Available online at www.sciencedirect.com
ScienceDirect
Theriogenology 73 (2010) 48–55
Theriogenology
www.theriojournal.com

Photonic characteristics and *ex vivo* imaging of *Escherichia coli*-Xen14 within the bovine reproductive tract

J. Curbelo [a], K. Moulton [a], S. Willard [a,b,*]

[a] *Department of Animal and Dairy Sciences and the Mississippi State University, Mississippi State, Mississippi, USA*
[b] *Department of Biochemistry and Molecular Biology, Mississippi State University, Mississippi State, Mississippi, USA*

Received 6 April 2009; received in revised form 8 July 2009; accepted 30 July 2009

Abstract

The objectives of this study were to (1) characterize the photonic properties of *Escherichia coli*-Xen14 and (2) conduct photonic imaging of *E. coli*-Xen14 within bovine reproductive tract segments (RTS) ex vivo (*Bos indicus*). *E. coli*-Xen14 was grown for 24 h in Luria Bertani medium (LB), with or without kanamycin (KAN). Every 24 h, for an 8-d interval, inoculums were imaged and photonic emissions (PE) collected. Inoculums were subcultured and plated daily to determine the colony forming units (CFU) and ratio of photon emitters to nonemitters. In the second objective, abattoir-derived bovine reproductive tracts (n = 9) were separated into posterior and anterior vagina, cervix, uterine body, and uterine horns. Two concentrations (3.2×10^8 and 3.2×10^6 CFU/200 μL for relative [High] and [Low], respectively) of *E. coli*-Xen14 were placed in translucent tubes for detection of PE through RTS. The CFU did not differ ($P = 0.31$) over time with or without KAN presence; they remained stable with 99.93% and 99.98% photon emitters, respectively. However, PE were lower ($P < 0.0001$) in cultures containing KAN than in those containing no KAN (629.8 ± 117.7 vs. 3012.0 ± 423.5 relative lights units per second [RLU/sec], respectively). On average, the percentage of PE between RTS, for both concentrations, was higher ($P < 0.05$) in the uterine body. In summary, *E. coli*-Xen14 remained stable with respect to the proportions of photon emitters with or without KAN (used to selectively culture *E. coli*-Xen14). However, KAN presence suppressed photonic activity. The ability to detect PE through various segments of the reproductive tract demonstrated the feasibility of monitoring the presence of *E. coli*-Xen14 in the bovine reproductive tract ex vivo.

Keywords: Biophotonics; *Escherichia coli*; Reproductive tract; Uterus

1. Introduction

The rate of uterine infections in cattle has been estimated to be from 2.2% to 37.3% [1], with economic losses as high as $236 per case [2]. These economic losses include reduced fertility, decreased milk yield, increased treatment costs, and an increased culling rate [1]. *Escherichia coli* (*E. coli*) is the most common isolated bacteria in postpartum uterine infections in cattle [3] and one of the few uterine pathogens associated with clinical signs [4]; specifically, inflammation of all layers of the uterine wall, edema, myometrial degeneration, and infiltration by leukocytes [5]. In addition, chronic infections caused by *E. coli* can retard ovarian follicular development, decrease plasma estradiol concentrations, and suppress formation of a competent corpus luteum [4].

Conventional models to estimate bacterial populations and invasiveness in vivo typically require

* Corresponding author. Tel.: +1 (262) 325 2640; fax: +1 (662) 325 8664.
E-mail address: swillard@bch.msstate.edu (S. Willard).

0093-691X/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2009.07.020

extensive sampling procedures at each sampling time-point, considerable time and labor to establish cultures, and so forth. Another approach has been the use of luciferases (e.g., photon-emitting indicators) to label pathogens and monitor their progress during infections in small and/or large animals [6–9]. This feature is being used as a detection system for various kinds of expression models, acting as a reporter for the activity of regulatory elements that control its expression [10]. The use of biophotonic imaging allows biological processes of an infective state of eukaryotic cells to be monitored longitudinally, in vitro and in vivo, in real time and noninvasively [11]. The *lux* operon has been modified for bacterial incorporation using plasmids, via transponson mutagenesis and chromosomal insertion, and routinely carries antibiotic resistance selection markers for cultivation as monocultures under controlled environmental conditions [12]. For example, *E. coli* has been conferred with bioluminescent properties by incorporating the *lux* genes of *Photorhabdus luminescences* on its chromosome (e.g., lux operon in *E. coli*-Xen14; Caliper Life Sciences, Alameda, CA, USA) which can then be selected for, using antibiotics to maintain pure cultures. This lux operon system encodes all proteins required for photon emissions, including the luciferase, substrate, and substrate-regenerating enzymes, which can be expressed within the bacterial host [13]. This feature, compared with plasmid integration, exhibits a continuous and stable luciferase expression over time and without antibiotic pressure; theoretically, the lux genes incorporated in the bacterial chromosome should be a stable as its native chromosome genes. [12]. The use of bioluminescent bacteria, such *E. coli*-Xen14, coupled with photonic imaging technologies may represent an efficient model for achieving a greater understanding of the pathogenesis of uterine infections. Some studies have established positive relationships between photonic emissions (PE) and bacterial populations for gram-negative bacteria ($r = 0.99$ [14]), and our laboratory has previously described and validated both in vitro [15] and ex vivo [9] bioluminescent bacterial models.

Studies have examined, for example, models for infection in the mouse [16], ex vivo imaging of gestating ewes [9], real-time monitoring of *Salmonella* in swine [7], and screening of pathogens in living fish [17]. Monitoring *lux*-transformed bacteria through tissues of small animals (e.g., rats or mice) is feasible due to the thin and semitransparent nature of mammalian tissue [6]. Any obstruction of photonic transference generated from the *lux*-transformed bacteria as signals pass through tissues is likely due to the scattering and absorption of photons associated with tissue thickness and the type of tissue acting as the barrier [18]. To date, this technology has not been reported in large animals (e.g., bovine) in vivo or ex vivo. Thus, the objectives of this study were to (1) characterize the photonic properties of bioluminescent *E. coli*-Xen14 in vitro with respect to operon stability and bacterial growth curves and (2) conduct photonic imaging of *E. coli*-Xen14 from within the bovine reproductive tract ex vivo (*Bos indicus*) as a first step toward development of an in vivo large animal model.

2. Materials and methods

2.1. Operon stability evaluation

To evaluate the stability of the *lux* operon in *E. coli*-Xen14, one colony was inoculated in 20 mL Luria Bertani medium (LB) and grown at 37 °C in an orbital shaker at 180 rpm for 24 h to produce fresh inoculums. Aliquots (100 μL) were transferred to tubes (three tubes/treatment) containing fresh 29.9 mL LB or LB plus kanamycin (KAN; Sigma-Aldrich, Inc. St. Louis, MO, USA) as the selective agent (30 μg/mL; i.e., KAN resistance is a characteristic of the transformed *E. coli*-Xen14 line for selective growth in vitro) and incubated at 37 °C in an orbital shaker at 180 rpm for 24 h. Black 96-well plates ($n = 3$) were divided into two sections with $n = 8$ wells per treatment. Each well was filled with 100 μL aliquots and imaged (2 sec) to collect PE using the XR/MEGA-10Zero imaging system (Stanford Photonics, Inc, Palo Alto, CA, USA) and analyzed using Image J software (National Institutes of Health, Bethesda, MD, USA) in which pixel intensities associated with PE were quantified within regions of interest (e.g., individual wells of a 96-well plate, designated areas over segments of the reproductive tract, etc.) and standardized to relative lights units per second (RLU/sec). After imaging, the content of a well was used to reinoculate the corresponding tube containing fresh LB for the next day's imaging. The content of the other seven wells were serially diluted in 900 μL LB or LB plus KAN and plated on LB or LB plus KAN agar plates (i.e., inoculums grown in KAN were plated on agar containing KAN) to allow the number of colony forming units (CFU) to be compared with levels of PE activity. Furthermore, each plate was imaged using the Nightowl imaging system (Berthold Technologies, Oak Ridge, TN, USA) to determine the ratio of non–photon-emitting versus photon-emitting colonies. This entire procedure was conducted over eight consecutive days and independently repeated three times.