Chapter 31

Identification of Effectors: Precipitation of Supernatant Material

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Abstract

Bacterial secretion systems allow the transport of proteins, called effectors, as well as external machine components in the extracellular medium or directly into target cells. Comparison of the secretome, i.e. the proteins released in the culture medium, of wild-type and mutant cells provides information on the secretion profile. In addition, mass spectrometry analyses of the culture supernatant of bacteria grown in liquid culture under secreting conditions allows the identification of secretion system substrates. Upon identification of the substrates, the secretion profile serves as a tool to test the functionality of secretion systems. Here we present a classical method used to concentrate the culture supernatant, based on trichloroacetic acid precipitation.

Key words Supernatant, TCA precipitation, Secretome

1 Introduction

Bacterial secretion systems are macromolecular machines dedicated to the transport of proteins across the cell envelope. These secretion systems deliver effectors outside the cell, either in the medium (T1SS, T2SS, T5SS, T9SS) or directly into target cells (T3SS, T4SS, T6SS) [1]. Secretion of effector proteins into the milieu can be observed in these systems, and the analysis of secretion supernatant has been widely used either to identify new secreted effectors or to probe the functionality of secretion systems. For contact-dependent systems such as the T3SS, in vitro secretion in the medium can be observed under certain conditions (e.g. Ca^{2+} depletion, acidic pH) [2, 3]. As it is not always possible to predict effectors by bioinformatics approaches (*see* Chapter 2), analysis of the content of the culture media, the so-called secretome, using global proteomic approaches has been widely used to identify secretion system substrates in T2SS [4–7], T6SS [8–10], T3SS [11] and T9SS [12].

Upon the identification of substrates, the secretion profile is used to test the functionality of the secretion system using sodium

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