Transport and Catabolism of Carbohydrates by *Neisseria meningitidis*

Meriem Derkaoui^{a, b} Ana Antunes^b Jamila Nait Abdallah^{a, b} Sandrine Poncet^a
Alain Mazé^a Que Mai Ma Pham^a Abdelhamid Mokhtari^{a, c}
Ala-Eddine Deghmane^b Philippe Joyet^a Muhamed-Kheir Taha^b
Josef Deutscher^{a, d}

^aMicalis Institute, INRA, AgroParisTech, Université Paris-Saclay, Jouy-en-Josas, and ^bInstitut Pasteur, Unité des Infections Bactériennes Invasives, Paris, France; ^cDepartment of Biology, 8 May 1945 University, Guelma, Algeria; ^dCentre National de la Recherche Scientifique, UMR8261 (affiliated with Université Paris Diderot, Sorbonne Paris Cité), Expression Génétique Microbienne, Institut de Biologie Physico-Chimique, Paris, France

Key Words

Neisseria meningitidis · Glucose · Maltose · Gluconate · Virulence

Abstract

We identified the genes encoding the proteins for the transport of glucose and maltose in *Neisseria meningitidis* strain 2C4-3. A mutant deleted for *NMV_1892 (glcP)* no longer grew on glucose and deletion of *NMV_0424 (malY)* prevented the utilization of maltose. We also purified and characterized glucokinase and α -phosphoglucomutase, which catalyze early catabolic steps of the two carbohydrates. *N. meningitidis* catabolizes the two carbohydrates either via the Entner-Doudoroff (ED) pathway or the pentose phosphate pathway, thereby forming glyceraldehyde-3-P and either pyruvate or fructose-6-P, respectively. We purified and characterized several key enzymes of the two pathways. The genes required for the transformation of glucose into gluconate-6-P and its further catabolism via the ED pathway are organized in two adjacent operons. *N. meningitidis* also contains genes encoding proteins which exhibit similarity to the gluconate transporter (*NMV_2230*) and gluconate kinase (*NMV_2231*)

of Enterobacteriaceae and Firmicutes. However, gluconate might not be the real substrate of *NMV_2230* because *N. meningitidis* was not able to grow on gluconate as the sole carbon source. Surprisingly, deletion of *NMV_2230* stimulated growth in minimal medium in the presence and absence of glucose and drastically slowed the clearance of *N. meningitidis* cells from transgenic mice after intraperitoneal challenge.

© 2016 S. Karger AG, Basel

Introduction

The β -proteobacterium *Neisseria meningitidis* is a strictly human pathogen. In about 10% of any population, this commensal microorganism colonizes the nasopharynx [Yazdankhah and Caugant, 2004], where it resides asymptotically [Caugant and Maiden, 2009]. For reasons not yet understood it can occasionally become virulent and cross the epithelial cell layer and enter the blood

M.D. and A.A. contributed equally to this work.

stream [Campsall et al., 2013]. In order to explain this dual lifestyle, the hypothesis of the existence of carriage and invasive strains has been put forward [Caugant and Maiden, 2009; Taha et al., 2002]. Only the latter are assumed to cause severe infections leading to septicemia and meningitis, sometimes with a fatal outcome [Zarantonelli et al., 2008]. The current concept of meningococcal pathogenesis is based on the polygenic nature of meningococcal virulence comprising differences in genes involved in diverse aspects of meningococcal biology [Schoen et al., 2008]. However, the genetic and physiological differences between carriage and invasive strains are not clearly understood, but are proposed to be related to amino acid metabolism and oxidative stress response. In addition, differences in carbon catabolism are also thought to be decisive whether a strain is invasive or not [Schoen et al., 2014]. For example, the type of available carbon sources affects meningococcal complement resistance [Exley et al., 2005b].

In contrast to most bacteria, *N. meningitidis* is known to utilize only a small number of carbon sources, including a few amino acids, such as glutamate [Mallavia and Weiss, 1970], and the two carbohydrates maltose and glucose [Beno et al., 1968]. The tricarboxylic acid cycle intermediate succinate is also taken up and catabolized by meningococci [Weiss, 1970]. In addition, these bacteria can grow on L-lactate as well as pyruvate as the sole carbon source. Interestingly, glucose and lactate are present in millimole per liter amounts in the nasopharynx [Exley et al., 2005a], the usual habitat of *N. meningitidis*. While the transporter for lactate has been identified [Exley et al., 2005b], the permease involved in glucose uptake is not known. In many bacteria, glucose is efficiently transported via the phosphoenolpyruvate:carbohydrate phosphotransferase system (PTS) [Deutscher et al., 2006]. However, *N. meningitidis* possesses only an incomplete PTS composed of enzyme I (EI), HPr and two EIIA's, but lacks known EIIB's and the membrane components EIIC [Boël et al., 2003]. Nevertheless, it contains the metabolite-controlled HPr kinase, which phosphorylates HPr at Ser-46 [Derkaoui et al., 2016]. In Firmicutes, the resulting P-Ser-HPr is involved in numerous regulatory processes [Deutscher et al., 2006]. Although little is known about the role of the *N. meningitidis* incomplete PTS, it was suggested to carry out regulatory functions, possibly in meningococcal virulence [Poncet et al., 2009]. Indeed, the meningococcal *ptsI* and *ptsH* genes, which encode the general PTS proteins EI and HPr, were reported to be essential for experimental infection in an infant rat model of meningococcal bacteremia [Sun et al., 2000]. In addition,

the PTS component HPr was found to interact with the transcription regulator CrgA and its deletion affects virulence and capsule synthesis of *N. meningitidis* [Derkaoui et al., 2016].

Several bacteria transport glucose not only by the PTS, but also at lower efficiency via ion symport permeases, such as GlcP of *Bacillus subtilis* [Paulsen et al., 1998] or GlcU of *Staphylococcus xylosus* [Fiegler et al., 1999] and *Listeria monocytogenes* [Aké et al., 2011]. These permeases usually have a broad substrate specificity for hexoses [Paulsen et al., 1998]. The gene *NMV_1892* of *N. meningitidis* strain 2C4-3 encodes a protein resembling GlcP of Firmicutes. This protein was therefore a likely candidate for the glucose transporter of this pathogen.

Meningococci are not only devoid of PTS-catalyzed transport but also of fructose-6-P 1-kinase [Baart et al., 2010], a key enzyme of the Embden-Meyerhof-Parnas (EMP) pathway, which uses ATP to transform fructose-6-P into fructose-1,6-bisphosphate (FBP). Heterologous expression of a *pfk* gene in *N. meningitidis*, which allowed the metabolism of glucose via the EMP pathway, had no positive effect on the growth of the recombinant strain [Baart et al., 2010]. In contrast, it caused a reduction in biomass production. It was therefore proposed that for preferably oxidative organisms, such as *N. meningitidis*, catabolism via the EMP pathway would not represent an advantage. Meningococci catabolize glucose mainly via the Entner-Doudoroff (ED) pathway (70%) [Baart et al., 2007], and to a minor extent via the pentose phosphate pathway (PPP) [Schoen et al., 2014]. The transcription regulator HexR has recently been shown to control the expression of several genes presumed to encode enzymes of the PPP and the ED pathway [Antunes et al., 2015]. Part of the pyruvate formed via both pathways or from lactate utilization or taken up from the medium is either further metabolized via the tricarboxylic acid cycle or transformed into acetate, which is mostly secreted into the culture medium [Leighton et al., 2001].

N. meningitidis can also utilize maltose [Beno et al., 1968], which contains two glucose molecules connected via an α -1,4 glycosidic bond. Most bacteria transport the disaccharide either via an ABC transporter [Boos and Shuman, 1998; Monedero et al., 2008] or via a PTS [Mokhtari et al., 2013; Schönert et al., 2006]. As already mentioned, meningococci contain an incomplete PTS unable to transport carbohydrates. In addition, they lack genes encoding the membrane components of a carbohydrate-specific ABC transporter. Nevertheless, we found that *N. meningitidis* contains a presumed operon encoding three enzymes likely involved in maltose utilization. A fourth

gene encodes a LacY-type permease, which was a possible candidate for the maltose transporter.

In this study, we indeed identified the two permeases which catalyze the uptake of the carbohydrates glucose and maltose. We also characterized the enzymes, which catalyze their first catabolic steps as well as several key enzymes of the two carbon catabolic pathways used by *N. meningitidis*. Finally, we searched in the meningococcal genome for permeases transporting additional carbon sources and discovered a transport protein resembling gluconate permeases from Firmicutes and enterobacteria.

Results and Discussion

Identification of the Meningococcal Glucose Permease

N. meningitidis is known to utilize glucose. However, the enzymes catalyzing the uptake of glucose and the first catabolic steps have not been identified. Based on sequence similarity to bacterial glucose permeases, NMB0535 of *N. meningitidis* strain MC58 was suggested to function as a glucose transporter [Echenique-Rivera et al., 2011]. This protein is nearly identical to NMV_1892 of strain 8013 and exhibits 46% sequence similarity to *B. subtilis* GlcP, which has been shown to take up glucose and mannose [Paulsen et al., 1998]. In order to test whether NMV_1892, which is encoded by a presumably monocistronic gene, catalyzes glucose transport in *N. meningitidis*, we deleted the NMV_1892 gene by replacing it with a spectinomycin resistance cassette. The resulting mutant lost the capacity to grow in glutamine-free Roswell Park Memorial Institute (RPMI) medium containing glucose as the sole carbon source (fig. 1) and failed to acidify glucose-containing cystine trypticase agar (CTA) medium (Difco) (online suppl. fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000447093). To further confirm the glucose transport function of NMV_1892, we cloned its gene together with the ribosome binding site into plasmid pSU18c [Chandler, 1991], where it is transcribed from the *lac* promoter (see Materials and Methods). The resulting plasmid was subsequently used to transform the *Escherichia coli* Δ ptsHICrr mutant LJ140, which cannot utilize glucose [Aké et al., 2011; Pimentel-Schmitt et al., 2009]. However, when transformed with pSU18c containing the meningococcal NMV_1892 gene, it was able to ferment this sugar when grown on MacConkey agar plates (fig. 2a) similar to the *Escherichia coli* wild-type strain NM522 transformed with empty pSU18c (fig. 2a). LJ140 transformants carrying empty pSU18c were not able to ferment glucose (fig. 2a).

Fig. 1. Growth of the *N. meningitidis* wild-type (wt) strain 2C4-3 (dark gray/blue in the online version) and the *glcP* (light gray/red) and *gntP*-like (black/green) deletion mutants derived from it on RPMI medium without glutamine in the absence (dashed lines) or presence (solid lines) of 0.5% glucose. Deletion of *glcP* prevented growth on glucose. In contrast, in three independent experiments, the *gntP*-like mutant grew significantly faster on glucose and to higher cell densities than the wild-type strain. Slight growth of the *gntP*-like mutant within the first 2 h was also observed in the absence of glucose, whereas under this condition the wild-type strain and the *glcP* mutant exhibited no significant growth.

Characterization of Enzymes Involved in Glucose Catabolism via the ED Pathway

In most bacteria, glucose taken up via a non-PTS transporter is first phosphorylated to glucose-6-P by an enzyme called glucokinase (Glc) or hexokinase [Wagner et al., 1995]. Glc activity has previously been detected in crude extracts of *N. meningitidis*, but the enzyme has not been purified and characterized [Holten, 1974b]. In order to identify the *N. meningitidis* Glc, we carried out a BLAST search with Glc from *E. coli*. The search revealed that the *N. meningitidis* NMV_1004 gene encodes a protein exhibiting 62% sequence similarity to *E. coli* Glc. We purified His-tagged NMV_1004 as described in Materials and Methods and tested whether it can phosphorylate glucose. Indeed, NMV_1004 used ATP to transform D-glucose into D-glucose-6-P, and we therefore called it Glc (table 1).

NMV_1004 is part of an operon containing several other genes that, based on sequence similarities, probably encode enzymes involved in glucose metabolism (fig. 3a),

Fig. 2. Fermentation assays on MacConkey agar plates containing 0.5 mM IPTG and either 1% glucose (**a**) or 1% maltose (**b**). The meningococcal glucose permease GlcP restored glucose utilization in *E. coli* strain LJ140 and the maltose permease MalY maltose utilization in GSJ100. In **a** we tested strain LJ140 transformed with either empty plasmid pSU18c or plasmid pSU18c-glcP, in which the *N. meningitidis* *glcP* gene is expressed from the *lac* promoter. The strong red color (appears as dark gray in the print version) formed by LJ140[pSU18c-glcP] indicates that the synthesis of *N.*

meningitidis GlcP restores glucose utilization in strain LJ140. *E. coli* strain NM522[pSU18c] was used as a positive control. Similarly, in **b** we tested fermentation of maltose by strain GSJ100 transformed either with empty pSU18c or with pSU18c-malY. Again, the red color (dark gray) formed by GSJ100[pSU18c-malY] indicates that expression of the *N. meningitidis* *malY* gene restores maltose utilization in strain GSJ100. Strain NM522[pSU18c] was used as a positive control.

Table 1. Specific enzyme activities of purified proteins

Gene	Enzyme activity	Specific activity, $\mu\text{mol}/\text{min}$ and mg protein
<i>glk</i> (NMV_1004)	glucokinase	15
<i>gnd</i> (NMV_0016)	6-phosphogluconate dehydrogenase	2
<i>rpiA</i> (NMV_0882)	D-ribose-5-P isomerase A	3.66
<i>rpe</i> (NMV_1185)	D-ribulose-5-P 3-epimerase	2
<i>pgcM</i> (NMV_0427)	α -phosphoglucomutase	12.5
<i>edd</i> (NMV_1001)	gluconate-6-P dehydratase	2.5
<i>fbaA</i> (NMV_2059)	fructose-1,6-bisphosphate aldolase	56
<i>gntK</i> (NMV_2231)	gluconate kinase	150

such as: a glucose-6-P dehydrogenase (NMV_1002), which oxidizes glucose-6-P to 6-P-glucono- δ -lactone and a 6-P-gluconolactonase (NMV_1003), which transforms 6-P-glucono- δ -lactone into gluconate-6-P. Together with kinase (Glk), these enzymes transform glucose into gluconate-6-P, the branching point for the PPP and ED pathways. The operon also contains the gene for a P-glucose isomerase (NMV_1006), which interconverts glucose-6-P

and fructose-6-P. A protein of unknown function is encoded by NMV_1007. The expression of the operon has recently been shown to be controlled by the RpiR-like repressor HexR, the gene of which, NMV_1005, is also part of this operon [Antunes et al., 2015].

HexR was also reported to control the expression of the adjacent genes NMV_1000 and NMV_1001, which are divergently oriented to the NMV_1002 to NMV_1007

Fig. 3. Schematic presentation of the *N. meningitidis* genomic regions containing the genes encoding the enzymes necessary for the metabolism of glucose (**a**) and maltose (**b**) via the ED pathway. In **a**, the genes encoding the ED enzymes Eda and Edd are in dark gray. The maltose permease gene *maly* (**b**) is associated with the corresponding catabolic genes, whereas the glucose permease

gene *glcP* (NMV_1892) is located elsewhere on the genome. Although gluconate is not utilized by *N. meningitidis*, *gntP*- and *gntK*-like genes are present in 2C4-3 (**c**) and other meningococcal and gonococcal strains. The genes encoding the PPP enzymes are randomly distributed over the whole chromosome and are not shown here.

operon (fig. 3a). Based on sequence similarities, they were likely to encode the ED pathway enzymes gluconate-6-P dehydratase (Edd; NMV_1001), which converts gluconate-6-P into 2-keto-3-deoxy-6-P gluconate (KDPG) and KDPG aldolase (NMV_1000), which cleaves KDPG into glyceraldehyde-3-P and pyruvate. *N. meningitidis* catabolizes glucose mainly via the ED pathway (70%) [Baart et al., 2007]. We therefore confirmed the presumed Edd activity of NMV_1001 by carrying out a spectrophotometric assay as described in Materials and Methods. Purified NMV_1001 indeed converts gluconate-6-P into KDPG (table 1). Interestingly, in two γ -proteobacteria, KDPG controls HexR by dissociating and thus inactivating the oligomeric regulator [Daddaoua et al., 2009; Leyn et al., 2011]. However, no such effect of KDPG was observed for *N. meningitidis* HexR [Antunes et al., 2015].

Characterization of Enzymes Involved in Glucose Catabolism via the PPP

Catabolism via the PPP requires five additional enzymes. We carried out BLAST searches with the corresponding *E. coli* and *B. subtilis* proteins and could identify candidates for the five enzyme activities: gluconate-6-P dehydrogenase (Gnd; NMV_0016); D-ribulose-5-P 3-epimerase (Rpe; NMV_1185); D-ribose-5-P isomerase A (RpiA; NMV_0882); transketolase (NMV_0929), and transaldolase (NMV_0388; fig. 4). The genes for the presumed PPP enzymes are not organized in an operon, but are randomly distributed over the meningococcal chromosome. We purified three of the five enzymes and confirmed their activity. We found that NMV_0016 is a de-

carboxylating Gnd, which converts gluconate-6-P into ribulose-5-P (branching point to the PPP; fig. 4). This activity has previously been measured in *N. meningitidis* crude extracts, but the enzyme has not been purified and characterized [Holten, 1974a]. We confirmed that NMV_1185 transforms D-ribulose-5-P into D-xylulose-5-P, and that NMV_0882 isomerizes D-ribulose-5-P to D-ribose-5-P. During the metabolism of glucose via the PPP, three molecules of glucose are transformed into two molecules of fructose-6-P, one molecule of glyceraldehyde-3-P and three of CO₂. While glyceraldehyde-3-P is metabolized via the lower part of glycolysis, fructose-6-P is recycled to glucose-6-P by one of the presumed phosphoglucose isomerases, NMV_1006 (Pgi1) or NMV_0369 (Pgi2). *N. meningitidis* lacks fructose-6-P 1-kinase activity, which would allow the phosphorylation of fructose-6-P to FBP (fig. 4). Nevertheless, *N. meningitidis* can probably form FBP from glyceraldehyde-3-P, which is partly converted into dihydroxyacetone phosphate by a presumed triosephosphate isomerase (NMV_2075). Indeed, we could demonstrate that purified NMV_2059 functions as an FBP aldolase (Fba). It probably also catalyzes the reverse reaction by using the two triosephosphates to form FBP (fig. 4). In numerous organisms, FBP is an important allosteric regulator of metabolic enzymes, such as pyruvate kinase [Waygood and Sanwal, 1974], and of signal transduction proteins, such as HPr kinase [Derkaoui et al., 2016; Deutscher and Engelmann, 1984], which might explain why this glycolytic intermediate is synthesized in *N. meningitidis*, although it cannot be further metabolized.

glucose and maltose transport activities of GlcP and MalY, respectively, were confirmed by appropriate assays, the substrate of the GntP-like NMV_2230 remains obscure. Although NMV_2231 efficiently phosphorylates gluconate, *N. meningitidis* was not able to grow in MM containing gluconate as the sole carbon source. The substrate of NMV_2230 might therefore be one of the other 15 hexonates, which after its uptake is converted to gluconate.

N. meningitidis is also known to efficiently utilize the disaccharide maltose, but the enzymes that catalyze its uptake and catabolism have not been identified. Meningococci do not possess the components of an ABC transporter for maltose. Nevertheless, maltose phosphorylase activity had been detected in meningococcal crude extracts precipitated with ammonium sulfate [Fitting and Doudoroff, 1952]. Bacteria transporting the disaccharide via an ABC transporter usually use maltose phosphorylase to catalyze the first step of maltose catabolism, i.e. its phosphorolysis into D-glucose and D-glucose-1-P. We therefore carried out a BLAST search with the sequence of the *Lactobacillus casei* maltose phosphorylase MalP

(LCABL_11440) [Monedero et al., 2008]. We indeed identified a gene in the *N. meningitidis* strain 2C4-3, *NMV_0426*, which encodes a protein that exhibits strong sequence identity (59%) to *L. casei* maltose phosphorylase. In addition, the genes upstream and downstream from *NMV_0426* encode enzymes also potentially involved in maltose catabolism (fig. 3b): *NMV_0425* encodes a presumed aldose 1-epimerase, which transforms α -D-glucose into β -D-glucose, and *NMV_0427* encodes a presumed phosphoglucumutase, which interconverts D-glucose-1-P and D-glucose-6-P (fig. 4). *NMV_0427* is 46% identical to α -phosphoglucumutase (PgcM) from the *B. subtilis* maltodextrin operon [Schönert et al., 2006], which converts both α - and β -D-glucose-1-P into glucose-6-P [Mesak and Dahl, 2000]. However,

Fig. 5. Growth of the *N. meningitidis* wild-type (wt) strain 2C4-3 (black/blue in the online version) and the $\Delta malY$ mutant (gray/red) derived from it on RPMI medium lacking glutamine in the absence (dashed line) or presence (solid line) of 0.5% maltose. While the wild-type strain grew to a high cell density in the presence of maltose, the $\Delta malY$ mutant showed only slight growth.

NMV_0427 lacks the phosphorylation site signature sequence [T(P/A)SHNP] of bacterial phosphoglucomutases [Mehra-Chaudhary et al., 2011]. In order to test whether it nevertheless functions as phosphoglucomutase we purified His-tagged NMV_0427 as described in Materials and Methods. Purified NMV_0427 was able to convert α -D-glucose-1-P into D-glucose-6-P (table 1), but did not react with β -D-glucose-1-P. The activity of NMV_0427 therefore resembles PgcMs, such as PgmH of *Lactococcus lactis* [Neves et al., 2006]. However, NMV_0427 exhibits no sequence similarity to PgmH and we therefore preferred to call it PgcM based on its strong similarity to the *B. subtilis* enzyme, although the *Bacillus* enzyme reacts with α - and β -D-glucose-1-phosphate [Mesak and Dahl, 2000].

The three genes encoding the presumed enzymes for maltose catabolism probably form an operon together with a fourth gene, NMV_0424 (fig. 3b), which is annotated as a sucrose/H⁺ cotransporter-like transmembrane protein of the major facilitator superfamily. We suspected that NMV_0424 might in fact catalyze maltose uptake in *N. meningitidis*. Indeed, NMV_0424 exhibits strong sequence similarity (61%) to the protein MalY of the α -proteobacterium *Caulobacter crescentus*, which has been shown to function as a maltose transporter in this organism [Lohmiller et al., 2008]. In order to test whether NMV_0424 of *N. meningitidis* strain 2C4-3 is indeed a

maltose permease, we deleted the NMV_0424 gene by replacing it with a kanamycin resistance cassette. The resulting mutant grew normally on glucose (data not shown), but in contrast to the wild-type strain 2C4-3 grew very poorly in maltose-containing medium (fig. 5). The NMV_0424 mutant also lost the capacity to acidify maltose-containing CTA medium (online suppl. fig. 1). To further confirm that NMV_0424 functions as a maltose transporter, we cloned its gene together with the ribosome binding site into plasmid pSU18c [Bartolomé et al., 1991], where it is transcribed from the *lac* promoter (see Materials and Methods). The resulting plasmid was subsequently used to transform the *E. coli* *malE* mutant GSJ100 [Blau-deck et al., 2003], which lacks the periplasmic maltose binding protein MalE and, therefore, is not able to utilize maltose. In contrast, a resulting transformant, which contained pSU18c carrying NMV_0424, was able to ferment this disaccharide when grown on maltose-containing MacConkey agar plates (fig. 2b) similar to the *E. coli* wild-type strain NM522 transformed with empty pSU18c (fig. 2b). GSJ100 transformants carrying the empty plasmid pSU18c were not able to ferment maltose (fig. 2b).

We noticed that MalY-like transporters are present in lactobacilli that lack a maltose-specific ABC transporter, such as *L. salivarius* (LSL_1282) and *L. reuteri*. NMV_0424 exhibits up to 82% sequence identity to some of these *Lactobacillus* transporters. This unusually high sequence identity suggests that *N. meningitidis* probably acquired the maltose transporter gene by horizontal gene transfer from one of these lactobacilli. The donor could well be *L. salivarius*, which contains a maltose operon with the same gene order as *N. meningitidis* (*malY*, *galM*, *malP*, *pgcM*; fig. 3b). Even more important, these two bacteria share a common habitat – the nasopharynx. *N. gonorrhoeae*, which colonizes a different habitat, lacks the enzymes for maltose utilization.

In summary, *N. meningitidis* takes up maltose via the presumed H⁺ symport permease NMV_0424, which we dubbed MalY in agreement with the name given to the *C. crescentus* maltose transporter [Lohmiller et al., 2008]. Intracellular maltose is phosphorylated into α -D-glucose and α -D-glucose-1-P by the enzyme maltose phosphorylase, MalP (NMV_0426; fig. 4). The two products of this reaction, α -D-glucose and α -D-glucose-1-P, are subsequently converted into D-glucose-6-P either by the enzymes aldose 1-epimerase (GalM, NMV_0425) and Glk (NMV_1004), or by the PgcM (NMV_0427), respectively. Similar to glucose, maltose is therefore probably mainly metabolized via the ED pathway and to a minor extent via the PPP.

Fig. 6. Growth of a *B. subtilis* $\Delta gntP$ mutant and $\Delta gntP$ complemented with the *N. meningitidis* *gntP* gene in CSK MM containing 0.5% Na⁺-gluconate or 0.5% Na⁺-gluconate plus 1 mM IPTG. The $\Delta gntP$ mutant and the complemented strain were not able to grow on gluconate as the sole carbon source. However, when 1 mM of IPTG was added the complemented strain grew half as fast as the *B. subtilis* wild-type strain. Both strains reached a similar final OD₆₀₀.

N. meningitidis 2C4-3 Contains a GntP-Like Permease and Gluconate Kinase

In order to identify additional carbohydrate transporters in *N. meningitidis*, we carried out BLAST searches with various carbohydrate permeases and catabolic enzymes from *E. coli* and *B. subtilis*. We found that NMV_2230 exhibits significant similarity (54–59%) to hexonate permeases from *B. subtilis* and *E. coli* (GntP, GntT, GntU, and IdnT). In addition, the protein encoded by the downstream gene NMV_2231 (fig. 3c) showed 56% sequence similarity to gluconate kinase (GntK) from *E. coli*. However, the wild-type strain 2C4-3 was not able to grow on Na⁺-gluconate as the sole carbon source. Failure to utilize gluconate was also observed for the *N. meningitidis* serotype B strain MC58 (data not shown). It was possible that NMV_2230 or NMV_2231 contain a mutation leading to the inactivation of the encoded enzyme. To test this possibility, we purified His-tagged NMV_2231. The purified enzyme exhibited very strong GntK activity (table 1). We also tested whether *N. meningitidis* GntP can complement a *B. subtilis* *gntP* mutant. For that purpose, we constructed a *gntP* mutant derived from strain BSB168, which grew normally on CSK minimal medium (MM) containing glucose (data not shown), but was unable to utilize gluconate (fig. 6). We subsequently integrated the *N. meningitidis* NMV_2230 gene controlled by

the IPTG-inducible *spac* promoter into the genome of the *B. subtilis* *gntP* mutant. Surprisingly, although NMV_2230 does not allow the growth of *N. meningitidis* on gluconate, it partially restored growth of the *B. subtilis* *gntP* mutant on CSK MM containing IPTG and gluconate (fig. 6). A possible explanation might be that the true substrate of NMV_2230 is not gluconate, but one of the other 15 hexonates, such as L-idonate or D-galactonate, which after its uptake might be converted to gluconate via an epimerase or a dehydrogenase/reductase system. Such a system exists in *E. coli*, where the GntP-like IdnT takes up L-idonate, which is first oxidized by IdnD to 5-keto-gluconate, then reduced to gluconate by IdnO and finally converted to gluconate-6-P by the GntK IdnK [Bausch et al., 1998]. IdnK exhibits 53% sequence similarity to NMV_2231. In addition, IdnT, which shows 58% sequence similarity to NMV_2230, slowly transports gluconate. IPTG-induced overexpression of NMV_2230 in the *B. subtilis* *gntP* mutant might allow slow growth on gluconate, whereas in *N. meningitidis* the permease might not be sufficiently produced to allow growth on the hexonate. Unfortunately, L-idonate and most other hexonates are not commercially available and we therefore could not test this hypothesis.

Surprisingly, deletion of the NMV_2230 gene affected growth in glucose-containing MM. The NMV_2230 mutant reproducibly grew faster and to a higher cell density than the wild-type strain (fig. 1). Compared to the other strains, the NMV_2230 mutant also exhibited slight growth during the first 2 h in the absence of glucose, suggesting that NMV_2230 deletion allows a more efficient utilization of compounds present in the MM.

Deletion of the gntP-Like NMV_2230 Enhances *N. meningitidis* Virulence

We also tested the impact of the deletion of the *gntP*-like gene on meningococcal invasiveness into the blood after intraperitoneal challenge of BALB/c mice. We determined the colony-forming units per milliliter of *N. meningitidis* in the blood at 2, 6 and 24 h after infection with standardized inocula of 10⁷ CFU per mouse. Interestingly, compared to the wild-type strain, the NMV_2230 mutant exhibited a stronger increase of bacterial counts and subsequently persisted at higher levels (fig. 7). After 24 h the difference between the wild-type and the NMV_2230 mutant was more than three orders of magnitude. The enhanced resistance against clearance from the blood might partly be due to the observed faster growth of the *gntP*-like mutant in a glucose-containing environment (fig. 1).

Fig. 7. Enhanced virulence of the *N. meningitidis* Δ NMV_2230 (Δ gntP) mutant in mice. The virulence was evaluated by the levels of bacterial load in blood samples from BALB/c mice after intraperitoneal challenge with standardized inocula of 10^7 CFU. The Δ NMV_2230 mutant (filled gray squares) showed significantly higher virulence compared to the *N. meningitidis* wild-type strain 2C4-3 (filled black circles). Three mice were infected with the wild-type strain and 4 with the Δ NMV_2230 mutant. Survival of the wild-type strain 2C4-3 and the Δ NMV_2230 mutant was determined by bacterial counts from blood samples of infected mice 2, 6, and 24 h after intraperitoneal infection. Data were calculated from two independent experiments and mean values together with standard deviations (error bars) are presented.

Conclusions

The availability of carbon sources is an important factor for the colonization by bacterial pathogens of epithelial surfaces in humans and animals. For example, *N. meningitidis* strains unable to utilize lactate cannot colonize the nasopharynx [Exley et al., 2005a]. Glucose and maltose are also present in the nasopharynx [Iyer et al., 2005]. *N. meningitidis* seems to have adapted to this specific environment because lactate, glucose and maltose belong to the few carbon sources utilized by this pathogen. We here establish that the two carbohydrates, glucose and maltose, are taken up by the ion-driven symporters GlcP and MalY, respectively. Most striking is the high degree of sequence identity (around 80%) between MalY and presumed maltose symporters of some oral lactobacilli (*L. salivarius*, 82%; *L. fermentum*, 78%; *L. oris*, 77%). This finding suggests that *N. meningitidis* acquired the maltose operon from an oral *Lactobacillus* strain. Several enzymes catalyzing the catabolism of glucose and maltose via the PPP or the ED pathway have also been

characterized. Finally, we also identified a novel transporter resembling gluconate permeases. However, *N. meningitidis* is not able to utilize gluconate and the substrate of this permease might therefore be another hexonate. Surprisingly, deletion of the presumed hexonate transporter increased the survival rate of *N. meningitidis* in a mouse infection model.

Experimental Procedures

Bacterial Strains and Growth Conditions

The meningococcal strain 2C4-3, isolated as clone 12 from the serogroup C, class 1 strain 8013 [Nassif et al., 1991] has the relevant phenotype P⁺, Opa⁺, Opc⁺, PilC1⁺/PilC2⁺. This strain, as well as the *glcP*, *malY*, and *gntP* deletion mutants derived from it, was grown either in GC medium base (Difco) containing the supplements previously described [Kellogg et al., 1963], in RPMI medium, and in RPMI medium supplemented with serum. Growth studies of *N. meningitidis* strains were carried out in glucose-, maltose- or Na⁺-gluconate-containing RPMI medium lacking glutamate. When appropriate, kanamycin or spectinomycin was added at a final concentration of 100 or 75 µg/ml, respectively. Growth of the *B. subtilis* BSB168-derived *gntP* mutant and its transformant containing pIC634-gntP-2C4-3 was measured in C MM containing 25 mM of Na⁺-gluconate in the presence or absence of 1 mM of IPTG. The *E. coli* strains NM522 (Stratagene) or NM522 transformed with plasmid pREP4-GroES/EL [Amrein et al., 1995] were used for protein overproduction. Strains harboring derivatives of the His-tag expression vector pQE30 (Qiagen) were grown at 37°C under agitation in LB medium supplemented with 100 µg/ml ampicillin for NM522 and with 50 µg/ml ampicillin and 12.5 µg/ml kanamycin for NM522[pREP4-GroES/EL].

Plasmids Used in This Study

The plasmid pSU18c is a pACYC184-derived vector containing the multiple cloning site and the *lacZa* reporter gene of pUC18 [Bartolomé et al., 1991]. Plasmid pQE30 (Qiagen) was used for the overproduction of His-tagged proteins. For proteins forming inclusion bodies it was used in combination with pREP4-GroES/EL [Amrein et al., 1995], which expresses the genes for the chaperone GroES/EL. The pIC634 plasmid is a derivative of pDG1664 [Guérout-Fleury et al., 1996], which contains the *E. coli lacI* gene and the *spac* promoter inserted at the *Bam*HI/*Hind*III/*Eco*RI restriction sites. It also contains two *B. subtilis* DNA fragments, allowing its integration by double recombination in the *thrC* locus of this organism.

Purification of His-Tagged Proteins

The genes encoding the proteins Glk, PgcM, GntK, Gnd, Edd, Rpe, RpiA, and Fba were PCR amplified by using *N. meningitidis* 2C4-3 genomic DNA as the template and the corresponding primer pairs (table 2). The amplicons were cut with the appropriate restriction enzymes and cloned into the His-tag expression vector pQE30 (Qiagen), cut with the same enzymes. The correct sequence of each insert was confirmed by DNA sequencing. The resulting plasmids were used to transform *E. coli* strains NM522 or NM522[pREP4-GroES/EL]. His-tagged proteins were purified on Ni-NTA columns (Qiagen) as previously described [Galinié et al.,

Table 2. List of primers used in this study

Name	Used for	Sequence (5' to 3')
glcPBamHI	N.m. <i>ΔglcP</i>	CACGGATCCGCGGAAGTGAAGGCGGAAA
glcPEcoRV1		GGGGATATCAAGACGACCAATGGGGACG
glcPEcoRV2		CCCGATATCTTCAGACGGCCCGGTGTACTGTGTACCGCG
glcPHindIII		ATTAAGCTTCAACGCGCCTATCATGCCC
specSmaIFor		ATACCCGGGGATCCGGTGATTGATTGAGC
specSmaIRev		CGGCCCCGGGTGAACGAATTGTTAGAC
malYBamHI	N.m. <i>ΔmalY</i>	GGTGGATCCGCCCCGCGCGTTCAAACCG
malYEcoRV1		ATCGATATCGTGCTTTT GCCAGCGCGG
malYEcoRV2		ATCGATATCGCTTGGGCGGGCATTATC
malYKpnI		ACGGGTACCGGCCAGCCCGTGCGAACC GC
KanSmaI1		GGGCCCCGGGCCAGCGAACCATTGAGG
KanSmaI2		AAACCCGGGTTCAGACGGCTACTAAAACAATTCATCCAG
AMgntP2C-BamF	N.m. <i>ΔgntP</i>	GCACGGATCCTGCCGCTGCCTGAAATACCGTCC
AMgntP2C-ECORIR		AAGCGAATTCTGTAAATAAAAGTACAAAATTGTCAGG
KangntP2C-ECORIF		GGGGGGAATTCCCCAGCGAACCATTGAGG
KangntP2C-HindR		CTAAAAAGCTTTTCAGACGGCTACTAAAACAATTCATCCAG
AvgntP2C-HindF		CATCAAGCTTCAGACGGAAAGGATAGTAAATG
AvgntP2C-SalIR		GGGGTTCGACGGCTTCAGACGGCATATTGCTTC
DeltagntP-F	<i>ΔgntP</i> verificat.	GCCCCCACCACGTGGGCATC
DeltagntP-F1		CTTGCACAAACCCCTG
DeltagntP-R		GGTGCTTCAGCACCTTAGAGAATC
KanF1		GATGTGGAACGGGAAAAGGACATGATG
KanR1		TCTTCATACTCTTCCGAGCAAAGGACGCC
amont-gntP-D-ApaI	BSB168 <i>ΔgntP</i>	CGTGGGCCCCAAATGGGAATCGATCCGGAAACGCC
amont-gntP-R-SacII		ACGTCCGCGGCAAGTGCAACGATGATTAACGGCATGG
aval-gntP-D-SpeI		ACGTACTAGTAAGTTTAGTTGTATGAAATTAAGAAGG
aval-gntP-R-SalI		ACGTGTCGACAAGAGCCTGTCTGACGTATTCAATCC
amont-D-Kana	<i>aphA3</i>	CGTTGCACTTGCCGCGGACGTGTTCTTGAAAACGGCGGAGAAGC
aval-R-Kana		CAACTAAACTTACTAGTACGTCCCATGTAAAGGAATTCTGTACGG
GlkNmBaFor	pQE30-glcK	GTGAGGATCCATGTCTTCTACGCCGAAT
GlkNmHiRev		ATATAAGCTTCGATAATGCAGCCCCGCTG
GntKNmBaHiFor	pQE30-gntK	AGGAGGATCCATGACTACGCATTTTGTCTG
GntKNmBaHiRev		TGTCAAGCTTCAGACGGCATATTGCTTCAA
Gnd8013KpnIF	pQE30-gnd	GCTTGGTACCATGAAAGGCGATATTGGTG
Gnd8013HindR		CGGCAAGCTTATCAAATATCGTAGG
rpiA8013BamF	pQE30-rpiA	GGACGGATCCATGACGACACAAGACGAACTCAAGCG
rpiA8013HindR		GACGGAAGCTTAACGTCAGGATTACCCTTGGCAGGG
Rpe8013BamF	pQE30-rpe	GGAGGATCCATGACCGCCTACCGTATCGCACCCAG
Rpe8013KpnIR		CTGAAGGTACCTAATTTTCAGACGGCATTTCTATTTC
Edd8013BamF	pQE30-edd	GAGAGGATCCGTGAACACTACTCCTATCCACTCC
Edd8013HindR		TGAAAAGCTTGTCTGAAACGCGCATCAG
FbaNmBaHiFor	pQE30-fba	GGAGGGATCCATGGCACTCGTATCCATGCG
FbaNmBaHiRev		AAGCAAGCTTTTATTTGACGATTTGGTTC
pgmB8013BamF	pQE30-pgcM	GGGGGATCCATGACGTTTACCGCAGTGCTCTTCG
pgmB8013HindR		CTTAAAGCTTTATCTGACGCGTTTACCTGCCC
edaFcoliBam	pQE30-edaEc	AAAGGATCCATGAAAAACTGGAAAACAAGTG
edaRcoliSal		ATCGTTCGACCGGGCATTGTTGACTTTTACAG
SP219	pSU18c-glcP	CGCGGATCCTAGATAGTAATATTCGACACC
SP220		CTCAAGAAAGCTTTTATTTGTCCGCCC
SP236	pSU18c-malY	CGCGGATCCTAGATACGAGGCAACGCCGTACTGG
SP237		TTCCCAAGCTTTCAAACCCCGCCGTGTGTTG
D-GntP-HindIII	pIC634-gntP	ACGTAAGCTTACATAAGGAGGAAGTACTATGGACGGCTGGACACAGACGC
R-GntP-SphI		ACGTGCATGCTCAGACGATGGCGAACAGCAGTGC

Restriction sites are in bold letters and the *N. meningitidis* DNA uptake sequence is written in italics.

1997]. To purify *E. coli* 2-dehydro-3-deoxyphosphogluconate aldolase, the *eda* gene was PCR amplified by using genomic DNA of *E. coli* strain NM522 as the matrix and the primers *edaFcoliBam* and *edaRcoliSal* (table 2). The resulting amplicon was cut with *Bam*HI and *Sal*I, inserted into pQE30 cut with the same enzymes and subsequently cloned into *E. coli* strain NM522. For one clone, the correct sequence of the insert was confirmed by DNA sequencing. Subsequent purification of the enzyme was carried out as previously described [Galinier et al., 1997]. Maltose phosphorylase of *Enterococcus faecalis* and ribitol-5-P 2-dehydrogenase from *L. casei* were purified as described in Mokhtari et al. [2013] and Bourand et al. [2013], respectively.

Spectrophotometric Enzyme Assays

To measure the activity of the purified enzymes, we carried out appropriate spectrophotometric assays. The tests were performed in a total volume of 500 μ l and the change in OD₃₄₀ was continuously followed with a Kontron Bio-Tek spectrophotometer using the Autorate program. All mixtures (except for PgcM) contained 50 mM of Tris/HCl, pH 7.4, and 10 mM of MgCl₂. To measure the activity of the various enzymes we added the following: for Glk, 10 mM of glucose, 1 mM of NADP, 1 mM of ATP, 10 μ g of glucose-6-phosphate 1-dehydrogenase (Sigma), and 8 μ g of purified Glk; for GntK, 1 mM of gluconate, 1 mM of NADP, 1 mM of ATP, 25 μ g of 6-phosphogluconate dehydrogenase (Sigma), and 2 μ g of purified GntK; for Gnd, 1 mM of gluconate-6-P, 1 mM of NADP, and 10 μ g of purified Gnd; for Edd, 1 mM of gluconate-6-P, 1 mM of NADH, 30 μ g of *E. coli* 2-dehydro-3-deoxyphosphogluconate aldolase, 20 μ g of triosephosphate isomerase (Sigma), 24 μ g of glycerol-3-P dehydrogenase (Sigma), and 40 μ g of purified Edd; for Rpe, 0.5 mM of D-xylulose-5-P, 1 mM of NADH, and 18 μ g of *L. casei* D-ribitol-5-P 2-dehydrogenase [Bourand et al., 2013], and 10 μ g of purified Rpe; for D-ribose-5-P isomerase, 1 mM of ribose-5-P, 1 mM of NADH, 18 μ g of *L. casei* ribitol-5-P 2-dehydrogenase, and 30 μ g of purified D-ribose-5-P isomerase; for FBP aldolase, 5 mM of FBP, 1 mM of NADH, 20 μ g of triosephosphate isomerase, 24 μ g of glycerol-3-P dehydrogenase and 5 μ g of purified FBP aldolase. For PgcM we used 50 mM of phosphate buffer, pH 7.4, and either 10 mM of β -D-glucose-1-P (Sigma) or 10 mM of maltose and 4 μ g of maltose phosphorylase from *E. faecalis* [Mokhtari et al., 2013] to form α -D-glucose-1-P. For both substrates we added 1 mM of NADP, 10 μ g of glucose-6-phosphate 1-dehydrogenase, and 20 μ g of purified PgcM. All different assay mixtures were incubated for 5–10 min at ambient temperature before the reaction was started by adding the enzyme to be tested.

Construction of *N. meningitidis* Δ glcP, Δ malY and Δ gntP Mutants

The three deletion mutants were constructed by first amplifying upstream and downstream regions of the targeted gene by using the corresponding primer pairs listed in table 2. The two upstream and downstream DNA fragments of the *glcP* and the *malY* genes were cloned into plasmid pQE30 cut with *Bam*HI/*Hind*III for *glcP* and *Bam*HI/*Kpn*I for *malY*. The *glcP*-related fragments were cut with *Bam*HI/*Eco*RV and *Eco*RV/*Hind*III and the *malY* fragments with *Bam*HI/*Eco*RV and *Eco*RV/*Kpn*I. In each case, the simultaneous insertion of the two amplified fragments into pQE30 created an *Eco*RV site. A spectinomycin cassette was inserted in the *Eco*RV site of the *glcP*-related fragments and a kanamycin cassette in the *Eco*RV site of the *malY* construct. The two antibiotic resistance

cassettes were amplified with primers, which created *Sma*I sites at their ends and thus allowed blunt end ligation. For *gntP* deletion, a *Bam*HI/*Eco*RI upstream fragment was first cloned into pBluescript KS+ followed by a kanamycin resistance cassette amplified with *Eco*RI and *Hind*III sites. Finally, the downstream *gntP* fragment containing *Hind*III and *Sal*I sites was added. The three deletion plasmids also contained the uptake sequence for *N. meningitidis* allowing the transformation of strain 2C4-3. Transformants with the correct antibiotic resistance were selected and the correct deletion of each gene (*glcP*, *gntP*, and *malY*) was verified by PCR with appropriate primers and subsequent DNA sequencing of the amplicon.

Glucose and Maltose Utilization Assays

The *N. meningitidis* wild-type strain 2C4-3 and the *glcP* mutant derived from it were grown in CTA medium containing 1% glucose. Similarly, the wild-type strain and the *malY* mutant were grown in CTA medium containing 1% maltose. The medium also contained 17 mg/l of phenol red as a pH indicator, which changes its color from red to yellow owing to the acidification of the medium during utilization of the added sugar [Sáez-Nieto et al., 1982].

Complementation of a Glucose-Negative *E. coli* Strain with the NMV_1892 Gene

Heterologous complementation assays were carried out following a previously described method [Pimentel-Schmitt et al., 2009]. First, the *N. meningitidis* *glcP* gene NMV_1892 together with its ribosome binding site was amplified by PCR with *N. meningitidis* 2C4-3 genomic DNA as the template and the primers SP219 and SP220, which introduce *Bam*HI and *Hind*III restriction sites (table 2). The resulting PCR fragment was digested with the indicated restriction enzymes and ligated into vector pSU18c in the same orientation as the 5'-part of *lacZ* encoding the α -fragment of β -galactosidase [Chandler, 1991], thus putting the NMV_1892 gene under control of *Plac*. In order to avoid fusion of NMV_1892 to the N-terminus of the α -fragment, the 5' oligonucleotides were chosen in such a way that they created a stop codon located upstream from the ribosome binding site. The stop codon was in frame with the NMV_1892 start codon. The correct sequence of the insert was confirmed by DNA sequencing. The resulting plasmid was introduced into *E. coli* strain LJ140, which carries a *ptsHICrr* deletion and therefore exhibits very poor fermentation of glucose [Lévy et al., 1990]. Transformants were plated on MacConkey agar, which contained 1 mM IPTG and 1% glucose in order to test for heterologous complementation of the glucose-negative phenotype. Empty pSU18c was used to transform *E. coli* strains NM522 and LJ140, thus providing positive and negative controls, respectively, for glucose fermentation.

Complementation of an *E. coli* *malE* Mutant with the NMV_0424 Gene

The approach described above was also used to complement the *E. coli* strain GSJ100 [Blaudeck et al., 2003], which lacks the periplasmic maltose binding protein MalE and therefore cannot utilize maltose, with the *N. meningitidis* *malY* gene. The *malY* gene together with its ribosome binding site was amplified by PCR with the primers SP236 and SP237. The amplicon was cloned into pSU18c cut with *Bam*HI and *Hind*III. The resulting plasmid was used to transform the *E. coli* *malE* mutant GSJ100 [Blaudeck et al., 2003]. Fermentation assays with the complemented mutant and control strains were carried out on MacConkey agar as described above.

Complementation of a *B. subtilis* *gntP* Mutant with the NMV_2230 Gene

In order to test whether the GntP-like NMV_2230 can complement a *gntP* mutant, we deleted the *gntP* gene of *B. subtilis* strain BSB168. First, the upstream region of *gntP* and the *aphA3* kanamycin resistance gene were amplified by using *B. subtilis* genomic DNA and plasmid pGEM-T(*aphA3*) [Joyet et al., 2010], respectively, as the template and appropriate primer pairs (table 2). The forward primer for *aphA3* overlapped the reverse primer for the *gntP* upstream region, which in the second step allowed the fusion of the two DNA fragments by PCR amplification by using the two amplicons as the template and the two distal oligonucleotides as primers. The fused DNA was cut with *ApaI* and *SpeI* and inserted into plasmid pGEM-T Easy (Promega) cut with the same enzymes. The downstream region of *B. subtilis* *gntP* was subsequently amplified by using genomic *B. subtilis* DNA as the template and appropriate primers (table 2). The resulting amplicon was cut with *SpeI* and *Sall* and cloned into pGEM-T Easy containing *aphA3* and the upstream region of *gntP*. The resulting plasmid pGEM-T-Δ*gntP*-Kan was used to transform the *B. subtilis* strain BSB168 and a double recombination allowed the replacement of *gntP* with the kanamycin-resistance gene *aphA3*. The correct deletion of *gntP* was confirmed by PCR amplification of the corresponding region and DNA sequencing of the amplicon.

In the second step, the *gntP*-like gene of *N. meningitidis* was PCR amplified by using genomic DNA as the template and the primers D-GntP-HindIII and R-GntP-SphI (table 2). The resulting DNA fragment was cut with *HindIII* and *SphI* and inserted into plasmid pIC634, which is a derivative of pDG1664 [Guérout-Fleury et al., 1996], containing the *E. coli* *lacI* gene and the *spac* promoter. In one of the resulting plasmids the correct sequence of the *gntP*-like gene, which is under control of the *spac* promoter, was verified by DNA sequencing. Plasmid pIC634 also contains the upstream and down-

stream regions of the *B. subtilis* *thrC* gene and thus allowed the simultaneous integration of *lacI*, *erm*, and the *Pspac*-controlled *gntP* gene at the *thrC* locus of the *B. subtilis* wild-type strain BSB168. Growth studies with the wild-type strain, *gntP* mutant and the complemented strain were carried out in CSK MM containing gluconate as the sole carbon source [Galinier et al., 1997].

Virulence Assays in Mice

The virulence of meningococcal strains was evaluated by their ability to provoke bacteremia in mice after intraperitoneal challenge. The experimental design was approved by the Institut Pasteur Review Board. Bacterial loads in infected 6-week-old female BALB/c mice (Janvier, France) were determined from blood samples taken at 2, 6 and 24 h after intraperitoneal challenge with standardized inocula of 10⁷ CFU per mouse for each tested strain. Bacterial counts were determined by plating serial dilutions of blood on GC medium base plates.

Acknowledgements

This research was supported by a grant from the Agence Nationale de la Recherche (ANR-05-MIIM-031) to J.D. and M.-K.T., and the 'Initiative d'Excellence' program of the French government (grant DYNAMO, ANR-11-LABX-0011). J.N.A. received a PhD fellowship from the INRA and A.M. from the Algerian government. We thank Dario Giorgini for his precious technical assistance, Mireille Larribe and Eliane Milohanic for fruitful discussions, Winfried Boos for sending us *E. coli* strain GSJ100, Knut Jahreis for providing *E. coli* strain LJ140 and plasmid pSU18c, Dominique LeCoq for plasmid pIC634 and Alexa Bourand for purifying *L. casei* D-ribitol-5-P 2-dehydrogenase.

References

- Aké FM, Joyet P, Deutscher J, Milohanic E: Mutational analysis of glucose transport regulation and glucose-mediated virulence gene repression in *Listeria monocytogenes*. *Mol Microbiol* 2011;81:274–293.
- Amrein KE, Takacs B, Stieger M, Molnos J, Flint NA, Burn P: Purification and characterization of recombinant human p50csk protein-tyrosine kinase from an *Escherichia coli* expression system overproducing the bacterial chaperones GroES and GroEL. *Proc Natl Acad Sci USA* 1995;92:1048–1052.
- Antunes A, Golfieri G, Ferlicca F, Giuliani MM, Scarlato V, Delany I: HexR controls glucose-responsive genes and central carbon metabolism in *Neisseria meningitidis*. *J Bacteriol* 2015;198:644–654.
- Baart GJ, Langenhof M, van de Waterbeemd B, Hamstra HJ, Zomer B, van der Pol LA, Beuvery EC, Tramper J, Martens DE: Expression of phosphofructokinase in *Neisseria meningitidis*. *Microbiology* 2010;156:530–542.
- Baart GJ, Zomer B, de Haan A, van der Pol LA, Beuvery EC, Tramper J, Martens DE: Modeling *Neisseria meningitidis* metabolism: from genome to metabolic fluxes. *Genome Biol* 2007;8:R136.
- Bartolomé B, Jubete Y, Martinez E, de la Cruz F: Construction and properties of a family of pACYC184-derived cloning vectors compatible with pBR322 and its derivatives. *Gene* 1991;102:75–78.
- Bausch C, Peekhaus N, Utz C, Blais T, Murray E, Lowary T, Conway T: Sequence analysis of the GntII (subsidiary) system for gluconate metabolism reveals a novel pathway for L-idonic acid catabolism in *Escherichia coli*. *J Bacteriol* 1998;180:3704–3710.
- Beno DW, Devine LF, Larson GL: Identification of *Neisseria meningitidis* carbohydrate fermentation patterns in Mueller-Hinton broth. *J Bacteriol* 1968;96:563.
- Blaudeck N, Kreutzenbeck P, Freudl R, Sprenger GA: Genetic analysis of pathway specificity during posttranslational protein translocation across the *Escherichia coli* plasma membrane. *J Bacteriol* 2003;185:2811–2819.
- Boël G, Mijakovic I, Mazé A, Poncet S, Taha M-K, Larribe M, Darbon E, Khemiri A, Galinier A, Deutscher J: Transcription regulators potentially controlled by HPr kinase/phosphorylase in Gram-negative bacteria. *J Mol Microbiol Biotechnol* 2003;5:206–215.
- Boos W, Shuman H: Maltose/maltodextrin system of *Escherichia coli*: transport, metabolism, and regulation. *Microbiol Mol Biol Rev* 1998;62:204–229.
- Bourand A, Yebra MJ, Boël G, Mazé A, Deutscher J: Utilization of D-ribitol by *Lactobacillus casei* BL23 requires a mannose-type phosphotransferase system and three catabolic enzymes. *J Bacteriol* 2013;195:2652–2661.
- Campsall PA, Laupland KB, Niven DJ: Severe meningococcal infection: a review of epidemiology, diagnosis, and management. *Crit Care Clin* 2013;29:393–409.
- Caugant DA, Maiden MC: Meningococcal carriage and disease – population biology and evolution. *Vaccine* 2009;27S:B64–B70.

- Chandler MS: New shuttle vectors for *Haemophilus influenzae* and *Escherichia coli*: P15A-derived plasmids replicate in *H. influenzae* Rd. Plasmid 1991;25:221–224.
- Daddaoua A, Krell T, Ramos JL: Regulation of glucose metabolism in *Pseudomonas*: the phosphorylative branch and Entner-Doudoroff enzymes are regulated by a repressor containing a sugar isomerase domain. J Biol Chem 2009;284:21360–21368.
- Derkaoui M, Antunes A, Poncet S, Joyet P, Henry C, Nait-Abdallah J, Mazé A, Taha M-K, Deutscher J, Deghmane A-E: Interaction of the *Neisseria meningitidis* phosphocarrier protein HPr with CrgA regulates capsule production, cell adhesion and virulence. Mol Microbiol 2016;100:788–807.
- Deutscher J, Engelmann R: Purification and characterization of an ATP-dependent protein kinase from *Streptococcus faecalis*. FEMS Microbiol Lett 1984;23:157–162.
- Deutscher J, Francke C, Postma PW: How phosphotransferase system-related protein phosphorylation regulates carbohydrate metabolism in bacteria. Microbiol Mol Biol Rev 2006;70:939–1031.
- Echenique-Rivera H, Muzzi A, Del Tordello E, Seib KL, Francois P, Rappuoli R, Pizza M, Seruto D: Transcriptome analysis of *Neisseria meningitidis* in human whole blood and mutagenesis studies identify virulence factors involved in blood survival. PLoS Pathog 2011;7:e1002027.
- Exley RM, Goodwin L, Mowe E, Shaw J, Smith H, Read RC, Tang CM: *Neisseria meningitidis* lactate permease is required for nasopharyngeal colonization. Infect Immun 2005a;73:5762–5766.
- Exley RM, Shaw J, Mowe E, Sun YH, West P, Williamson M, Botto M, Smith H, Tang CM: Available carbon source influences the resistance of *Neisseria meningitidis* against complement. J Exp Med 2005b;201:1637–1645.
- Fiegler H, Bassias J, Jankovic I, Brückner R: Identification of a gene in *Staphylococcus xylosum* encoding a novel glucose uptake protein. J Bacteriol 1999;181:4929–4936.
- Fitting C, Doudoroff M: Phosphorolysis of maltose by enzyme preparations from *Neisseria meningitidis*. J Biol Chem 1952;199:153–163.
- Galinier A, Haiech J, Kilhoffer M-C, Jaquinod M, Stülke J, Deutscher J, Martin-Verstraete I: The *Bacillus subtilis* *crh* gene encodes a HPr-like protein involved in carbon catabolite repression. Proc Natl Acad Sci USA 1997;94:8439–8444.
- Guérout-Fleury AM, Frandsen N, Stragier P: Plasmids for ectopic integration in *Bacillus subtilis*. Gene 1996;180:57–61.
- Holten E: 6-Phosphogluconate dehydrogenase and enzymes of the Entner-Doudoroff pathway in *Neisseria*. Acta Pathol Microbiol Scand B Microbiol Immunol 1974a;82:207–213.
- Holten E: Glucokinase and glucose 6-phosphate dehydrogenase in *Neisseria*. Acta Pathol Microbiol Scand B Microbiol Immunol 1974b;82:201–206.
- Iyer R, Baliga NS, Camilli A: Catabolite control protein A (CcpA) contributes to virulence and regulation of sugar metabolism in *Streptococcus pneumoniae*. J Bacteriol 2005;187:8340–8349.
- Joyet P, Derkaoui M, Poncet S, Deutscher J: Control of *Bacillus subtilis* *mtl* operon expression by complex phosphorylation-dependent regulation of the transcriptional activator MtlR. Mol Microbiol 2010;76:1279–1294.
- Kellogg DS, Peacock WL, Deacon WE, Brown L, Pirkle CI: *Neisseria gonorrhoeae*. I. Virulence genetically linked to clonal variation. J Bacteriol 1963;85:1274–1279.
- Leighton MP, Kelly DJ, Williamson MP, Shaw JG: An NMR and enzyme study of the carbon metabolism of *Neisseria meningitidis*. Microbiology 2001;147:1473–1482.
- Lévy S, Zeng GQ, Danchin A: Cyclic AMP synthesis in *Escherichia coli* strains bearing known deletions in the *pts* phosphotransferase operon. Gene 1990;86:27–33.
- Leyn SA, Li X, Zheng Q, Novichkov PS, Reed S, Romine MF, Fredrickson JK, Yang C, Osterman AL, Rodionov DA: Control of proteobacterial central carbon metabolism by the HexR transcriptional regulator: a case study in *Shewanella oneidensis*. J Biol Chem 2011;286:35782–35794.
- Lohmiller S, Hantke K, Patzer SI, Braun V: TonB-dependent maltose transport by *Caulobacter crescentus*. Microbiology 2008;154:1748–1754.
- Mallavia LP, Weiss E: Catabolic activities of *Neisseria meningitidis*: utilization of glutamate. J Bacteriol 1970;101:127–132.
- Mehra-Chaudhary R, Mick J, Tanner JJ, Henzl MT, Beamer LJ: Crystal structure of a bacterial phosphoglucomutase, an enzyme involved in the virulence of multiple human pathogens. Proteins 2011;79:1215–1229.
- Mesak LR, Dahl MK: Purification and enzymatic characterization of PgcM: a β -phosphoglucomutase and glucose-1-phosphate phosphodismutase of *Bacillus subtilis*. Arch Microbiol 2000;174:256–264.
- Mokhtari A, Blancato VS, Repizo GD, Henry C, Pikis A, Bourand A, de Fatima Álvarez M, Immel S, Mechakra-Maza A, Hartke A, Thompson J, Magni C, Deutscher J: *Enterococcus faecalis* utilizes maltose by connecting two incompatible metabolic routes via a novel maltose 6'-phosphate phosphatase (MapP). Mol Microbiol 2013;88:234–253.
- Monedero V, Yebra MJ, Poncet S, Deutscher J: Maltose transport in *Lactobacillus casei* and its regulation by inducer exclusion. Res Microbiol 2008;159:94–102.
- Nassif X, Puaoi D, So M: Transposition of Tn1545- Δ 3 in the pathogenic *Neisseriae*: a genetic tool for mutagenesis. J Bacteriol 1991;173:2147–2154.
- Neves AR, Pool WA, Castro R, Mingote A, Santos F, Kok J, Kuipers OP, Santos H: The α -phosphoglucomutase of *Lactococcus lactis* is unrelated to the α -D-phosphohexomutase superfamily and is encoded by the essential gene *pgmH*. J Biol Chem 2006;281:36864–36873.
- Paulsen IT, Chauvaux S, Choi P, Saier MH Jr: Characterization of glucose-specific catabolite repression-resistant mutants of *Bacillus subtilis*: identification of a novel hexose:H⁺ symporter. J Bacteriol 1998;180:498–504.
- Pimentel-Schmitt EF, Jahreis K, Eddy MP, Amon J, Burkovski A, Titgemeyer F: Identification of a glucose permease from *Mycobacterium smegmatis* mc² 155. J Mol Microbiol Biotechnol 2009;16:169–175.
- Poncet S, Milohanic E, Mazé A, Nait Abdallah J, Aké F, Larribe M, Deghmane A-E, Taha M-K, Dozot M, De Bolle X, Letesson JJ, Deutscher J: Correlations between carbon metabolism and virulence in bacteria. Contrib Microbiol 2009;16:88–102.
- Sáez-Nieto JA, Fenoll A, Vazquez J, Casal J: Prevalence of maltose-negative *Neisseria meningitidis* variants during an epidemic period in Spain. J Clin Microbiol 1982;15:78–81.
- Schoen C, Blom J, Claus H, Schramm-Glück A, Brandt P, Müller T, Goesmann A, Joseph B, Konietzny S, Kurzai O, Schmitt C, Friedrich T, Linke B, Vogel U, Frosch M: Whole-genome comparison of disease and carriage strains provides insights into virulence evolution in *Neisseria meningitidis*. Proc Natl Acad Sci USA 2008;105:3473–3478.
- Schoen C, Kischkies L, Elias J, Ampattu BJ: Metabolism and virulence in *Neisseria meningitidis*. Front Cell Infect Microbiol 2014;4:114.
- Schönert S, Seitz S, Krafft H, Feuerbaum EA, Andernach I, Witz G, Dahl MK: Maltose and maltodextrin utilization by *Bacillus subtilis*. J Bacteriol 2006;188:3911–3922.
- Sun Y, Bakshi S, Chalmers R, Tang C: Functional genomics of *Neisseria meningitidis* pathogenesis. Nat Med 2000;6:1269–1273.
- Taha M-K, Deghmane A-E, Antignac A, Zarantonelli ML, Larribe M, Alonso JM: The duality of virulence and transmissibility in *Neisseria meningitidis*. Trends Microbiol 2002;10:376–382.
- Wagner E, Marcandier S, Egeter O, Deutscher J, Götz F, Brückner R: Glucose kinase-dependent catabolite repression in *Staphylococcus xylosum*. J Bacteriol 1995;177:6144–6152.
- Waygood EB, Sanwal BD: The control of pyruvate kinases of *Escherichia coli*. I. Physicochemical and regulatory properties of the enzyme activated by fructose 1,6-diphosphate. J Biol Chem 1974;249:265–274.
- Weiss E: Catabolic activities of *Neisseria meningitidis*: utilization of succinate. J Bacteriol 1970;101:133–137.
- Yazdankhah SP, Caugant DA: *Neisseria meningitidis*: an overview of the carriage state. J Med Microbiol 2004;53:821–832.
- Zarantonelli ML, Lancellotti M, Deghmane A-E, Giorgini D, Hong E, Ruckly C, Alonso JM, Taha M-K: Hyperinvasive genotypes of *Neisseria meningitidis* in France. Clin Microbiol Infect 2008;14:467–472.