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Review

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Potential and utilization of thermophiles and thermostable enzymes in biorefining

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Abstract

In today's world, there is an increasing trend towards the use of renewable, cheap and readily available biomass in the production of a wide variety of fine and bulk chemicals in different biorefineries. Biorefineries utilize the activities of microbial cells and their enzymes to convert biomass into target products. Many of these processes require enzymes which are operationally stable at high temperature thus allowing *e.g.* easy mixing, better substrate solubility, high mass transfer rate, and lowered risk of contamination. Thermophiles have often been proposed as sources of industrially relevant thermostable enzymes. Here we discuss existing and potential applications of thermophiles and thermostable enzymes with focus on conversion of carbohydrate containing raw materials. Their importance in biorefineries is explained using examples of lignocellulose and starch conversions to desired products. Strategies that enhance thermostablity of enzymes both *in vivo* and *in vitro* are also assessed. Moreover, this review deals with efforts made on developing vectors for expressing recombinant enzymes in thermophilic hosts.

Background

Thermostable enzymes and microorganisms have been topics for much research during the last two decades, but the interest in thermophiles and how their proteins are able to function at elevated temperatures actually started as early as in the 1960's by the pioneering work of Brock and his colleagues [1]. Microorganisms are, based on their optimal growth temperatures, divided into three main groups, *i.e.* psychrophiles (below 20°C), mesophiles (moderate temperatures), and thermophiles (high temperatures, above 55°C) [2]. Only few eukaryotes are known to grow above this temperature, but some fungi grow in the temperature range 50 – 55°C [3]. Several years ago Kristjansson and Stetter [4], suggested a further division of the thermophiles and a hyperthermophile boundary (growth at and above 80°C) that has today reached general acceptance. Most thermophilic bacteria characterised today grow below the hyperthermophilic boundary (with some exceptions, such as *Thermotoga* and *Aquifex* [5]) while hyperthermophilic species are dominated by the Archaea.

Use and development of molecular biology techniques, permitting genetic analysis and gene transfer for recombinant production, led to dramatically increased activities in the field of thermostable enzymes during the 1990's. This also stimulated isolation of a number of microbes from thermal environments in order to access enzymes that could significantly increase the window for enzymatic bioprocess operations. One of the early successful commercialised examples was analytical use of a thermostable enzyme, *Taq*-polymerase, in polymerase chain