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ORIGINAL ARTICLE

Growth inhibitory properties of lactose fatty acid esters

Seung-Min Lee [a], Guneev Sandhu [b], Marie K. Walsh [a,*]

[a] Department of Nutrition, Dietetics, and Food Sciences, Utah State University, 8700 Old Main Hill, Logan, UT 84322-4415, USA
[b] Kerry Ingredients, 160 Terminal Ave., Clark, NJ 07066, USA

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Abstract Sugar esters are biodegradable, nonionic surfactants which have microbial inhibitory properties. The influence of the fatty acid chain length on the microbial inhibitory properties of lactose esters was investigated in this study. Specifically, lactose monooctanoate (LMO), lactose monodecanoate (LMD), lactose monolaurate (LML) and lactose monomyristate (LMM) were synthesized and dissolved in both dimethyl sulfoxide (DMSO) and ethanol. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were determined in growth media. LML was the most effective ester, exhibiting MIC values of < 0.05 to < 5 mg/ml for each Gram-positive bacteria tested (*Bacillus cereus*, *Mycobacterium KMS*, *Streptococcus suis*, *Listeria monocytogenes*, *Enterococcus faecalis*, and *Streptococcus mutans*) and MBC values of < 3 to < 5 mg/ml for *B. cereus*, *M. KMS*, *S. suis*, and *L. monocytogenes*. LMD showed MIC and MBC values of < 1 to < 5 mg/ml for *B. cereus*, *M. KMS*, *S. suis*, *L. monocytogenes*, and *E. faecalis*, with greater inhibition when dissolved in ethanol. LMM showed MIC and MBC values of < 1 to < 5 mg/ml for *B. cereus*, *M. KMS*, and *S. suis*. LMO was the least effective showing a MBC value of < 5 mg/ml for only *B. cereus*, though MIC values for *S. suis* and *L. monocytogenes* were observed when dissolved in DMSO. *B. cereus* and *S. suis* were the most susceptible to the lactose esters tested, while *S. mutans* and *E. faecalis* were the most resilient and no esters were effective on *Escherichia coli O157:H7*. This research showed that lactose esters esterified with decanoic and lauric acids exhibited greater microbial inhibitory properties than lactose esters of octanoate and myristate against Gram-positive bacteria.

Abbreviations: DMSO, dimethyl sulfoxide; ETOH, ethanol; MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration; LMO, lactose monooctanoate; LMD, lactose monodecanoate; LML, lactose monolaurate; LMM, lactose monomyristate

* Corresponding author. Tel.: +1 435 797 2177; fax: +1 435 979 2379.
E-mail addresses: choco1628@gmail.com (S.-M. Lee), guneevsandhu@gmail.com (G. Sandhu), marie.walsh@usu.edu (M.K. Walsh).

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1. Introduction

Sugar esters are nonionic surfactants used in a variety of applications in the food, pharmaceutical, and personal care industries. The microbial inhibitory activity of sugar esters has been studied. Although it has been shown that sugar esters inhibit bacterial growth, there is a lack of consensus as to which bacteria are most susceptible. While some studies have shown inhibitory effects of Gram-negative bacteria (Ferrer et al., 2005; Habulin et al., 2008; Zhang et al., 2014; Smith et al., 2008), others have shown inhibition of only Gram-positive bacteria (Wagh et al., 2012; Piao et al., 2006). Studies have shown that esters containing laurate were inhibitory against both Gram-positive and Gram-negative bacteria (Smith et al., 2008; Nobmann et al., 2009; Zhang et al., 2014). A study on the microbial inhibitory activity of lactose monolaurate showed low minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) for *Listeria monocytogenes* and *Mycobacterium* sp. strain KMS, and no inhibitory activity against Gram-negative bacteria (Wagh et al., 2012).

The nature and number of fatty acid chains esterified to sugars can be variable, yielding a broad range of hydrophilic-lipophilic balances and microbial inhibitory activities (Szuts and Szabó-Révész, 2012). Previous research showed that fatty acid derivatives such as monolaurin are highly inhibitory and more inhibitory than lauric acid (Smith et al., 2008; Nobmann et al., 2009). Others have reported that sugar monoesters of decanoic, myristic and palmitic acids were microbial inhibitory (Piao et al., 2006; Habulin et al., 2008; Zhang et al., 2014). There was one study investigating the microbial inhibition of sugar octanoate esters which showed no inhibitory effects (Zhang et al., 2014).

Of the carbohydrate fatty acid esters previously investigated, sucrose esters have been the most thoroughly studied (Nobmann et al., 2009). Other oligosaccharide esters of laurate, including maltose, fructose and galactose have been synthesized and have generally been shown to be very effective microbial inhibitory agents (Nobmann et al., 2009; Watanabe et al., 2000; Devulapalle et al., 2004; Habulin et al., 2008), whereas hexose laurate did not suppress microbial growth significantly (Watanabe et al., 2000).

While many studies examine the microbial inhibition of sugar esters in terms of MIC values, few studies have determined the MBC values of sugar esters. In this study we synthesized novel lactose esters including lactose monooctanoate (LMO), lactose monodecanoate (LMD) and lactose monomyristate (LMM). The microbial inhibitory properties of these esters (MIC and MBC) in microbial growth media, and the previously synthesized ester, lactose monolaurate (LML) (Wagh et al., 2012) were determined against Gram-positive (*Bacillus cereus*, *Mycobacterium KMS*, *Streptococcus suis*, *L. monocytogenes*, *Enterococcus faecalis* and *Streptococcus mutans*) bacteria and the Gram-negative bacteria, *Escherichia coli* O157:H7. Furthermore, we also determined MIC and MBC values of the esters dissolved in two solvents, DMSO and ethanol. This allowed us to ascertain the role of the solvents in the microbial inhibitory activity.

Table 1 Microorganisms and growth media used in this study.

Microorganism	ATCC no./serovar	Gram reaction[a]	Growth medium
Bacillus cereus	13061	+	BHI
Mycobacterium sp. strain KMS	NA	+	LB
Streptococcus suis	89/1591	+	BHI
Listeria monocytogenes	EGDe	+	BHI
Listeria monocytogenes	FSL J1-177	+	BHI
Listeria monocytogenes	FSL N3-013	+	BHI
Listeria monocytogenes	FSL R2-499	+	BHI
Listeria monocytogenes	FSL N1-227	+	BHI
Enterococcus faecalis	V538	+	BHI
Streptococcus mutans	FSL R2-499	+	BHI
Escherichia coli O157:H7	EDL 931	−	LB

NA = not available.
[a] +, positive; −, negative.

2. Materials and methods

2.1. Bacterial strains

Bacterial strains used are listed in Table 1. *E. faecalis* V538 and *L. monocytogenes* EGDe were received from Dr. Andy Benson of the University of Nebraska, Lincoln. Different clinical isolates of *Listeria* (FSL J1-177, FSL N3-013, FSL R2-499 and FSL N1-227) were obtained from Dr. Martin Wiedmann, director of the international Life Sciences Institute North American Database at Cornell University. *S. suis* 89/1591 was received from Dr. Richard Higgins of University of Montreal, Qubec, Canada. *M. KMS* was isolated by Utah State University from treatment soils in Champion International Superfund Site, Libby, Montana. *B. cereus* ATCC 13061, *S. mutans* ATCC 25175 and *E. coli* O157:H7 EDL 931 stains were obtained from ATCC (Manassas, VA).

2.2. Materials and equipment

Materials and equipment included a high-performance liquid chromatography (HPLC) (Beckman System Gold 125 Solvent Module, Ontario, Canada) equipped with Luna 5 μm C18 100 Å (250 mm × 4.6 mm, Phenomenex, Torrance, CA, USA) and an evaporative light scattering detector (Agilent Technologies, Santa Clara, CA, USA), incubator shaker, spectrophotometer (Beckman, USA), 48 microtitre well plates (Becton Dickinson, NJ, USA), brain–heart infusion (BHI) media, Lauria–Bertani (LB) media, granulated agar (BD, New Jersey, USA), lactose (Proliant, Iowa, USA), vinyl laurate, vinyl myristate, vinyl decanoate, vinyl octanoate (TCI, Portland OR, USA), lipase TM2 (immobilized from *Thermomyces lanuginose*), Tween 80, Whatman glass microfiber filters, molecular sieves (3A), 2-methyl-2-butanol (2M2B) (dried using 10% 3A molecular sieves), dimethyl sulfoxide (DMSO) (Sigma Aldrich, MO, USA), ethanol, and acetonitrile (HPLC grade, Thermo Fisher, PA, USA).