ELSEVIER

FEMS Microbiology Reviews 29 (2005) 703–717

FEMS MICROBIOLOGY Reviews

www.fems-microbiology.org

Lessons from signature-tagged mutagenesis on the infectious mechanisms of pathogenic bacteria

Nicolas Autret, Alain Charbit *

INSERM U-570, CHU Necker-Enfants Malades, 156, rue de Vaugirard, 75730 Paris Cedex 15, France

Received 1 June 2004; received in revised form 18 September 2004; accepted 21 October 2004

First published online 26 November 2004

Abstract

Studies on the genetic basis of bacterial pathogenicity have been undertaken for almost 30 years, but the development of new genetic tools in the past 10 years has considerably increased the number of identified virulence factors. Signature-tagged mutagenesis (STM) is one of the most powerful general genetic approaches, initially developed by David Holden and colleagues in 1995, which has now led to the identification of hundreds of new genes requested for virulence in a broad range of bacterial pathogens. We have chosen to present in this review, the most recent and/or most significant contributions to the understanding of the molecular mechanisms of bacterial pathogenicity among over 40 STM screens published to date. We will first briefly review the principle of the method and its major technical limitations. Then, selected studies will be discussed where genes implicated in various aspects of the infectious process have been identified (including tropism for specific host and/or particular tissues, interactions with host cells, mechanisms of survival and persistence within the host, and the crossing of the blood brain barrier). The examples chosen will cover intracellular as well as extracellular Gram-negative and Gram-positive pathogens.

Keywords: Bacterial pathogenesis; Virulence; Signature-tagged mutagenesis; Transposons

Contents

* Corresponding author. Tel.: +33 1 40 61 53 76; fax: +33 1 40 61 55 92.
E-mail address: charbit@necker.fr (A. Charbit).

0168-6445/$22.00
doi:10.1016/j.femsre.2004.10.006

1. Introduction

Pathogenic bacteria have evolved complex molecular mechanisms to invade and survive within their hosts. One can define a virulence gene as a gene whose product is necessary for survival and persistence within the host [1]. Virulence factors in bacterial pathogens can act either directly on the infectious process, like toxins and adhesins, or indirectly, by participating in regulatory processes or because they are required for bacterial survival. Since virulence genes participate in various stages of the infectious process, their inactivation may lead in some cases to a complete loss of virulence or more generally to intermediate phenotypes corresponding to variable degrees of attenuation. A number of genetic methods like signature-tagged mutagenesis (STM) or in vivo expression technology (IVET) were developed to discover such new genes, especially those that cannot be identified by computer-assisted genomic predictions, or by subtractive DNA–DNA hybridization techniques (see for example [2]). STM, initially described by the group of David Holden [3], has now been applied to a variety of bacterial pathogens. The studies published over the past 10 years establish that STM is one of the most powerful and versatile large-scale genetic approaches to identify virulence determinants and can be therefore considered as a functional genomic approach.

However, careful examination of the publications reveals a very important qualitative and quantitative heterogeneity of the data. This heterogeneity is in part due to the variety of the organisms studied, of the models, and sizes of the screens. Therefore, we revaluated the data from STM studies, focusing on those that provided the most significant information on the role of the genes involved in bacterial pathogenicity.

We will first recall below the major parameters that need to be set-up to perform an efficient STM screen and some of the restraints that may hinder the identification of attenuated candidates. The general features of the mutants identified through STM carried out on Gram-positive and Gram-negative microorganisms will be summarized. Then, selected examples will be organized into four categories: (i) STM studies on *Mycobacterium tuberculosis* which provided, unlike most other STM studies, overlapping information on the role of lipid biosynthesis in mycobacterial virulence; (ii) STM screens performed in several Gram-positive and Gram-negative pathogens (*Streptococcus pneumoniae*, *Staphylococcus aureus* and members of the genus *Yersinia*) where pathogenicity was evaluated simultaneously in different hosts or tissues, to understand the molecular bases of host tropism; (iii) studies addressing the mechanisms of survival and persistence within the host, including adaptation to stress and nutritional deficiencies, and factors involved in colonization processes; and finally, (iv) STM screens aimed at identifying genes responsible for the traversing of physiological barriers.

2. Signature-tagged mutagenesis

2.1. Principle

Transposable elements have been widely used to study microorganisms [4]. STM is an evolution of traditional transposon mutagenesis that allows the large-scale analysis of transposon-insertion mutants for the identification of virulence genes in pathogenic bacteria. This method has two major advantages over other classical – targeted or random – gene inactivation approaches: (i) conceptually, STM is based on a negative selection of the mutants, i.e., mutants, which have lost the capacity to survive in a given host (see below), allowing the discovery of virulence genes without prior indication of their nature or function; (ii) technically, many mutants can be screened at the same time (the mutants assembled within pools are easily identified by a unique sequence – or tag – carried by the inserted transposon), which allows, in principle, a rapid and exhaustive analysis of virulence factors in a given organism. It is worth mentioning that the principle of applying STM does not necessarily require a transposon. DNA tags can be included during allelic replacement (signature-tagged allele replacement) on a systematic genome-wide scale [5]. This has been applied to *Saccharomyces cerevisiae*, also called bar-coding [6].