Electronic Journal of Biotechnology 28 (2017) 95–100

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Review

Mannheimia haemolytica growth and leukotoxin production for vaccine manufacturing — A bioprocess review

Tobias Oppermann [a,b], Nadine Busse [b], Peter Czermak [a,b,c,*]

[a] Fraunhofer Institute for Molecular Biology and Applied Ecology, Project Group Bioresources, Winchesterstrasse 2, 35394 Giessen, Germany
[b] Institute of Bioprocess Engineering and Pharmaceutical Technology, University of Applied Sciences Mittelhessen, Wiesenstrasse 14, 35390 Giessen, Germany
[c] Department of Chemical Engineering, Kansas State University, Durland Hall 1005, Manhattan, KS 66506-5102, USA

ARTICLE INFO

Article history:
Received 10 April 2017
Accepted 5 June 2017
Available online 10 June 2017

Keywords:
Bovine respiratory disease
Cell growth
Commensal flora of cattle
Enzootic calf pneumonia
Facultatively anaerobic
Gram-negative
Leukotoxin production
Mastitis in sheep
Operational parameters
Mannheimia haemolytica
Rod-shaped bacteria

ABSTRACT

Mannheimia haemolytica leukotoxin (LKT) is a known cause of bovine respiratory disease (BRD) which results in severe economic losses in the cattle industry (up to USD 1 billion per year in the USA). Vaccines based on LKT offer the most promising measure to contain BRD outbreaks and are already commercially available. However, insufficient LKT yields, predominantly reflecting a lack of knowledge about the LKT expression process, remain a significant engineering problem and further bioprocess optimization is required to increase process efficiency. Most previous investigations have focused on LKT activity and cell growth, but neither of these parameters defines reliable criteria for the improvement of LKT yields. In this article, we review the most important process conditions and operational parameters (temperature, pH, substrate concentration, dissolved oxygen level, medium composition and the presence of metabolites) from a bioprocess engineering perspective, in order to maximize LKT yields.

Contents

* Corresponding author.
E-mail address: peter.czermak@lse.thm.de (P. Czermak).
Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

http://dx.doi.org/10.1016/j.ejbt.2017.06.001

1. Introduction

Bovine respiratory disease (BRD) is economically the most important disease in the cattle industry although it also affects other wild and domestic ruminants [1,2,3,4]. The high morbidity and up to 50% mortality result in considerable losses [5] often approaching $US 1 billion per year in the US cattle industry alone [6,7,8,9].

BRD is a complex multifactorial disease causing a severe form of pneumonia. A BRD outbreak typically occurs after transportation to feedlots, hence the common name for the disease is 'shipping fever' [4,10]. Although the mechanism of infection and the complex interactions among the host, pathogen and environment are not fully understood, *Mannheimia haemolytica* leukotoxin (LKT) is the predominant virulence factor [6,11,12,13,14,15].

M. haemolytica is a Gram-negative, facultative anaerobic, non-motile, opportunistic pathogen [16]. As a commensal organism of the upper respiratory tract and nasopharynx of healthy ruminants, it can colonize the lower respiratory tract of stressed or immunocompromised animals and overcome their innate immunity, causing pneumonia [7,16]. LKT is a 105-kDa, soluble, heat-labile protein that belongs to the repeat-in-toxin (RTX) family, and it has a dose-dependent effect. At low concentrations, LKT induces bovine cells to undergo a respiratory burst and degranulation thus causing inflammatory cytokine production. At higher concentrations, LKT induces apoptosis and the formation of transmembrane pores, the latter resulting in necrosis and the breakdown of the pulmonary immune system [8,13,17]. LKT is closely related to *Escherichia coli* α-hemolysin and is similarly encoded by a four-gene polycistronic operon (*lkt*CABD). The *lkt*A gene encodes the inactive proLKT protein, whereas *lkt*B and *lkt*D encode proteins that promote secretion [18,19,20,21], and *lkt*C encodes the enzyme that activates LKT by acylation [19]. The expression and activation of LKT has been comprehensively reviewed [12,19,22,23,24,25,26,27].

More than 20 *M. haemolytica* serotypes, subdivided into two biotypes (A and T), have been identified thus far, revealing a high degree of amino acid sequence diversity for LKT due to the complex gene mosaic structure [3,8,28,29,30]. The most relevant forms from a veterinary perspective are biotype A serotype 1 in cattle and biotype A serotype 2 in sheep [28,31]. The treatment of BRD typically involves aggressive antimicrobial therapy, combined with improved feedlot management and vaccination to prevent further outbreaks [6,8]. Although antimicrobials are widely used, they are becoming less effective due to the spread of antibiotic resistance [2,8,15,32]. The demand for BRD vaccines is therefore rising, and currently-available vaccines based on LKT as the predominant antigen are highly effective [33,34,35,36]. The role of several other virulence factors of *M. haemolytica* such as the capsule, outer membrane proteins (e.g. PIpE), neuraminidase, adhesins, and lipopolysaccharides have also been investigated for vaccine formulation [8,28,37,38,39,40]. A PIpE-LKT fusion protein as antigen showed a significant protection against a bacterial challenge [39,41,42]. Nevertheless, LKT provided as *M. haemolytica* supernatant is still the most relevant and successfully applied antigen for vaccination. However, the yields of LKT are often low [43,44] and it is unclear whether the rising demand for the vaccine can be met by current processes. This review article therefore focuses on the optimization of LKT yields in *M. haemolytica* from a bioprocess engineering perspective. Major process parameters such as temperature, dissolved oxygen concentration and media composition are considered based on the hypothesis that *M. haemolytica* experiences comparably dramatic changes in its physical environment during the course of infection.

2. The expression profile of LKT

LKT expression should occur during the log phase of cell growth but the precise expression profile remains uncharacterized [12,19,45,46,47,48]. Moreover, higher growth rates and more biomass do not necessarily lead to higher LKT yields [44,49,50]. However, previous investigations often focused on *M. haemolytica* growth and LKT activity, and there is little correlation between the total amount of LKT in the culture supernatant and LKT activity [45]. One potential reason for this is the strong dependence of LKT activity on temperature. The complex and non-standardized preparation of samples for current LKT activity assays can lead to the rapid thermal inactivation of LKT, resulting in high standard errors [51]. Furthermore, there is high strain-dependent variability in terms of optimum LKT expression, making it difficult to generalize previous investigations [30,43,47,48,49]. As a result, cell growth rate and LKT activity are not strictly reliable as criteria for the optimization of LKT expression, and a clear differentiation among optimal cell growth, LKT activity and LKT expression is therefore necessary. The Enzyme-linked Immunosorbent Assay (ELISA) is the most common and well established method to quantify LKT expression [38,52].

3. Process and kinetic parameters

The available data concerning *M. haemolytica* media and process requirements for cell growth and LKT production are limited and often contradictory (Table 1). However, *M. haemolytica* experiences dramatic changes in its physical environment during the course of infection, including changes in temperature, oxygen levels and nutrient availability. Therefore, critical factors such as media composition, pH, dissolved oxygen, inoculum density and their effects on cell growth and LKT expression are discussed in more detail below, including the impact of acetic acid as the major metabolic byproduct (Table 2, Table 3).

3.1. Medium requirements and supplements

LKT production usually involves a two-stage batch process including a change in the medium composition [44,47]. The most common media for LKT production are brain heart infusion (BHI) broth and chemically

Table 1
Critical medium components affecting *M. haemolytica* cell growth, LKT activity and LKT expression.

		Inhibitory/sub-optimal	Beneficial/essential
Complex media supplements	Growth	N/A	Yeast extract
	LKT expression	Yeast extract	N/A
	LKT activity	N/A	BSA, FCS
Carbon source	Growth	Galactose, glycerol, sucrose, lactate	Glucose
	LKT expression	N/A	N/A
Amino acids	Growth	L-Methionine	L-Alanine, L-Isoleucine
	LKT expression	The absence of amino acids	N/A
Vitamins	Growth	N/A	Calcium pantothenate, nicotinamide, thiamine
	LKT expression	N/A	N/A
Trace elements	Growth	BSA + Fe^{3+}+Mg^{2+}, Ca^{2+}	Fe^{3+}, Mg^{2+}
	LKT expression	BSA + Fe^{3+}+Mg^{2+}, Ca^{2+}	Mn^{2+}+Fe^{3+}
	LKT activity	BSA + Fe^{3+}+Mg^{2+}, Ca^{2+}	N/A