International Journal of Medical Microbiology 301 (2011) 133–139

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.de/ijmm

GamA is a eukaryotic-like glucoamylase responsible for glycogen- and starch-degrading activity of *Legionella pneumophila*

Vroni Herrmann[a], Anja Eidner[a], Kerstin Rydzewski[a], Inga Blädel[b,1], Matthieu Jules[c], Carmen Buchrieser[c], Wolfgang Eisenreich[d], Klaus Heuner[a,b,*]

[a] *Robert Koch-Institut, Research Group P26, Nosocomial Infections of the Elderly, Nordufer 20, 13353 Berlin, Germany*
[b] *Institut für Molekulare Infektionsbiologie, Universität Würzburg, Röntgenring 11, 97070 Würzburg, Germany*
[c] *Institut Pasteur, Biologie des Bactéries Intracellulaires and CNRS URA 2171, 28 Rue du Dr Roux, 75724 Paris, France*
[d] *Lehrstuhl für Biochemie, Technische Universität München, Lichtenbergstr. 4, 85747 Garching, Germany*

ARTICLE INFO

Article history:
Received 26 May 2010
Received in revised form 17 August 2010
Accepted 21 August 2010

Keywords:
Legionella
Glucoamylase
Glycogen
Starch
Metabolism
Glucose
A. castellanii

ABSTRACT

Legionella pneumophila (*Lp*) is the causative agent of Legionnaires' disease, an atypical pneumonia. *Lp* is found in freshwater habitats and replicates within different protozoa (amoebae). It is known that *Lp* uses amino acids as primary energy and carbon sources for replication. However, very recently it was reported that *Lp* is able to metabolize also carbohydrates (glucose). Here, we present for the first time experimental evidence that the *lpp0489* [*gamA*] gene encodes a eukaryotic-like glucoamylase (GamA) responsible for the glycogen- and starch-degrading activities of *Lp*. Although not essential for intra- and extracellular growth, we showed that GamA is expressed and active during intracellular replication in *Acanthamoeba castellanii*, suggesting that *Lp* is degrading glycogen during intracellular replication. Altogether, these findings indicate that *Lp* is indeed able to degrade exogenous polysaccharides and to utilize carbohydrates (glucose).

Introduction

Legionella pneumophila (*Lp*) is the causative agent of Legionnaires' disease, an atypical pneumonia. *Lp* is found in freshwater habitats where it replicates within different protozoa (amoebae). *Lp* is transmitted to humans by *Lp*-contaminated aerosols. After entering the human lung, *Lp* is phagocytosed by alveaolar macrophages wherein *Lp* is able to replicate leading to Legionnaires' disease. Many virulence factors of *Lp* are already known, however, less is known about nutrition of *Lp* within the environment or during intracellular replication (Hoffman, 2008; Rowbotham, 1986; Swanson and Hammer, 2000).

Legionella species seem to have an obligate requirement for a host cell (in vivo) for replication in the environment, but nearly nothing is known about the life of legionellae present in biofilms. *Lp* survives within amoebae and macrophages due to its ability to establish a replication vacuole derived from the endoplasmic reticulum. At the end of the intracellular growth phase, *Lp* differentiates into a spore-like form (MIF), also called the transmissive form, which seems to be metabolically nearly dormant (Faulkner and Garduno, 2002; Garduno et al., 2002; Greub and Raoult, 2003; Hammer et al., 2002; Molofsky and Swanson, 2004; Swanson and Hammer, 2000). These transmissive forms of *Lp* may be able to persist in the environment for long periods.

It is known that *Lp* uses amino acids as primary energy and carbon sources (Pine et al., 1979; Reeves et al., 1981; Ristroph et al., 1981; Sauer et al., 2005; Tesh et al., 1983; Wieland et al., 2005). In line with this is the observation that amino acid transporters of the host cell and of *Lp* are essential for intracellular replication (Sauer et al., 2005; Wieland et al., 2005) and that the resective genes are highly upregulated (Brüggemann et al., 2006). However, recently, it was reported, that *Lp* secretes an endoglucanase (CelA), which is able to degrade carboxymethyl cellulose (Debroy et al., 2006). In addition, some studies have indicated that *Lp* is able to utilize carbohydrates (Tesh et al., 1983; Weiss et al., 1980) and only recently, it was demonstrated that the Entner–Doudoroff (ED) pathway is necessary for intracellular growth of *L. peumophila* (Eylert et al., 2010; Harada et al., 2010). Moreover, the available 4 genome sequences of *Lp* [*Lp* Philadelphia (Chien et al., 2004), *Lp* Paris, *Lp* Lens (Cazalet et al., 2004), and *Lp* Corby (Glöckner et al., 2008)] show that the *Lp* genome contains genes that code for all proteins of the Embden–Meyerhof–Parnas (EMP) pathway, the

* Corresponding author at: P 26, Nosocomial Infections of the Elderly, Robert Koch-Institute, Nordufer 20, 13353 Berlin, Germany. Tel.: +49 0 30 18754 2226; fax: +49 0 30 18754 2328.
E-mail address: heunerk@rki.de (K. Heuner).
[1] Present address: Universitätsklinikum Münster, ZMBE Institut für Infektiologie, Von-Esmarch-Str. 56, 48149 Münster, Germany.

1438-4221/$ – see front matter © 2010 Elsevier GmbH. All rights reserved.
doi:10.1016/j.ijmm.2010.08.016

Table 1
Primers and plasmids used in this study.

Name	Sequence or characteristics	Reference
Oligonucleotides		
PCR		
lpp0489_for	AAGAGATTTTCTTACGCAGT	This study
lpp0489_rev	AATTTTTCCTCTTCATGTCC	This study
lpp0489_inv_BamHI_for	CGCGGATCCGCGTTGTTTGGTCGTTATCCTGG	This study
lpp0489_inv_BamHI_rev	CGCGGATCCGCGGTTTTTTATATCGAGCAGATTGGC	This study
Kan_BamHI_for	CGGGATCCCGCTATCTGGACAAGGGAAAAC	This study
Kan_BamHI_rev	CGGGATCCCGGAAGAACTCCAGCATGAGAT	This study
lpp0488-gam-fwd	AATCATGTTGCCTGGCTATCTTATT	This study
Gam-rev	ACTCATTTCCTGTACTATGGCGA	This study
trp-rev	CTTACGCAGTCTCAATCAGTTTGTA	This study
RT-PCR		This study
RTgam-trp-fwd	GCTTCATACCATGTCTCAACCAATC	This study
RTgam-trp-rev	AGCTGAAGCTATAGGCATTACTGAAGC	This study
RT0490-0491F	GTCTTCAGTGGATTGATACTCAAGAAG	This study
RT0490-0491R	GTCAACGTATCGCAAGCATCAC	This study
RT0491-0492F	GTTGATAACAGGGAGTGCCATCC	This study
RT0491-0492R	CATTGTGGTCTCCTGCCCATC	This study
RT0492-0493F	CGGGATAGTTGGTAAGTCTATTACTC	This study
RT0492-0493R	GACAGGCGGTCACCTATGATC	This study
cspD-F	GCTATTTCTTCTTGTTCGGCG	This study
cspD-R	GCTAGAGGCGAAGTCAAGTG	This study
Plasmids		
pBCKS+	Commercial vector for subcloning	Promega
pIB_gam_1	1884-bp PCR fragment (primers lpp0488-gam-fwd and gam-rev; complete *gamA* gene) in pGMTeasy	This study
pIB1	*Eco*RI fragment of pIB_gam_1 cloned into pBCKS	This study
pIB_gam_2	2282-bp PCR fragment (primers lpp0488-gam-fwd and trp-rev; complete *gamA* and *yozG* genes) in pGMTeasy	This study
pIB2	*Eco*RI fragment of pIB_gam_2 cloned into pBCKS	This study

complete ED, and the pentose phosphate (PP) pathway (Fonseca et al., 2008; Hoffman, 2008). There are also various ABC-type transport systems, and some of them seem to be involved in sugar uptake. Very recently, we demonstrated by isotopoloque profiling studies that *Lp* strain Paris uses glucose as a carbon source (Eylert et al., 2010). Specifically, ^{13}C-label from [U-$^{13}C_6$]glucose was found in various amino acids and in the energy and carbon storage compound poly-3-hydroxybutyrate (PHB) (Eylert et al., 2010). This leaves the question about the source for glucose in the natural environments of *Lp*.

In the 1980s, it was shown that *Legionella* exhibits a weak starch hydrolysis activity (Hébert et al., 1980; Morris et al., 1980; Thorpe and Miller, 1981). Indeed, analysis of the genome sequences showed that *Lp* possesses putative systems for degradation of trehalose, cellulose and chitin as well as a eukaryotic-like glucoamylase (*lpp0489* [*gamA*]) putatively involved in the degradation of starch or glycogen (Brüggemann et al., 2006). It was also demonstrated by microarray analysis that *gamA* and other genes required for carbohydrate metabolism are induced during the intracellular replication in *A. castellanii* (Brüggemann et al., 2006). In this study, we thus analyzed the function of GamA further.

Materials and methods

Strains, mutant construction, plasmids, and oligonucleotides

Escherichia coli DH5α was used for cloning of recombinant plasmid DNA. Experiments were done with *Lp* Paris [CIP 107629 (Cazalet et al., 2004)], *Lp* Corby (Jepras et al., 1985), and the Δ*lspDE* mutant strain of *Lp* Corby (Schunder et al., 2010). The *lpp0489* (Δ*gamA*) single mutant strain of *Lp* Paris was constructed as described previously (Brüggemann et al., 2006; Heuner et al., 2002). In brief, the gene *lpp0489* was inactivated by insertion/deletion of a kanamycin resistance (kan^R) cassette into the chromosomal gene. The chromosomal region containing the *lpp0489* gene was PCR-amplified with the primers lpp0489_for and lpp0489_rev, and the product (2188 bp) was cloned into the pGEM-T easy vector (Promega). On this template, an inverse PCR was performed using the primers lpp0489_inv_BamHI_for and lpp0489_inv_BamHI_rev and the resulting PCR product (4531 bp), corresponding to the pGEM backbone and the flanking regions of *lpp0489*, was BamHI-digested, and ligated to the kan^R cassette (1210 bp) amplified via PCR from the plasmid pGEM-Kan^R (kanamycin resistance cassette subcloned into a pGEM-T easy vector), using primers containing *Bam*HI restriction sites at the ends (Kan_BamHI_for and Kan_BamHI_for). Primer sequences are given in Table 1. For chromosomal recombination, the construct was introduced into *Lp* Paris strain by transformation. For complementation of the Δ*gamA* mutant strain, the primers lpp0488-gam-fwd and gam-rev (Table 1) were used to amplify a product of 1884 bp containing the *gamA* gene. The fragment was ligated into pGEMTeasy, digested with *EcoRI*, and subcloned in pBCKS resulting in pIB1. An alternative complementation vector was constructed using the primers lpp0488-gam-fwd and trp-rev containing the *gamA* gene and the upstream *yozG* gene. The fragment (2282 bp) was ligated into pGEMTeasy, digested with *EcoRI* and subcloned in pBCKS resulting in pIB2. Plasmids were introduced into the Δ*gamA* mutant strain by electroporation (2.3 kV, 100 Ω, and 25 mF; Bio-Rad gene pulser). Plasmids and oligonucleotides used in this study are listed in Table 1.

Infection, growth conditions, and media

Lp strains were cultured in ACES-buffered yeast extract (AYE) broth [10 g N-(2-Acetamido)-2-amino-ethanesulfonamid Acid (ACES), 10 g yeast extract, 0.4 g L-cysteine, 0.25 g Fe-pyrophosphate, ad 1000 ml dH_2O, then adjusted to pH 6,8 with 3 M KOH and sterile filtrated] or on ACES-buffered charcoal-yeast (BCYE) extract agar at 37 °C. *A. castellanii* ATCC30010 was cultured in PYG 712 medium [2% proteose peptone, 0.1% yeast extract, 0.1 M glucose, 4 mM $MgSO_4$, 0.4 M $CaCl_2$, 0.1% sodium citrate dihydrate, 0.05 mM $Fe(NH_4)_2(SO_4)_2 \times 6H_2O$, 2.5 mM NaH_2PO_3, 2.5 mM K_2HPO_3] at 20 °C.