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Research review paper

# Recombinant protein secretion in *Escherichia coli*

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**Abstract**

The secretory production of recombinant proteins by the Gram-negative bacterium *Escherichia coli* has several advantages over intracellular production as inclusion bodies. In most cases, targeting protein to the periplasmic space or to the culture medium facilitates downstream processing, folding, and in vivo stability, enabling the production of soluble and biologically active proteins at a reduced process cost.

This review presents several strategies that can be used for recombinant protein secretion in *E. coli* and discusses their advantages and limitations depending on the characteristics of the target protein to be produced.


*Keywords:* Recombinant proteins; *Escherichia coli*; Secretion; Periplasm; Type II

**Contents**



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## 1. Introduction

Most bacteria secrete proteins such as degradative enzymes, toxins, and other pathogenicity factors into the extracellular environment (Fernandez and Berenguer, 2000). In Gram-negative bacteria, secreted proteins have to cross the two membranes of the cell envelope, which differ substantially in both composition and function (Koebnik et al., 2000).

The type I, II, III, IV, and V secretion pathways are widespread among Gram-negative bacteria and their mechanisms differ significantly. Despite these differences, the systems have, in common, a need to recognise specifically their cognate substrates and promote secretion without compromising the barrier function of the cell envelope (Koster et al., 2000). This review discusses the type I and type II mechanisms that are used most commonly for recombinant protein secretion in *Escherichia coli* K-12 or B strains. The type III secretion pathway is characteristic of several pathogenic Gram-negative bacteria and has been reviewed by Cornelis and Van Gijsegem (2000). Type IV secretion comprises those pathways usually found in bacterial conjugation systems (Pallen et al., 2003) and has been reviewed by Christie (2001). The type V mechanism includes the autotransporter and the two-partner secretion systems (Pallen et al., 2003), and has been reviewed by Jacob-Dubuisson et al. (2001).

Finally, protein secretion to the culture medium may also occur by leakage of periplasmic contents, and thus is not always mediated by specific transport mechanisms as will be discussed in this review.

## 2. Recombinant protein secretion

Secretion of recombinant proteins to the culture medium or periplasm of *E. coli* has several advantages over intracellular production. These advantages include simplified downstream processing, enhanced biological activity, higher product stability and solubility, and N-terminal authenticity of the expressed peptide (Cornelis, 2000; Makrides, 1996; Mergulhão et al., 2004b).

As *E. coli* does not naturally secrete high amounts of proteins (Sandkvist and Bagdasarian, 1996), recovery of a recombinant gene product can be greatly simplified by a secretion strategy that minimises contamination from host proteins. Additionally, if the product is secreted to the culture medium cell disruption is not required for recovery and,