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(54) Title: ELECTROTRANSFORMATION OF CLOSTRIDIUM PASTEURIANUM

(57) Abstract: By this invention, for the first time, a method for high-efficiency genetic transformation of the anaerobic bacterium *Clostridium pasteurianum* is provided. *Clostridium pasteurianum* is a bacterium of substantial industrial importance, due to its selectivity and high productivity of the biofuel and biochemical n-butanol, and its ability to grow on a wide variety of inexpensive substrates. Notable among the substrates that it can utilize as a sole source of carbon and energy is glycerine, which is produced in increasing quantities globally as a by-product of biodiesel processing. The industrial exploitation of *Clostridium pasteurianum* has previously been impeded by the lack of genetic engineering tools for this bacterium. This invention provides such tools for the first time. Included in the invention is a means for protecting newly introduced DNA from degradation by a restriction enzyme within C. *pasteurianum*. Then, a detailed protocol is given, which enables high-efficiency transformation of C. *pasteurianum* via a series of treatments and electroporation conditions which successfully negotiate the resistant cell wall of C. *pasteurianum*. Finally, the invention discloses selection markers and vector components, which round out the tools required to successfully perform genetic engineering in C. *pasteurianum* for the first time.