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Role of cell surface composition and lysis in static biofilm formation by *Lactobacillus plantarum* WCFS1

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ABSTRACT

Next to applications in fermentations, *Lactobacillus plantarum* is recognized as a food spoilage organism, and its dispersal from biofilms in food processing environments might be implicated in contamination or re-contamination of food products. This study provides new insights into biofilm development by *L. plantarum* WCFS1 through comparative analysis of wild type and mutants affected in cell surface composition, including mutants deficient in the production of Sortase A involved in the covalent attachment of 27 predicted surface proteins to the cell wall peptidoglycan (Δ*srtA*) and mutants deficient in the production of capsular polysaccharides (CPS1–4, Δ*cps1–4*). Surface adhesion and biofilm formation studies revealed none of the imposed cell surface modifications to affect the initial attachment of cells to polystyrene while biofilm formation based on Crystal Violet (CV) staining was severely reduced in the Δ*srtA* mutant and significantly increased in mutants lacking the *cps1* cluster, compared to the wild-type strain. Fluorescence microscopy analysis of biofilm samples pointed to a higher presence of extracellular DNA (eDNA) in *cps1* mutants and this corresponded with increased autolysis activity. Subsequent studies using Δ*acm2* and Δ*lytA* derivatives affected in lytic behaviour revealed reduced biofilm formation measured by CV staining, confirming the relevance of lysis for the build-up of the biofilm matrix with eDNA.

1. Introduction

Lactobacilli are Gram positive, generally non-motile bacteria which can be found in a diverse range of habitats. They are widely used in the food industry as probiotics (Boesten and de Vos 2008; Turroni et al. 2014) and starter cultures for the production of fermented food products (Caplice and Fitzgerald 1999; Leroy and De Vuyst 2004). However, besides their desired properties, they are also associated with food spoilage (Bartowsky and Henschke 2008; Bjorkroth and Korkeala 1996; Lyhs et al. 2001; Samelis et al. 2000). One contamination route with lactic acid bacteria is via the presence of biofilms in the food production environment (Somers et al. 2001). Biofilms are defined as microorganisms attached to a surface embedded in a matrix of extracellular polymeric substances (O'Toole et al. 2000). *L. plantarum* has been shown to form submerged biofilms, both as single species but also in multispecies biofilms (Fernández Ramírez et al. 2015; Kubota et al. 2008; Kubota et al. 2009; Metselaar et al. 2015; van der Veen and Abee 2011). Several stages in biofilm development can be recognized including surface adhesion, microcolony formation, biofilm growth, matrix formation, and biofilm dispersion as the final stage (Abee et al. 2011; Watnick and Kolter 2000).

Surface components of the cell envelope of bacteria have been shown important for interaction with the environment (Kleerebezem et al. 2010; O'Toole et al. 2000; Pratt and Kolter 1998). The main constituents of the Gram positive cell envelope are peptidoglycan, teichoic acids, proteins and polysaccharides (Kleerebezem et al. 2010; Silhavy et al. 2010). Especially, polysaccharides were found to play a role in biofilm formation contributing to the formation of the biofilm matrix (Branda et al. 2005; Stewart and Franklin 2008). Four different gene clusters encoding capsular polysaccharide biosynthesis are located on the *L. plantarum* WCFS1 genome and the role of these cell surface polysaccharides in probiotic functionality was studied in deletion mutants lacking either individual or multiple *cps* gene clusters (Remus et al. 2012). In addition, some of the cell surface proteins present in *L.*

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Table 1
Strains used in the present study.

Strain	Description	Notation	Reference
WCFS1	*L. plantarum* NCIMB8826		(Kleerebezem et al. 2003; Siezen et al. 2012)
NZ3548Cm	Cm^R; WCFS1 derivative; chromosomal replacement of the Δ*cps1A-I* gene cluster	Δ*cps1*	(Remus et al. 2012)
NZ3533ACm	Cm^R; WCFS1 derivative; chromosomal replacement of the Δ*cps2A-J* gene cluster	Δ*cps2*	
NZ3549Cm	Cm^R; WCFS1 derivative; chromosomal replacement of the Δ*cps3A-J* gene cluster	Δ*cps3*	
NZ3534Cm	Cm^R; WCFS1 derivative; chromosomal replacement of the Δ*cps4A-J* gene cluster	Δ*cps4*	(Andre et al. 2011)
NZ3550Cm	Cm^R; WCFS1 derivative; chromosomal replacement of the Δ*cps1A-3J* cluster	Δ*cps1–3*	
NZ3680Cm	Cm^R; WCFS1 derivative; chromosomal replacement of the Δ*cps1A-3J*, Δ*cps4A-J* cluster	Δ*cps1–4*	
NZ3513Cm	Δ*srtA*	Δ*srtA*	(Remus et al. 2013)
NZ3557	Cm^R; WCFS1 derivative; *acm2::cat*	Δ*acm2*	(Fredriksen et al. 2012)
TR0011	NZ7100 derivative; *lp_3093* (*lys2*)::lox72	Δ*lys2*	(Rolain et al. 2012)
TR006	NZ7100 derivative; lp_*3421* (*lytA*)::lox66-P32-cat-lox71	Δ*lytA*	

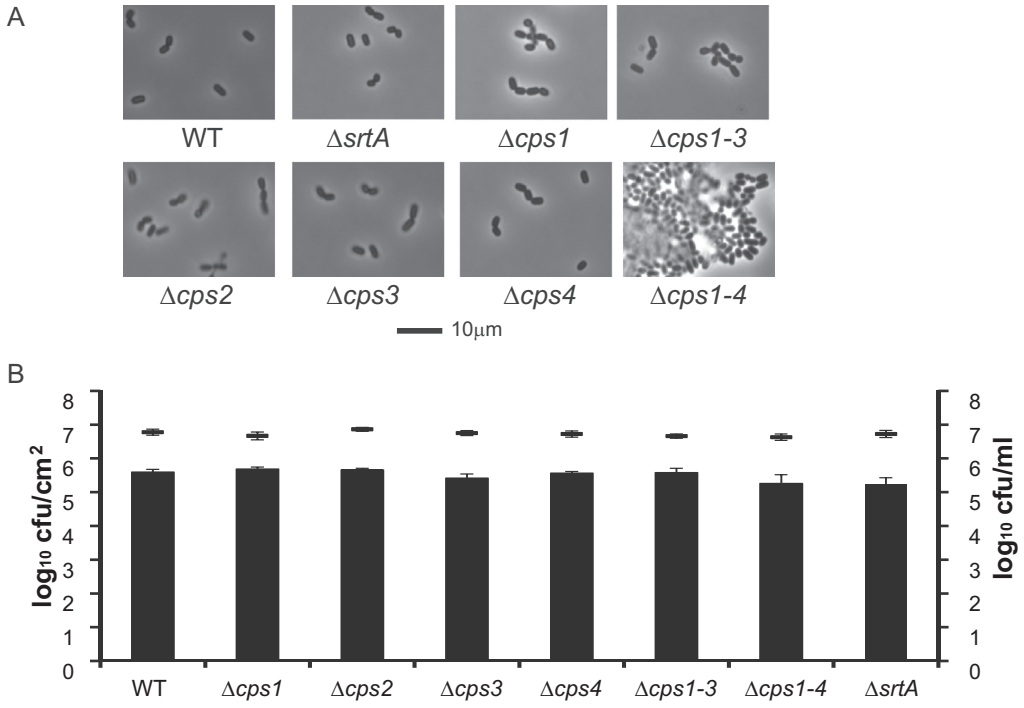

Fig. 1. Morphology and initial attachment of *Lactobacillus plantarum* WCFS1 and its mutants affected in cell surface properties (A) Morphology of *L. plantarum* WCFS1 (WT) and mutants affected in cell surface properties grown in BHIMnG at 30 °C for 18 h. (B) The number of cells attached to polystyrene after 1 h incubation at 30 °C in BHIMnG was determined and represented by the columns with the lines on top of each columns marking the initial inoculum (log_{10} cfu/ml). The error bars display the standard deviation of the experiment. The experiment was performed in triplicate and with three biological replicates. No significant difference from the WT was found (ANOVA, $P < 0.001$).

plantarum, such as mannose-specific adhesins, have been reported to play a role in attachment to biotic surfaces including host epithelial cells (Pretzer et al. 2005). Notably, the major sortase SrtA mediates covalent binding of 27 predicted cell-surface proteins encoded in the *L. plantarum* WCFS1 genome, including mucus-binding proteins, a hydrolase, as well as mannose-specific and collagen-binding adhesins (Boekhorst et al. 2005; Kleerebezem et al. 2010). Among these, the function of three sortase dependent proteins (SDPs) in microbe-host interactions has been experimentally validated (Du et al. 2015; Pretzer et al. 2005; Sturme et al. 2005). The deletion of sortase A in *Enterococcus faecalis* resulted in a lower initial attachment and to defective biofilm formation under static and dynamic conditions, showing its relevance in initial attachment and biofilm formation (Guiton et al. 2009). Additionally, Malik et al. (2013) reported that the highly autoaggregative, adhesive and biofilm forming phenotype of the vaginal *Lactobacillus plantarum* Strain CMPG5300 is sortase-dependent. Although the exact matrix composition of *L. plantarum* biofilms remains to be determined, enzyme treatments have shown proteins and eDNA to be part of the biofilm matrix (Fernández Ramírez et al. 2015). DNA can be released into the biofilm matrix either by active secretion or cell lysis (Jakubovics et al. 2013). The latter has been reported for different species including enterococci (Guiton et al. 2009; Thomas et al. 2008) and staphylococci (Qin et al. 2007; Rice et al. 2007) conceivably caused by autolysins that play a role in cell wall degradation (Bayles 2007; Frese et al. 2013).

The current study focuses on the role of capsular polysaccharides and cell-wall associated proteins in biofilm formation of *L. plantarum* WCFS1. Comparative analysis of *L. plantarum* WCFS1 (wild type) and selected mutants affected in cell surface composition (Δ*srtA*, Δ*cps1–4*) and cell lysis activity (Δ*acm2*, Δ*lytA*) provided evidence that cell wall autolysis and release of DNA are major determinants of *L. plantarum* WCFS1 static biofilm formation.

2. Materials and methods

2.1. Strains and media

The bacterial strains used in the present study are listed in Table 1. The strains were streaked from a −80 °C glycerol stock on De Man-Rogosa-Sharpe (MRS) agar (Merck) plates and incubated for 48 h at 30 °C. A single colony was inoculated in 10 ml of MRS at 30 °C for 18 h to prepare a starting culture. The media were supplemented with 10 μg/ml chloramphenicol when appropriate. Biofilms were grown in brain heart infusion (BHI; Becton Dickinson) supplemented with 0.005% manganese sulphate and 2% glucose (Merck) (BHIMnG) as it has been shown previously that this medium favours biofilm formation of *L.*