24.1 Introduction

The only absolute criterion of purity for a bacterial culture is that it has been derived from the progeny of a single cell. Failure to apply this criterion may lead to much effort in proving the purity of a culture. All strains upon which research is to be based should therefore be rigorously purified before starting to investigate the properties of individual organisms (Johnstone 1969). Ecologically oriented wine microbiologists are especially faced with the problem of how to obtain a pure culture of certain microbial strains from their densely populated natural habitats. The used methods comprise thereby a range from simple devices up to very complex machines. Most approaches to identify and enumerate microbes in wine use enrichment techniques (Fugelsang and Edwards 2007). Such indirect methods do not enumerate the original cell number in the sample, but their progeny, as enriched in a specific medium. Fugelsang and Edwards (2007) describe both general and selective growth media for plating yeasts and bacteria from wine. Unfortunately, plating and enrichment procedures are time consuming as colonies for some wine-related microbes take up to a week or more to appear on a Petri dish. Additionally, once colonies appear on a plate, the identification of the microbes requires further testing. Moreover, sublethally injured or viable but nonculturable cells, common in wine, may fail to grow on plates but are metabolically active. As a rule, culture-based techniques typically underestimate the size and diversity of a population (Kell et al. 1998; Millet and Lonvaud-Funel 2000). For monitoring the succession of a microbiota, cultivation-free molecular biological approaches were applied which give a more realistic view of a population. These spatiotemporal “snapshots” are often presented in the form of gel electrophoretic pattern of PCR amplicons or
pictures of fluorescence in situ hybridization (FISH) which allow a simultaneous visualization of the main role players within a population on species level (Amann et al. 1995; Mills et al. 2002; Hirschhäuser et al. 2005; Röder et al. 2007a, b). Unfortunately, there are no cultivable-free techniques available that could represent and monitor populations on strain level. Up to now, all methods that fit the strain level are culture dependent. With focus on a single cell, micromanipulation techniques are alternative methods to traditional cultivation approaches and a useful tool when complex habitats are investigated without cultivation (Fröhlich 2002; Fröhlich and König 1998; 1999a, b; 2000; Fröhlich et al. 2002). After the isolation of a single cell, different methods for identification on species or strain level can be applied, which are described below.

24.2 Micromanipulation Techniques

24.2.1 Historical Perspective

Since the beginning of the twentieth century, several attempts have been made to improve the management of single prokaryotic and eukaryotic cells by using micromanipulator techniques. Thereby, a suspension of an adjusted concentration of microorganisms was aspirated into a simple capillary tube, so that a single cell was transferred statistically in a defined volume (Harbeck and Rothenberg 1995).

Moreover, a survey of the chief methods devised for single organism cultures was presented by Johnstone (1969, 1973). These include the block cut method for the selection of an isolated organism on a lightly inoculated nutrient gel, formation of droplets with micropipettes, which are searched for those containing single organisms, and isolation by carrying the selected organisms across the sterile gel surface with a microneedle. Because of technical problems and disadvantages, these methods were not adopted for routine isolation.

Other attempts to improve the management of single microbial cells by using micromanipulator techniques have been described in the literature. Either microneedles or microcapillaries were used for the separation of single bacterial cells (Skerman 1968; Bakoss 1970; Johnstone 1973; Thomsen et al. 2004). The techniques suggested more than 30 years ago were based on the state of the art at that time. They were faced with several technical disadvantages, which hampered routine usage of the isolation techniques for a broad spectrum of prokaryotes in a microbiological laboratory. The magnification was limited and a transfer of single cells was hardly possible (Skerman 1968). It was designed for use with low power objectives (e.g., 10×) with a working distance of 7 mm or more. The instrument consists of a lens collar and magnetic tool carrier. The lens collar was clamped onto the objective and it contained two steel slides which permitted the magnet tool carrier to slide along freely. Knobs or microloops were the most useful tools for the isolation of cells from colonies on solid agar plates. By several operations, cells