05 ± 0.01	58.0 ± 0.1
	50	1.0 ± 0.4	0.24 ± 0.01	0.04 ± 0.01	51.4 ± 0.1
	100	1.0 ± 0.1	0.26 ± 0.02	0.05 ± 0.01	54.5 ± 0.1
50	25	2.0 ± 0.2	0.25 ± 0.04	0.04 ± 0.01	38.4 ± 0.1
	50	1.7 ± 0.5	0.26 ± 0.01	0.05 ± 0.01	35.1 ± 0.1
	100	1.5 ± 0.2	0.21 ± 0.02	0.04 ± 0.01	30.4 ± 0.1

The final biomass concentrations reached [given as g of cell dry weight (CDW) per L], maximal specific growth rates of the first (μ_{max1}) and second growth phase (μ_{max2}), and the biomass yield coefficients of cell dry weight formed per used substrate used ($Y_{X/S}$) are given as means with standard deviations.

FIGURE 3 | Specific maximal growth rates of *C. glutamicum* Δnan*R PCQ* as function of the used MurNAc concentrations. Values and error bars represent means and standard deviations of triplicates.

A sub-millimolar Monod constant is typical for PTS mediated uptake.

Comparative Analysis of Growth With MurNAc and/or GlcNAc

Growth of recombinant *C. glutamicum* with MurNAc and/or GlcNAc as sole carbon sources was compared (**Figure 4** and **Table 3**). With 25 mM MurNAc *C. glutamicum* Δ*nanR PCQnE* grew to a biomass concentration of 3.0 ± 0.1 gCDW/L, while the maximal biomass concentration was only 2.4 ± 0.1 gCDW/L with GlcNAc. The higher biomass concentration observed with MurNAc in comparison to GlcNAc indicated that lactate released from MurNAc by MurQ contributed to biomass formation. However, the biomass yield was higher with GlcNAc (0.44 ± 0.01 g·g^{-1}) than with MurNAc (0.39 ± 0.02 g·g^{-1}). GlcNAc catabolism was faster than MurNAc catabolism as the maximal growth rates and the specific substrate uptake rates were lower with MurNAc (0.22 ± 0.10 h^{-1} and

1.80 ± 0.10 mmol·g^{-1}·h^{-1}) than with GlcNAc (0.30 ± 0.01 h^{-1} and 3.00 ± 0.10 mmol·g^{-1}·h^{-1}) as shown in **Table 3**.

Unlike *E. coli and B. subtilis,* it is typical for *C. glutamicum* to simultaneously co-utilize carbon substrates present in blends (Blombach and Seibold, 2010). Therefore, *C. glutamicum* strain Δ*nanR PCQnE* was constructed by transforming strain Δ*nanR PCQnE* with plasmid pEC-XT99A-*nagE* for expression of the gene for the GlcNAc-specific PTS uptake system to establish whether MurNAc and GlcNAc are co-utilized or utilized sequentially. *C. glutamicum* strain Δ*nanR PCQnE* were grown with 25 mM MurNAc and/or 25 mM GlcNAc (**Figure 4**). With the blend of MurNAc and GlcNAc *C. glutamicum* Δ*nanR PCQnE* grew to a biomass concentration of 3.8 ± 0.1 gCDW/L, while a biomass concentration of only 2.1 ± 0.1 g/L was reached in the absence of *nagE*. Determination of the residual substrate concentrations revealed sequential utilization of GlcNAc before MurNAc (**Figure 4C**). Thus, unlike many growth substrates MurNAc and GlcNAc were not co-utilized.
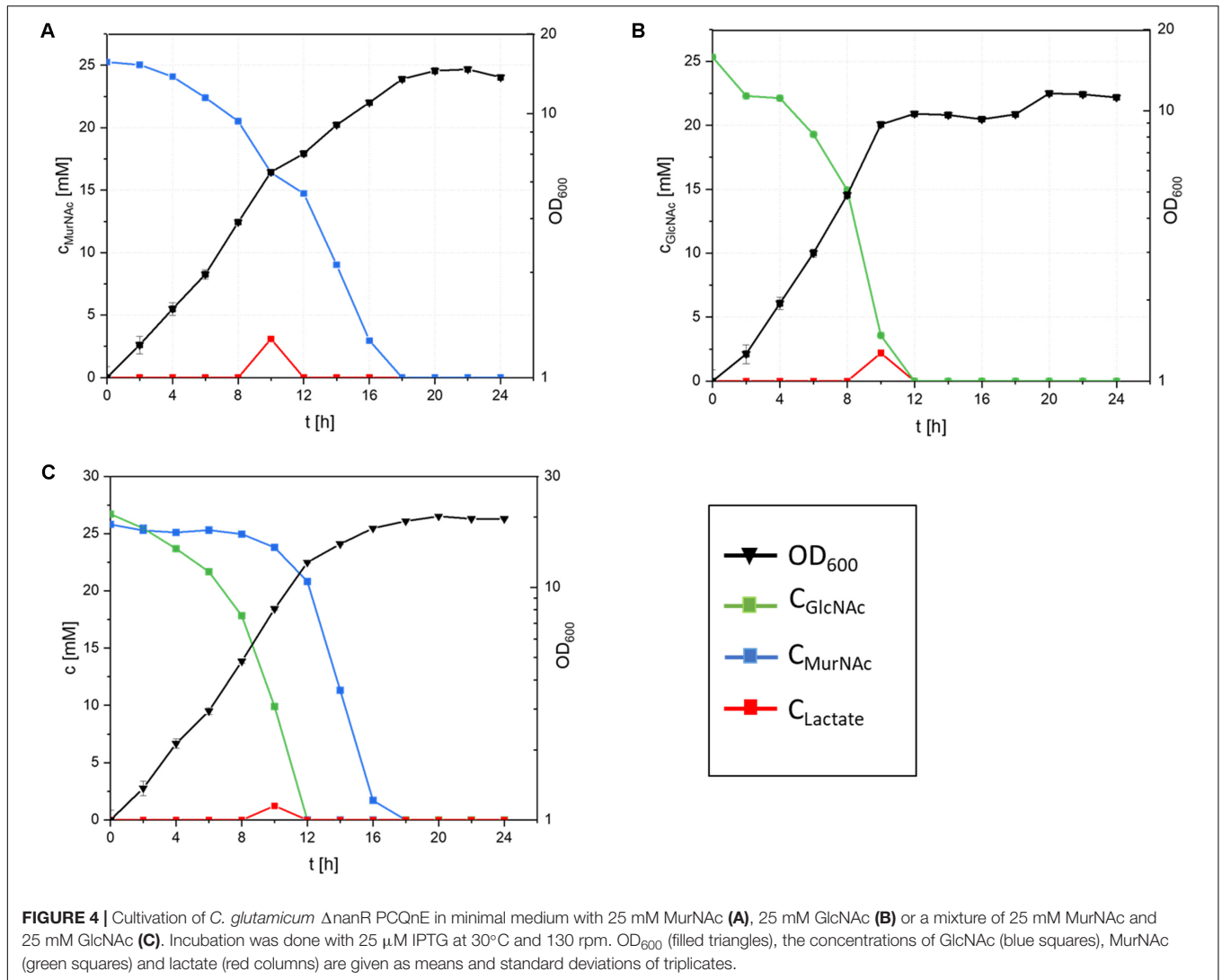
FIGURE 4 | Cultivation of *C. glutamicum* ΔnanR PCQnE in minimal medium with 25 mM MurNAc **(A)**, 25 mM GlcNAc **(B)** or a mixture of 25 mM MurNAc and 25 mM GlcNAc **(C)**. Incubation was done with 25 μM IPTG at 30°C and 130 rpm. OD$_{600}$ (filled triangles), the concentrations of GlcNAc (blue squares), MurNAc (green squares) and lactate (red columns) are given as means and standard deviations of triplicates.

TABLE 3 | Cultivation parameters of *C. glutamicum* Δ*nanR PCQnE* growing on MurNAc (25 mM), GlcNAc (25 mM) or a MurNAc-GlcNAc-mixture (both 25 mM).

	MurNAc	**GlcNAc**	**MurNAc-GlcNAc mix**
ΔOD$_{600}$ [−]	12.0 ± 0.1	9.6 ± 0.1	17.7 ± 0.1
ΔS MurNAc [mM]	25.3 ± 0.1	–	25.8 ± 0.1
ΔS GlcNAc[mM]	–	25.3 ± 0.1	26.7 ± 0.1
$Y^1_{X/S}$ [g·mmol^{-1}]	0.14 ± 0.01	0.09 ± 0.01	0.11 ± 0.10
μmax^1 [h^{-1}]	0.18 ± 0.01	0.22 ± 0.01	0.18 ± 0.01
qS^1 [mmol·g^{-1}·h^{-1}]	1.27 ± 0.10	2.42 ± 0.10	1.68 ± 0.10
$Y^2_{X/S}$ [g·mol^{-1}]	0.11 ± 0.01	–	0.08 ± 0.10
μmax^2 [h^{-1}]	0.10 ± 0.01	–	0.07 ± 0.20
qS^2 [mmol·g^{-1}·h^{-1}]	0.87 ± 0.10	–	0.90 ± 0.20

The parameters given are ΔOD$_{600}$ for biomass formed and ΔS for substrate used. Monophasic growth was observed with GlcNAc. With MurNAc, two phases were observed, and biomass yield coefficients of cell dry weight formed per used substrate used ($Y_{X/S}$), maximal specific growth rates (μmax) and specific substrate uptake rates (qS) are given for the phase 1 and phase 2. With MurNAc+GlcNAc, $Y_{X/S}$, μmax and qS are reported for the phase where GlcNAc was utilized exclusively (phase 1; 0–8 h) as well as for the phase when MurNAc was utilized exclusively (phase 2; 12–18 h).

TABLE 4 | Parameters describing concentration in mM and production yield ($Y_{P/S}$) of L-lysine, L-PA and 1,5-diaminopentane after 72 h with either 25 mM GlcNAc, either 25 mM MurNAc or 25mM GlcNAc of indicated strains and glutamate production after 48 h from either 25 mM MurNAc either a mixture of GlcNAc and MurNAc, each 25 mM.

Product	Strain ID	$C_{Substrate}$ [mM]	$C_{product}$ [mM]	$Y_{P/S}$ [mmol·mmol^{-1}]
Lycopene	Δ*crtYEb* Δ*nanR* PCQ	25 mM MurNAc	–	0.04 ± 0.01 mg (gCDW)$^{-1}$
	Δ*crtYEb* Δ*nanR* PCQnE	25mM GlcNAc +		0.10 ± 0.01 mg (gCDW)$^{-1}$
		25 mM MurNAc		
L-lysine	DM1729Δ*nanR* PCQnE	25 mM GlcNAc	7.6 ± 0.3	0.30 ± 0.10
	DM1729Δ*nanR* PCQnE	25 mM GlcNAc +	10.6 ± 0.6	0.21 ± 0.10
		25 mM MurNAc		
	DM1729Δ*nanR*	25mM MurNAc	6.9 ± 0.6	0.27 ± 0.05
1,5-diaminopentane	DM1729Δ*nanR* PCQ *ldcC*	25 mM MurNAc	4.3 ± 0.1	0.30 ± 0.10
L-PA	DM1729Δ*nanR* PCQ LPA	25 mM MurNAc	4.2 ± 0.2	0.35 ± 0.10
Glutamate	Δ*nanR* PCQ	25 mM MurNAc	1.0 ± 0.1	0.03 ± 0.00
	Δ*nanR* PCQnE	25mM GlcNAc +	1.9 ± 0.1	0.04 ± 0.00
		25 mM MurNAc		

MurNAc-Based Production of Food and Feed Additives and Derived Chemicals

MurNAc was expected not only to support growth of recombinant *C. glutamicum* strains, but also production of value-added compounds. Therefore, MurNAc was tested as sole carbon source or in blends with GlcNAc for production of the amino acids L-lysine and L-glutamate, the diamine 1,5-diaminopentane, the cyclic non-proteinogenic amino acid L-pipecolic acid, and the carotenoid lycopene.

The lycopene accumulating strain *C. glutamicum* Δ*crtYEb* Δ*nanR* (Matano et al., 2014) was transformed with the plasmids pVWEx1_*murP_crr,* pEC-XT99A_*nagE* and pCXE50_*murQ* as described above and the resulting strains were named Δ*crtYEb* Δ*nanR* PCQ and Δ*crtYEb* Δ*nanR* PCQnE. Cells of both strains accumulated lycopene when grown in MurNAc containing minimal medium. Strain Δ*crtYEb* Δ*nanR* PCQ showed a lycopene content of 0.04 mg ± 0.01 (g CDW)$^{-1}$ in MurNAc minimal medium. Growth of *C. glutamicum* Δ*crtYEb* Δ*nanR* PCQnE with a MurNAc/GlcNAc blend led to a lycopene content of 0.10 ± 0.01 mg (g CDW)$^{-1}$.

The L-lysine producing strains *C. glutamicum* DM1729Δ*nanR* PCQ and DM1729Δ*nanR* PCQnE were constructed based on DM1729Δ*nanR* as described above for lycopene accumulating strains. DM1729Δ*nanR* PCQ produced 7 ± 1 mM L-lysine ($Y_{P/S}$ 0.27 ± 0.05 mmol mmol^{-1}) and DM1729Δ*nanR* PCQnE produced 11 ± 1 mM L-lysine ($Y_{P/S}$ 0.21 ± 0.10 mmol mmol^{-1}) in minimal medium with either 25 mM MurNAc or a combination of 25 mM MurNAc and 25 mM GlcNAc., whereas 7.6 ± 0.3 mM L-lysine ($Y_{P/S}$ 0.30 ± 0.01 mmol mmol^{-1}) have been produced from DM1729PCQnE with 25 mM GlcNAc (**Table 4**).

To test if the L-lysine derived compounds 1,5-diaminopentane and L-pipecolic acid can also be produced from MurNAc, strain DM1729Δ*nanR* PCQ was transformed with either pEC-XT99A-*ldcC* or pEC-XT99A-*lysDH-proC*. 1,5-Diaminopentane can be generated from L-lysine by lysine decarboxylase LdcC and L-pipecolic acid can be generated from L-lysine in a three-step pathway by L-lysine-6-dehydrogenase (encoded by

lysDH from *S. pomeroyi*), spontaneous ring formation and by pyrroline 5-carboxylate reductase (encoded by endogenous *proC*) (Pérez-García et al., 2016, 2017). Although the strains showed poor growth, *C. glutamicum* Δ*nanR* DM1729*PCQ ldcC* was able to produce 4.3 ± 0.1 mM of 1,5-diaminopentane ($Y_{P/S}$ 0.30 ± 0.10 mmol mmol^{-1}) and *C. glutamicum* Δ*nanR* DM1729*PCQ LPA* produced 4.0 ± 0.2 mM of L-pipecolic acid ($Y_{P/S}$ 0.35 ± 0.10 mmol mmol^{-1}) from MurNAc as sole carbon source as shown in **Table 4**.

L-Glutamate production was accomplished by *C. glutamicum* Δ*nanR* PCQ and *C. glutamicum* Δ*nanR* PCQnE using penicillin G as trigger. *C. glutamicum* Δ*nanR* PCQ accumulated 1 ± 0 mM of L-glutamate from 25 mM MurNAc after 48 h, whereas *C. glutamicum* Δ*nanR* PCQnE produced 2 ± 0 mM of L-glutamate under these conditions.

DISCUSSION

In this work, production of food and feed additives by *C. glutamicum* from MurNAc, an alternative carbon source without competing use in human and animal nutrition, has been established. The food amino acid L-glutamate, the feed amino acid L-lysine and the feed additive lycopene were produced from MurNAc, GlcNAc and blends of both hexosamines.

Metabolic engineering of *C. glutamicum* for access to MurNAc relied on *E. coli* genes. Its MurNAc PTS system was active in *C. glutamicum* and strains expressing *crr* in addition were able to grow faster and yield more biomass than the strains without heterologous Crr (**Figure 2**). Crr is a glucose-family specific EIIA component, but it can interact with the PTS-EIIBC components of several members of the glucose PTS family (Barabote and Saier, 2005). *C. glutamicum* has two complete PTS^{Glc} systems (Barabote and Saier, 2005). The finding that MurNAc could be utilized without heterologous Crr indicated that a PTS component of *C. glutamicum* made up for its absence. Moreover, the low Monod constant found for growth of the recombinant with MurNAc (**Figure 3**) indicated

that the MurNAc PTS catalyzed high-affinity MurNAc uptake in *C. glutamicum,* although the MurNAc PTS seems to have a lower affinity for its substrate than, e.g., the heterologous expressed GlcNAc specific PTS system NagE from *Corynebacterium glycinophilum* which showed a KM value 3.8 ± 0.6 μM (Ferenci, 1996; Matano et al., 2014). The K_M for the etherase MurQ in *E. coli* found in literature had a similar range of value (1.2 mM) (Hadi et al., 2008) with the K_M value found experimentally in this study for the MurNAc PTS (0.9 ± 0.1 mM) system, making the two enzymatic steps of up taking, phosphorylation and esterification balanced.

Growth of recombinant *C. glutamicum* with MurNAc was biphasic and lactate accumulated during the interphase (**Figure 4A**). MurNAc differs from GlcNAc only by one additional lactoyl group that is hydrolysed to lactate by etherase MurQ. Although *C. glutamicum* can utilize lactate as sole carbon source (Eikmanns, 2005), lactate accumulated. Utilization of D-lactate requires *dld* encoding quinone-dependent D-lactate dehydrogenase (Stansen et al., 2005; Kato et al., 2010). Utilization of L-lactate requires quinone-dependent L-lactate dehydrogenase which is encoded in the LldR repressed operon *cg3227-lldD* (Engels et al., 2008; Georgi et al., 2008). L-Lactate is secreted by *C. glutamicum* under certain conditions, e.g., during growth with glucose when oxygen is limiting, but is quickly re-utilized once *cg3227-lldD* is derepressed (Eggeling and Bott, 2005; Stansen et al., 2005; Georgi et al., 2008).

Corynebacterium glutamicum co-utilizes glucose simultaneously with many different carbon sources including those that required introduction of heterologous pathways, for example, xylose (requiring xylose isomerase gene from *E. coli* (Kawaguchi et al., 2006), arabinose (requiring the *araBAD* operon from *E. coli* (Kawaguchi et al., 2008; Schneider et al., 2011), cellobiose (requiring β-glucosidase) or glycerol (requiring *E. coli* glycerol kinase and glycerol-3-phosphate dehydrogenase), (Rittmann et al., 2008; Sasaki et al., 2008; Zahoor et al., 2012; Meiswinkel et al., 2013; Wendisch et al., 2016). Only rarely, glucose repression has been observed, for example, during sequential utilization of glucose before ethanol (due to catabolite repression of the alcohol dehydrogenase gene *adhA*) (Gerstmeir et al., 2004) or before glutamate (due to catabolite repression of the operon *gluABCD* encoding the glutamate uptake system) (Wendisch et al., 2000; Blombach and Seibold, 2010). The preferential utilization of GlcNAc before MurNAc by *C. glutamicum* Δ*nanR PCQnE* may be explained by an offset between fast uptake and hydrolysis of MurNAc yielding GlcNac-6-P and D-lactate followed by fast utilization of GlcNAc-6-P, but accumulation of D-lactate. It is conceivable that overexpression of *dld* would accelerate D-lactate catabolism precluding transient D-lactate accumulation during growth with MurNAc.

A proof-of-concept for MurNAc-based fermentative production of food and feed additives was reached. The engineered strain DM1729Δ*nanR* PCQ and DM1729Δ*nanR* PCQnE showed comparable L-lysine production as observed previously for GlcN and GlcNAc (Matano et al., 2014). Similarly, lycopene production from MurNAc by *C. glutamicum*Δ*crtYEb*Δ*nanR* PCQ was comparable to that observed by similar strains with 100 mM glucose [30 ± 10 μg·g (CDW)$^{-1}$] and 100 mM GlcNAc [29.6 ± 4.5 μg·g (CDW)$^{-1}$] (Heider et al., 2012; Matano et al., 2014). To establish viable production processes with MurNac as sole or combined carbon source, more work to increase titres, yields and volumetric productivities is needed. Conceptually, however, this work laid the foundation for recycling the cell wall fraction of bacterial biomass from large-scale production processes as substrate for fermentative production of food and feed additives.

CONCLUSION

Corynebacterium glutamicum was successfully metabolically engineered for utilization of the amino sugar MurNAc as alternative carbon source for growth and production of relevant value-added compounds, specifically L-lysine, L-glutamate and lycopene, from this carbon source lacking competing uses in human and animal nutrition.

AUTHOR CONTRIBUTIONS

VW conceived the study. VW and ES planned the experiments. ES and LB performed and analyzed the experiments. ES and LB drafted the manuscript. VW finalized the manuscript. All the authors agreed to the final version of the manuscript.

FUNDING

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2018.02046/full#supplementary-material

REFERENCES

Arndt, A., and Eikmanns, B. J. (2008). "Regulation of carbon metabolism in *Corynebacterium glutamicum*," in *Corynebacteria: Genomics and Molecular Biology*, ed. A. Burkovski (Wymondham: Caister Academic Press).

Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., et al. (2006). Construction of *Escherichia coli* K-12 in-frame, single-gene knockout mutants: the Keio collection. *Mol. Syst. Biol.* 2, 1–11.

Barabote, R. D., and Saier, M. H. Jr. (2005). Comparative genomic analyses of the bacterial phosphotransferase system. *Microbiol. Mol. Biol. Rev.* 69, 608–634.

Blombach, B., and Seibold, G. M. (2010). Carbohydrate metabolism in *Corynebacterium glutamicum* and applications for the metabolic engineering of l-lysine production strains. *Appl. Microbiol. Biotechnol.* 86, 1313–1322. doi: 10.1007/s00253-010-2537-z

Borisova, M., Gaupp, R., Duckworth, A., Schneider, A., Dalügge, D., Mühleck, M., et al. (2016). Peptidoglycan recycling in gram-positive bacteria is crucial for survival in stationary phase. *mBio* 7:e00923-16. doi: 10.1128/mBio.00923-16

Chen, J.-K., Shen, C.-R., and Liu, C.-L. (2010). N-Acetylglucosamine: production and applications. *Mar. Drugs* 8, 2493–2516. doi: 10.3390/md8092493

Cramer, A., and Eikmanns, B. J. (2007). RamA, the transcriptional regulator of acetate metabolism in *Corynebacterium glutamicum*, is subject to negative autoregulation. *J. Mol. Microbiol. Biotechnol.* 12, 51–59.

Curtis, S. J., and Epstein, W. (1975). Phosphorylation of d-glucose in *Escherichia coli* mutants defective in glucosephosphotransferase, mannosephosphotransferase, and glucokinase. *J. Bacteriol.* 122, 1189–1199.

Dahl, U., Jaeger, T., Nguyen, B. T., Sattler, J. M., and Mayer, C. (2004). Identification of a phosphotransferase system of *Escherichia coli* required for growth on N-Acetylmuramic Acid. *J. Bacteriol.* 186, 2385–2392.

Dominguez, H., Cocaign-Bousquet, M., and Lindley, N. D. (1997). Simultaneous consumption of glucose and fructose from sugar mixtures during batch growth of *Corynebacterium glutamicum*. *Appl. Microbiol. Biotechnol.* 47, 600–603.

Domsch, K. H. (1982). *Soil Biochemistry: Books in Soils and the Environment Series*, Vol. 5, eds E. A. Paul and J. N. Ladd. New York, NY: Marcel Dekker Inc., 96–97.

Eggeling, L., and Bott, M. (2005). *Handbook of Corynebacterium glutamicum*. Boca Raton, FL: CRC Press.

Eikmanns, B. J. (2005). "Central metabolism: Tricarboxylic acid cycle and anaplerotic reactions," in *Handbook on Corynebacterium glutamicum*, eds L. Eggeling and M. Bott (Boca Raton, FL: CRC Press), 241–276.

Engels, V., Lindner, S. N., and Wendisch, V. F. (2008). The global repressor SugR controls expression of genes of glycolysis and of the L-lactate dehydrogenase LdhA in *Corynebacterium glutamicum*. *J. Bacteriol.* 190, 8033–8044. doi: 10.1128/JB.00705-08

Ferenci, T. (1996). Adaptation to life at micromolar nutrient levels: the regulation of *Escherichia coli* glucose transport by endoinduction and cAMP. *FEMS Microbiol. Rev.* 18, 301–317.

Georgi, T., Engels, V., and Wendisch, V. F. (2008). Regulation of L-lactate utilization by the FadR-type regulator LldR of *Corynebacterium glutamicum*. *J. Bacteriol.* 190, 963–971.

Gerstmeir, R., Cramer, A., Dangel, P., Schaffer, S., and Eikmanns, B. J. (2004). RamB, a novel transcriptional regulator of genes involved in acetate metabolism of *Corynebacterium glutamicum*. *J. Bacteriol.* 186, 2798–2809.

Gibson, D. G. (2011). Enzymatic assembly of overlapping DNA fragments. *Methods Enzymol.* 498, 349–361. doi: 10.1016/B978-0-12-385120-8.0 0015-2

Goodell, E. W., and Schwarz, U. (1985). Release of cell wall peptides into culture medium by exponentially growing *Escherichia coli*. *J. Bacteriol.* 162, 391–397.

Hadi, T., Dahl, U., Mayer, C., and Tanner, M. E. (2008). Mechanistic studies on N-acetylmuramic acid 6-phosphate hydrolase (MurQ): an etherase involved in peptidoglycan recycling. *Biochemistry* 47, 11547–11558. doi: 10.1021/bi8014532

Hanahan, D. (1983). Studies on transformation of *Escherichia coli* with plasmids. *J. Mol. Biol* 166, 557–580.

Heider, S. A., Peters-Wendisch, P., and Wendisch, V. F. (2012). Carotenoid biosynthesis and overproduction in *Corynebacterium glutamicum*. *BMC Microbiol.* 12:198. doi: 10.1186/1471-2180-12-198

Jaeger, T., Arsic, M., and Mayer, C. (2005). Scission of the lactyl ether bond of N-acetylmuramic acid by *Escherichia coli* "etherase". *J. Biol. Chem.* 280, 30100–30106.

Kato, O., Youn, J.-W., Stansen, K. C., Matsui, D., Oikawa, T., and Wendisch, V. F. (2010). Quinone-dependent D-lactate dehydrogenase Dld (Cg1027) is essential for growth of *Corynebacterium glutamicum* on D-lactate. *BMC Microbiol.* 10:321. doi: 10.1186/1471-2180-10-321

Kawaguchi, H., Sasaki, M., Vertes, A. A., Inui, M., and Yukawa, H. (2008). Engineering of an L-arabinose metabolic pathway in *Corynebacterium glutamicum*. *Appl. Microbiol. Biotechnol.* 77, 1053–1062.

Kawaguchi, H., Vertes, A. A., Okino, S., Inui, M., and Yukawa, H. (2006). Engineering of a xylose metabolic pathway in *Corynebacterium glutamicum*. *Appl. Environ. Microbiol.* 72, 3418–3428.

Kinoshita, S., Udaka, S., and Shimono, M. (1957). Studies on the amino acid fermentation. *Production of l-glutamic acid by various microorganisms. J. Gen. Appl. Microbiol.* 3, 193–205.

Kirchner, O., and Tauch, A. (2003). Tools for genetic engineering in the amino acid-producing bacterium *Corynebacterium glutamicum*. *J. Biotechnol.* 104, 287–299.

Lee, J. (2014). Development and characterization of expression vectors for *Corynebacterium glutamicum*. *J. Microbiol. Biotechnol.* 24, 70–79.

Matano, C., Kolkenbrock, S., Hamer, S. N., Sgobba, E., Moerschbacher, B. M., and Wendisch, V. F. (2016). *Corynebacterium glutamicum* possesses beta-N-acetylglucosaminidase. *BMC Microbiol.* 16:177. doi: 10.1186/s12866-016-0 795-3

Matano, C., Uhde, A., Youn, J. W., Maeda, T., Clermont, L., Marin, K., et al. (2014). Engineering of *Corynebacterium glutamicum* for growth and L-lysine and lycopene production from *N*-acetyl-glucosamine. *Appl. Microbiol. Biotechnol* 98, 5633–5643. doi: 10.1007/s00253-014-5676-9

Meiswinkel, T. M., Gopinath, V., Lindner, S. N., Nampoothiri, K. M., and Wendisch, V. F. (2013). Accelerated pentose utilization by *Corynebacterium glutamicum* for accelerated production of lysine, glutamate, ornithine and putrescine. *Microb Biotechnol* 6, 131–140. doi: 10.1111/1751-7915. 12001

Munoz, E., Ghuysen, J.-M., and Heymann, H. (1967). Cell Walls of Streptococcus pyogenes, Type 14. *C Polysaccharide-Peptidoglycan and G Polysaccharide-Peptidoglycan Complexes. Biochemistry* 6, 3659–3670.

Nuoffer, C., Zanolari, B., and Erni, B. (1988). Glucose permease of *Escherichia coli. The effect of cysteine to serine mutations on the function, stability, and regulation of transport and phosphorylation. J Biol Chem* 263, 6647–6655.

Pérez-García, F., Max Risse, J., Friehs, K., and Wendisch, V. F. (2017). Fermentative production of L-pipecolic acid from glucose and alternative carbon sources. *Biotechnol J* 12, 1600646. doi: 10.1002/biot.201600646

Pérez-García, F., Peters-Wendisch, P., and Wendisch, V. F. (2016). Engineering *Corynebacterium glutamicum* for fast production of L-lysine and L-pipecolic acid. *Appl. Microbiol. Biotechnol.* 100, 8075–8090. doi: 10.1007/s00253-016-7682-6

Peters-Wendisch, P. G., Schiel, B., Wendisch, V. F., Katsoulidis, E., Mockel, B., Sahm, H., et al. (2001). Pyruvate carboxylase is a major bottleneck for glutamate and lysine production by *Corynebacterium glutamicum*. *J. Mol. Microbiol. Biotechnol* 3, 295–300.

Plumbridge, J. (2009). An Alternative Route for Recycling of N-Acetylglucosamine from Peptidoglycan Involves the N-Acetylglucosamine Phosphotransferase System in *Escherichia coli*. *J. Bacteriol.* 191, 5641–5647. doi: 10.1128/JB.00 448-09

Reith, J., and Mayer, C. (2011). Peptidoglycan turnover and recycling in Gram-positive bacteria. *Appl. Microbiol. Biotechnol.* 92, 1–11. doi: 10.1007/s00253-011-3486-x

Rittmann, D., Lindner, S. N., and Wendisch, V. F. (2008). Engineering of a glycerol utilization pathway for amino acid production by *Corynebacterium glutamicum*. *Appl. Environ. Microbiol.* 74, 6216–6222. doi: 10.1128/AEM. 00963-08

Sasaki, M., Jojima, T., Inui, M., and Yukawa, H. (2008). Simultaneous utilization of D-cellobiose, D-glucose, and D-xylose by recombinant *Corynebacterium glutamicum* under oxygen-deprived conditions. *Appl. Microbiol. Biotechnol.* 81, 691–699. doi: 10.1007/s00253-008-1703-z

Schneider, J., Niermann, K., and Wendisch, V. F. (2011). Production of the amino acids L-glutamate, L-lysine, L-ornithine and L-arginine from arabinose by recombinant *Corynebacterium glutamicum*. *J. Biotechnol.* 154, 191–198. doi: 10.1016/j.jbiotec.2010.07.009

Shiio, I., Otsuka, S. I., and Takahashi, M. (1962). Effect of biotin on the bacterial formation of glutamic acid. I. Glutamate formation and cellular premeability of amino acids. *J Biochem* 51, 56–62.

Stansen, C., Uy, D., Delaunay, S., Eggeling, L., Goergen, J. L., and Wendisch, V. F. (2005). Characterization of a *Corynebacterium glutamicum* lactate utilization operon induced during temperature-triggered glutamate production. *Appl. Environ. Microbiol.* 71, 5920–5928.

Tchieu, J. H., Norris, V., Edwards, J. S., and Saier, M. H. Jr. (2001). The complete phosphotransferase system in *Escherichia coli*. *J. Mol. Microbiol. Biotechnol.* 3, 329–346.

Uehara, T., and Park, J. T. (2008). Growth of *Escherichia coli*: Significance of Peptidoglycan Degradation during Elongation and Septation. *J. Bacteriol.* 190, 3914–3922. doi: 10.1128/JB.00207-08

Uehara, T., Suefuji, K., Jaeger, T., Mayer, C., and Park, J. T. (2006). MurQ Etherase is required by *Escherichia coli* in order to metabolize anhydro-N-acetylmuramic acid obtained either from the environment or from its own cell wall. *J. Bacteriol.* 188, 1660–1662.

Uhde, A., Youn, J. W., Maeda, T., Clermont, L., Matano, C., Kramer, R., et al. (2013). Glucosamine as carbon source for amino acid-producing *Corynebacterium glutamicum*. *Appl. Microbiol. Biotechnol* 97, 1679–1687.

Wendisch, V. F. (2014). Microbial production of amino acids and derived chemicals: synthetic biology approaches to strain development. *Curr. Opin. Biotechnol* 30, 51–58. doi: 10.1016/j.copbio.2014.05.004

Wendisch, V. F., Brito, L. F., Gil Lopez, M., Hennig, G., Pfeifenschneider, J., Sgobba, E., et al. (2016). The flexible feedstock concept in Industrial Biotechnology: Metabolic engineering of *Escherichia coli*, *Corynebacterium glutamicum*, *Pseudomonas*, Bacillus and yeast strains for access to alternative carbon sources. *J. Biotechnol.* 234, 139–157. doi: 10.1016/j.jbiotec.2016.07.022

Wendisch, V. F., De Graaf, A. A., Sahm, H., and Eikmanns, B. J. (2000). Quantitative determination of metabolic fluxes during coutilization of two carbon sources: comparative analyses with *Corynebacterium glutamicum* during growth on acetate and/or glucose. *J. Bacteriol.* 182, 3088–3096.

Youn, J. W., Jolkver, E., Kramer, R., Marin, K., and Wendisch, V. F. (2008). Identification and characterization of the dicarboxylate uptake system DccT

in *Corynebacterium glutamicum*. *J. Bacteriol.* 190, 6458–6466. doi: 10.1128/JB.00780-08

Youn, J. W., Jolkver, E., Kramer, R., Marin, K., and Wendisch, V. F. (2009). Characterization of the dicarboxylate transporter DctA in *Corynebacterium glutamicum*. *J. Bacteriol.* 191, 5480–5488. doi: 10.1128/JB.00640-09

Zahoor, A., Lindner, S. N., and Wendisch, V. F. (2012). Metabolic engineering of *Corynebacterium glutamicum* aimed at alternative carbon sources and new products. *Computational and Structural Biotechnology Journal* 3, e201210004. doi: 10.5936/csbj.201210004

Zhang, J., and Yan, N. (2017). Production of Glucosamine from Chitin by Co-solvent Promoted Hydrolysis and Deacetylation. *ChemCatChem* 9, 2790–2796.