



J. Dairy Sci. 101:1–5
<https://doi.org/10.3168/jds.2017-13517>
 © American Dairy Science Association®, 2018.

Short communication: Roles of outer membrane protein W (OmpW) on survival and biofilm formation of *Cronobacter sakazakii* under neomycin sulfate stress

Yingwang Ye,*†^{1,2} Na Ling,†¹ Jina Gao,*¹ Maofeng Zhang,* Xiyang Zhang,* Liaowang Tong,* Dexin Ou,* Yaping Wang,* Jumei Zhang,† and Qingping Wu†²

*School of Food Science and Engineering, Hefei University of Technology, Hefei 230009, China

†State Key Laboratory of Applied Microbiology Southern China, Guangdong Provincial Key Laboratory of Microbiology Culture Collection and Application, Guangdong Institute of Microbiology, Guangzhou 510070, China

ABSTRACT

Cronobacter sakazakii is associated with severe infections including sepsis, neonatal meningitis, and necrotizing enterocolitis. Antibiotic resistance in *Cronobacter* species has been documented in recent years, but the genes involved in resistance in *Cronobacter* strains are poorly understood. In this study, we determined the role of outer membrane protein W (OmpW) on survival rates, morphologic changes, and biofilm formation between wild type (WT) and an OmpW mutant strain (Δ OmpW) under neomycin sulfate stress. Results indicated that the survival rates of Δ OmpW were significantly reduced after half minimum inhibitory concentration ($\frac{1}{2}$ MIC) treatment compared with the WT strain. Filamentation of *C. sakazakii* cells was observed after $\frac{1}{2}$ MIC treatment in WT and Δ OmpW, and morphologic injury, including cell disruption and leakage of cells, was more predominant in Δ OmpW. Under $\frac{1}{2}$ MIC stress, the biofilms of WT and Δ OmpW were significantly decreased, but decreasing rates of biofilm formation in mutant strain were more predominant compared with WT strain. This is the first report to determine the role of OmpW on survival, morphological changes, and biofilm formation in *C. sakazakii* under neomycin sulfate stress. The findings indicated that OmpW contributed to survival and reduction of morphological injury under neomycin sulfate stress. In addition, enhancing biofilm formation in Δ OmpW may be an alternative advantage for adaptation to neomycin sulfate stress.

Key words: *Cronobacter sakazakii*, outer membrane protein W (OmpW), neomycin sulfate, biofilm

Short Communication

Cronobacter sakazakii is considered an opportunistic foodborne pathogen associated with severe but rare infections with high mortality (Gurtler et al., 2005). Epidemiologic surveys suggested a positive correlation between powdered infant formula and *Cronobacter* infections (Muytjens et al., 1983, 1988; Biering et al., 1989; Clark et al., 1990; van Acker et al., 2001). Unfortunately, the true source of *Cronobacter* in powdered infant formula was not clearly understood. Consequently, *Cronobacter* posed a high risk to public health, especially for newborns.

In recent years, tolerance or resistance of foodborne pathogens to antibiotics is of great concern for human health. Although *Cronobacter* has been reported as being more sensitive than other *Enterobacter* species to some antibiotics, including aminoglycosides, ureidopenicillins, ampicillin, and carboxypenicillins (Gurtler et al., 2005), some strains are less sensitive to penicillin and cephalothin (Farmer et al., 1980). Outer membrane proteins (Omp) such as OmpW might be involved in tolerance to antibiotics in foodborne pathogens (Hu et al., 2005; Xu et al., 2006; Gil et al., 2007; Zhang et al., 2008; Beketskaia et al., 2014). OmpW is distributed in gram-negative bacteria, and its 3-dimensional structure consists of an 8-stranded β -barrel with a long and narrow hydrophobic channel (Hong et al., 2006). Neomycin sulfate, as an aminoglycoside antibiotic, can transfer through hydrophilic ion channels on the surface of bacteria to interfere with synthesis of proteins and further acquire antibacterial activity. However, the role of OmpW under neomycin sulfate stress in *Cronobacter* species is poorly understood.

In this study, we determined survival rates and morphologic changes of *C. sakazakii* cells under neomycin sulfate stress between the wild type (WT) and OmpW mutant (Δ OmpW) strains. We also assessed biofilm formation under neomycin sulfate stress using crystal

Received July 18, 2017.

Accepted September 4, 2017.

¹These authors contributed equally to the manuscript.

²Corresponding authors: yeyw04@mails.gucas.ac.cn and wuqp203@163.com

violet staining (CVS), scanning electron microscopy, and confocal laser scanning microscopy (CLSM).

Cronobacter sakazakii WT and $\Delta OmpW$ from Guangdong Microbiology Culture Center (GDMCC; Guangzhou, China) were incubated in unsupplemented neomycin sulfate Luria Bertani (LB) broth at 37°C for overnight. Then, 1% of the overnight culture was transferred into fresh LB containing different concentrations of neomycin sulfate for 16 h at 37°C. The MIC of neomycin sulfate were 8 $\mu\text{g}/\text{mL}$ and 7 $\mu\text{g}/\text{mL}$ for WT and $\Delta OmpW$. The 2 strains (10^6 cfu/mL) were transferred into normal LB and LB with 50% MIC for each for 6 h at 37°C. Survival rates were counted according to the following formula: Survival rate = number of cells in LB with $\frac{1}{2}$ MIC/No. of cells in normal LB.

Each experiment was performed in triplicate, and significance was determined using Origin pro 8.5.1 (OriginLab, Northampton, MA). The survival rate of the WT strain was higher ($P < 0.01$) than that of $\Delta OmpW$, as shown in Figure 1A. In *Escherichia coli*, the relatively high expression of OmpW was consistent with strong tolerance to tetracycline and ampicillin (Xu et al., 2006), indicating that OmpW functions as a porin to export antibiotics from the cell. In another study, survival capability significantly decreased in an OmpW mutant compared with a wild strain under tetracycline stress (Zhang et al., 2008). In contrast, a ceftriaxone-resistant *Salmonella* showed highly reduced expression of OmpW, suggesting reduction of ceftriaxone permeability (Hu et al., 2005). A dramatic decrease of OmpW synthesis in uropathogenic *E. coli* was observed under enrofloxacin stress (Piras et al., 2015). These studies indicate that the role of OmpW in antibiotic resistance depends on pathogen species and type of antibiotic.

We then assessed the morphological changes of *C. sakazakii* WT and $\Delta OmpW$ under neomycin sulfate stress. The procedure was described in detail by Wang et al. (2013), using scanning electron microscopy (Hitachi, Tokyo, Japan). In addition, we detected filamentation of *C. sakazakii* under neomycin sulfate treatment, and injury of mutant cells was more prevalent than that of the WT, as shown in Figure 1B.

Biofilm formation of 2 strains in LB broth and LB with $\frac{1}{2}$ MIC neomycin sulfate was determined using CVS described by Ye et al. (2015). For CVS detection, *C. sakazakii* WT and $\Delta OmpW$ strains were inoculated into 5 mL of LB and grown for 12 to 14 h at 37°C with constant shaking. Fifty microliters of culture (optical density at 600 nm = 0.5) was inoculated into 96-well polystyrene plates containing 250 μL of sterile LB broth with neomycin sulfate or without neomycin sulfate, and then incubated at 37°C for 24, 48, and 72 h. The plates were rinsed 3 times with deionized water, and the ad-

herent bacteria cells were stained with 1% crystal violet for 30 min. After being rinsed 3 times with deionized water, the crystal violet was liberated by 30% acetic acid following a 10-min incubation. The optical density values of each well were measured at 590 nm.

Furthermore, biofilm formation by *C. sakazakii* WT and $\Delta OmpW$ strains were determined using scanning electron microscopy. *Cronobacter sakazakii* were inoculated in LB broth at 37°C overnight and 0.05 mL of the culture transferred to 24-well plates (Baiyan, Shanghai, China) containing 5 mL of fresh LB with $\frac{1}{2}$ MIC neomycin sulfate. To test biofilm formation on glass, glass coverslips (Jingan, Shanghai, China) were immersed in LB broth and then inoculated with *C. sakazakii*. The coverslips were incubated in 24-well plates at 37°C for 24, 48, and 72 h, after which time bacterial biofilm formation was observed. The coverslips at different incubation times were rinsed in PBS

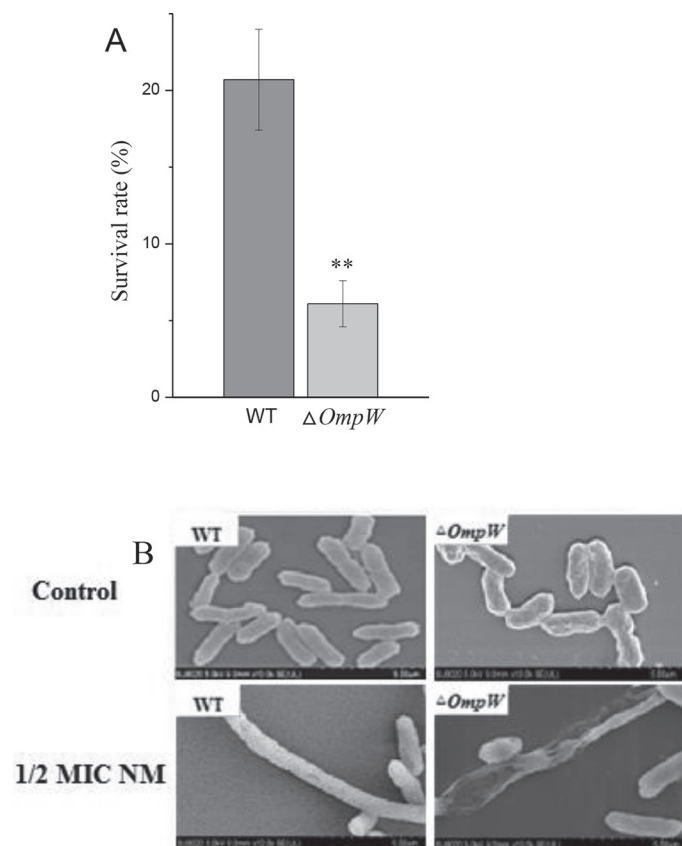


Figure 1. Survival rates (A) and morphological changes, including filamentation and disruption of cells under neomycin sulfate (B), of *Cronobacter sakazakii* wild type (WT) and an outer membrane protein mutant ($\Delta OmpW$) under neomycin sulfate (NM) stress (NM at $\frac{1}{2}$ MIC; the MIC were 8 and 7 $\mu\text{g}/\text{mL}$ for WT and $\Delta OmpW$, respectively). Survival rates = means \pm SD. ** $P < 0.01$ between WT and $\Delta OmpW$.

Download English Version:

<https://daneshyari.com/en/article/8501291>

Download Persian Version:

<https://daneshyari.com/article/8501291>

[Daneshyari.com](https://daneshyari.com)