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Propagation capacity of bacterial contaminants in platelet concentrates using a luciferase reporter system

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Introduction: Currently the use of molecular tools and techniques of Genetic Engineering in the study of microbial behavior in blood components has replaced the employment of classical methods of microbiology. This work focuses on the use of a novel *lux* reporter system for monitoring the contaminating propagation capacity of bacteria present in platelet concentrates under standard storage conditions in the blood bank.
Methods: A mini*Tn5* promotor probe carrying the *lux* operon from *Photorhabdus luminiscens* (pUTmini*Tn5luxCDABE*Km2) was used to construct four bacterial bioluminescent mutants: *Escherichia coli, Salmonella typhi, Proteus mirabilis* and *Pseudomonas aeruginosa*. Luminescent mutants were used for contamination tests with 20 CFU in platelet concentrates bags and were stored under standard storage conditions in the blood bank (100 rpm at 22 °C). The measurements of luminous activity and optical density were used to monitor bacterial proliferation during 7 days (168 h).
Results: During the exponential growth phase (log) of bacterial strains, a lineal correlation between luminous activity vs biomass was observed ($R^2 = 0.985, 0.976, 0.981$) for *E. coli*::*Tn5luxCDABE*Km2, *P. mirabilis*::*Tn5luxCDABE*Km2 and *P. auriginosa*::*Tn5luxCDABE*Km2, respectively. The above indicates that metabolic activity (production of ATP) is directly related to biomass in this phase of microbial growth. While conducting experiments, the inability to propagate *S. typhi*::*Tn5luxCDABE*Km2 was detected. We can speculate that platelet concentrates contain specific components that prevent the propagation of *S. typhi*.
Conclusion: The use of *luxCDABE* system for the quantification of luminous activity is a rapid and sensitive alternative to study the propagation and auto-sterilization of bacterial contaminants in platelet concentrates.

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1. Introduction

Transfusion of blood and blood components contaminated with bacteria can cause sepsis, which is considered a complication in transfusional medicine [1]. Among the hemocomponents more susceptible to microbial contamination is the platelet concentrates (PCs) due to the storage temperature (22 °C). Therefore is an excellent medium that promotes the propagation of these contaminants, particularly when the room temperature of the blood banks is greater and incubators of PCs are not available [2]. Some studies report 0.03% of PCs contaminated by bacteria, but although this percentage is low, the risk of transfusion-associated sepsis is present [3]. Contamination of PCs may be at the moment of venipuncture, cleansing of the phlebotomy site, bacteremia undetected in the donors or during blood fractionation [4]. *Staphylococcus* spp., *Pseudomonas* spp., *E. coli*, *Bacillus* spp. *Salmonella* spp., *Serratia*

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spp., *Enterobacter* spp., and others are examples of bacteria found in contaminated PCs [5]. Moreover, it has been suggested that compounds present in PCs such as leukocytes, preformed antibodies, complement proteins, lysozyme, lipoproteins and recently defensins identified on the surface of platelets, inhibit the growth of certain bacterial contaminants [6,7]. This phenomenon has been described as *"auto-sterilization"* [5,8,9]. The auto-sterilization of whole blood or blood components occurs since donation time to blood fractionation. In this step, phagocytes or soluble proteins act on the bacteria and eventually kill them. In some cases, the latter is not achieved, because some bacteria are able to evade the bactericidal action of these immunological proteins and can propagate in these hemocomponents. Traditional methods of Microbiology for the study of the propagation capacity of bacterial contaminants and auto-sterilization have been developed [8,9]. However, these methods have limitations due to the great amount of material and employee time. Currently, the bacterial bioluminescence offers great advantages direclty related to the metabolic activity (by ATP production) of microorganisms. Therefore, the measurement of bioluminescence provides a faster and simpler alternative methodology to monitor the proliferation and bacterial viability [10]. The aim of this work was to use a novel *luxCDABE* reporter system in the study of the propagation capacity of bacterial contaminants in platelet concentrates under standard storage conditions of blood banks by indirectly quantifying ATP production by luminometry.

2. Materials and methods

2.1. Bacterial strains and conditions growth

Bacterial strains and plasmid used in this study are listed in Table 1. These strains were genetically identified by 16S rRNA gene sequence analysis [11]. All strains were grown in Luria-Bertani (LB) broth (10 g/l tryptone, 5 g/l yeast extract, 5 g/l NaCl), or LB agar (supplemented with 1.5% agar). When necessary, media were supplemented with kanamycin (30 µg/ml).

2.2. *MiniTn5*luxCDABE*Km2 transposon mutagenesis*

Transposon-bearing suicide plasmid pUTmini*Tn5lux CDABE*Km2 was transferred from *E. coli* 17-1 λpir donor strain of the *Tn5* derivative containing the promoter-less *luxCDABE* full operon derived from *Photorabdus luminescens* and a kanamycin resistance gene "*ntpII*" [12] to recipient strains: *E. coli, S. typhi, P. mirabilis* and *P. aeruginosa* by bi-parental mating in solid media. This is a suicide plasmid that carries a R6K ɣ*ori* and replicates only in cells expressing the π protein. Donor and recipient strains were mixed (1:1) in logarithmic phase and incubated on the surface of the sterile 0.22 µm nitrocellulose filters (Millipore, Bedford, MA, USA) positioned on the surface of a LB agar plates (without kanamycin) and were incubated overnight at 37 and 30 °C (only for *P. aeruginosa*). To select the clones expressing the *lux* operon, cells were stripped from the filter, diluted in isotonic saline solution and plated onto LB plates containing 30 µg/ml of kanamycin and incubated overnight at 37 and 30 °C (only for *P. aeruginosa*) for 24 h. Light-emitting colonies were selected in a dark room. The stability of the mini*Tn5luxCDABE*Km2 transposon in all modified strains was assessed by repeated subculture in LB agar plates with and without kanamycin and visualized in a dark room. Luminescent strains were stored in 50% glycerol at −70 °C before use.

2.3. PCR amplifications

Genomic DNA was extracted using the QIAamp DNA Mini QIAcube Kit (QIAGEN, Germany). The reactions were performed in a Touchgene Gradient thermal cycler FTGRAD2D (TECHNE DUXFORT, Cambridge, UK) using MasterMix PCR 1 × (Roche Diagnostics, Germany), 200 pmol of each primer and 200 ng of template DNA. PCRs of the 16S rRNA gene were performed with universal primers 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′) and 1492R (5′-TAC GGY TAC CTT GTT ACG ACT T-3′) using the conditions recommended by DeSantis et al. [11]. The *luxE-nptII* segment in luminescent mutants was amplified with primers *luxE* Fwd (5′-CTTTCTTTGAGGATGAAATGC-3′) and *nptII* Rev (5′-GTCGGTCTTGACAAAAAGAAC-3′) using the conditions recommended by Bello-López et al. [13]. PCR products were analyzed on horizontals 1% agarose gels using 1× Tris-Acetate-EDTA buffer. The identity of amplicons was also determined by sequencing by Instituto de Biología-UNAM using an ABI PRISM® 310 Genetic Analyzer sequencer (Applied Biosystems, California, USA). Sequences were compared with the protein sequence database (GenBank) by means of the BlastX algorithm (http://blast.ncbi.nlm.nih.gov).

Table 1
Strains and plasmid used in this study.

Strain/Plasmid	Characteristics	Source
Escherichia coli S17-1 λpir	λ-*pir* lysogen of S17-1 (Tp^r Sm^r *thi pro hsd*R^- M^+ *recA* RP4::Mu-Km::*Tn7*)	[12]
Escherichia coli	Wild type	Wound
Salmonella tiphy	Wild type	Faeces
Proteus mirabilis	Wild type	Wound
Pseudomonas aeruginosa	Wild type	Wound
Escherichia coli::*Tn5luxCDABE*Km2	Chromosomal tagged::*Tn5luxCDABE*Km2	This work
Salmonella tiphy::*Tn5luxCDABE*Km2	Chromosomal tagged::*Tn5luxCDABE*Km2	This work
Proteus mirabilis::*Tn5luxCDABE*Km2	Chromosomal tagged::*Tn5luxCDABE*Km2	This work
Pseudomonas aeruginosa::*Tn5luxCDABE*Km2	Chromosomal tagged::*Tn5luxCDABE*Km2	This work
pUT mini*Tn5luxCDABE*Km2	Suicide vector, *ori* R6K, mini*Tn5luxCDABE*Km2 transposon, mob^+ (RP4); amp^r, Km^r	[12]

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