

# Evaluation of DNA isolation to the sensitivity of molecular diagnostics of Cms bacteria

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Molecular diagnostics of bacteria isolated from an environment is often difficult because of different factors and contaminants presented in the environmental samples. Diagnostics of *Clavibacter michiganensis* ssp. *sepedonicus*, the causal agent of quarantine disease called as ring rot of potato is particularly difficult. In the latent infection Cms bacteria occurs at low concentration in the potato tissue and often without causing symptoms on plants. In addition the gram-positive bacteria it is characterized by the presence of a cell wall with exopolysaccharide coat, which more or less surrounds the surface of the tested bacterial cells and can make the identification difficult. One of the most important factors enabling effective molecular diagnostics of Cms bacteria is the selection of an effective method of DNA isolation that allows to obtain a high sensitivity of the detection method. Therefore, the aim of the presented research was to develop and evaluate the effectiveness of the DNA isolation method used in the diagnosis of Cms strains with different degree of mucous and evaluation of the influence of bacterial mucous on the sensitivity of the PCR assay. The studies were carried out using different mucoid Cms strains. In the first step, the bacterial suspensions were standardized spectrophotometrically and after obtaining the bacterial colonies growing on the microbiological medium, the working suspensions  $10^8$  cfu/ml (colony forming units per ml) for each strain were prepared. Then the suspensions series with a concentration from  $10^6$  to  $10^0$  cfu/ml were prepared and used to isolation of bacterial DNA. Because of the high sensitivity of the classical molecular diagnosis methods on the environmental contaminants, the efficiency of the developed isolation method was evaluated using the classical PCR assay.