Use of terminal restriction fragment polymorphism analysis to profile the bacterial ecology of wine and wine fermentations.

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Winemakers require rapid and comprehensive monitoring of the bacteria at all stages of wine production. Since culture-dependent methods for bacterial identification are laborious and time-consuming, numerous culture-independent assays have been developed. This includes methods, such as real-time PCR, which are now routinely used in commercial wine service laboratories. However, comprehensive profiling techniques, such as the popular PCR-DGGE approach, have found little use in commercial service laboratories since the method is technically challenging and is relatively low throughput. To surmount this we adapted terminal restriction fragment length polymorphism (T-RFLP) analysis for high throughput determination of the bacterial composition of musts and wines. To accomplish this we generated a 16S rRNA gene T-RFLP database of wine-related bacteria, such as the class Bacilli, containing all members of the lactic acid bacteria and bacilli, and the family Acetobacteraceae, containing all members of the acetic acid bacteria. We performed in silico restriction digestions to elucidate which enzyme choices resulted in the best discrimination of wine related-bacteria based on the predicted terminal restriction fragments. The method was tested empirically on numerous wine-related bacteria to validate the predicted terminal restriction fragment lengths and assess strain to strain variability. T-RFLP was then used to profile numerous spoiled wines and fermentation samples. Our study suggests that T-RFLP analysis is a simple tool for rapid and high throughput determination of bacterial diversity in wines and winery environments.