Available online at www.sciencedirect.com
ScienceDirect
Veterinary Microbiology 127 (2008) 425–430
veterinary microbiology
www.elsevier.com/locate/vetmic

Short communication

Antibacterial activity of thionin Thi2.1 from *Arabidopsis thaliana* expressed by bovine endothelial cells against *Staphylococcus aureus* isolates from bovine mastitis

Alejandra Ochoa-Zarzosa, Heber Loeza-Ángeles, Eduardo Sagrero-Cisneros, Erik Villagómez-Gómez, Leticia Lara-Zárate, Joel E. López-Meza *

Centro Multidisciplinario de Estudios en Biotecnología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Michoacana de San Nicolás de Hidalgo, Apdo. Postal 53, Administración Chapultepec, C.P. 58262 Morelia, Michoacán, México

Received 26 May 2007; received in revised form 17 August 2007; accepted 21 August 2007

Abstract

Bovine mastitis is mainly caused by *Staphylococcus aureus* and antimicrobial therapy, commonly used for its control, has resulted in an increase in the frequency of resistant staphylococci in recent years. Thus, alternative therapies are desirable and the antimicrobial peptides represent attractive control agents. In this work, we expressed the antimicrobial peptide thionin *Thi2.1* cDNA from *Arabidopsis thaliana* in the bovine endothelial cell line BVE-E6E7 and evaluated its activity against bovine mastitis *S. aureus* isolates. A polyclonal population from BVE-E6E7 cells transfected with the pThi2.1 construct was obtained and thionin Thi2.1 expression was confirmed by RT-PCR. From this population, eight stably transfected cell clones were obtained and their conditioned media (CM) were evaluated against the *S. aureus* ATCC 27543 strain. Clones showed high antibacterial activity (>95%) relative to the activity of the polyclonal population. The C8 clone showed the highest antibacterial activity (>99%) and its CM was evaluated against eleven bovine mastitis *S. aureus* isolates. A 2.5 μg aliquot of total protein from the C8 clone's CM inhibited the growth of *S. aureus* isolates (>40%) relative to the CM from BVE-E6E7 cells used as control. Growth inhibition of *S. aureus* isolates was dose-dependent, showing a total inhibition at concentrations higher than 3.12 μg/ml. These results suggest that thionin Thi2.1 antimicrobial peptide could be use in the treatment of bovine mastitis.

Keywords: Bovine mastitis; *Staphylococcus aureus*; *Arabidopsis thaliana* thionin; Antimicrobial peptides

1. Introduction

Staphylococcus aureus is an important opportunistic pathogen that causes a variety of diseases in humans and animals. In cattle, *S. aureus* is the most frequently isolated pathogen causing clinical or

* Corresponding author. Tel.: +52 443 295 8029; fax: +52 443 295 8029.
E-mail address: elmeza@zeus.umich.mx (J.E. López-Meza).

0378-1135/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetmic.2007.08.031

subclinical mastitis worldwide (Kerro-Dego et al., 2002). Antimicrobial agents are widely used for the control of staphylococcal infections but their use is subject to considerable debate due to the emergence of antibiotic-resistant bacteria (Werckenthin et al., 2001).

Antimicrobial peptides are considered attractive, alternative therapies. They are produced by all complex organisms, as well as some microbes, and have diverse and complex antimicrobial activities which protect the host from invading bacteria, viruses and fungi (Ryan et al., 1998; Jenssen et al., 2006). Antimicrobial peptides from several origins have shown antibacterial activities against staphylococci (Ryan et al., 1998; Komatsuzawa et al., 2006; Capparelli et al., 2007). In particular, plant antimicrobial peptides are promising candidates for the treatment of *S. aureus* infections, including bovine mastitis.

Plants produce antimicrobial peptides, called thionins, which are small (~5 kDa) basic peptides stabilized by six to eight disulfide-linked cysteins. It has been suggested that thionins have an important role in the defense against pathogenic invaders, but their effects on pathogenic bacteria of mammals, such as *S. aureus*, are unknown (Thomma et al., 2002; Stec, 2006). Recently, we have reported that the expression of plant antimicrobial peptides in bovine endothelial cells is a useful tool to explore antibacterial, fungicidal and cytotoxic activities against a broad range of mammalian pathogens (Anaya-López et al., 2006b).

Thionin Thi2.1 from *Arabidopsis thaliana* is constitutively expressed or induced after pathogen attack but its antibacterial effects have only been evaluated against plant pathogenic bacteria (Epple et al., 1995; Chan et al., 2005). In this study, we assessed the antimicrobial activities of thionin *Thi2.1* cDNA expressed by bovine endothelial cells against *S. aureus* isolates from cases of bovine mastitis.

2. Materials and methods

2.1. S. aureus isolates

Eleven *S. aureus* isolates were collected from raw milk samples from cows with subclinical mastitis maintained at farms in Morelia, Michoacán, México (four from intensive and seven from extensive farms). *S. aureus* isolates were identified by standard biochemical tests and characterized by molecular amplification of the *nuc* gene encoding staphylococcal thermostable nuclease (Brakstad et al., 1992). All isolates were susceptible to 10 μg gentamicin (Sigma, St. Louis, MO, USA) as determined by the disk diffusion method (Bio-Rad, Richmond, CA, USA). Thus, gentamicin was selected to eliminate extracellular bacteria in invasion assays. The American Type Culture Collection (ATCC) *S. aureus* subsp. *aureus* 27543 strain, isolated from a case of clinical mastitis, was included as a positive control. Prior to invasion assays, bacteria were grown at 37 °C overnight in Luria–Bertani broth (LB; Difco, Detroit, MI, USA) and colony forming units (CFU) were adjusted by measuring the optical density at 560 nm.

2.2. Cell cultures

The bovine endothelial cell line BVE-E6E7, immortalized with the human papillomavirus type 16 E6E7 oncogen, was used to express *Thi2.1* cDNA (Cajero-Juárez et al., 2002). Cells were routinely cultured in Dulbecco's modified Eagles's medium (DMEM; Sigma) supplemented with 10% fetal calf serum (FCS; Equitech-Bio, Kerrville, TX, USA), 100 U/ml penicillin and streptomycin (Gibco, Gaithersburg, MD, USA) and grown in an atmosphere of 5% CO_2 at 37 °C.

The isolation of primary bovine mammary epithelial cells (bMEC) was performed on alveolar tissue from the udders of lactating cows, as described previously (Anaya-López et al., 2006a). Cells from passages 2–8 were cultured in Petri dishes (Corning-Costar, Cambridge, MA, USA) in growth medium (GM) composed by a DMEM medium/F-12 Ham nutrient mixture (DMEM/F-12, Sigma) supplemented with 10% FCS (Equitech-Bio), 10 μg/ml insulin (Sigma), 5 μg/ml hydrocortisone (Sigma), 100 U/ml penicillin and streptomycin (100 μg/ml) and 1 μg/ml amphotericin B (Invitrogen, Carlsbad, CA, USA).

2.3. Construction of expression vector and transfection of BVE-E6E7 cells

Thi2.1 cDNA from *A. thaliana* cloned into the pUC19 vector was kindly donated by Klaus Apel