A SIMPLE *IN SITU* TEST OF NAD(P)ase PHENOTYPE

Cultures were scored for NADase level by the colorimetric titration of acid release during NAD hydrolysis: Conidia and/or hyphae were suspended in water and 0.1 ml aliquants of such suspensions were mixed with 1 ml reaction mixtures; each reaction mixture contained 0.9 µmoles Tris-HCl, 1.37 mmoles glycerol, 40 nmoles phenol red, and 3.0 µmoles of NAD, and was adjusted to pH 8.4. At this pH, the reaction mixture was crimson red in color. Direct titration of the reaction mixture with dilute HCl indicated that the release of 0.92 µmoles of acid in a 1 ml reaction mixture would cause a color transition to brilliant yellow. Therefore, the relative NADase levels of different cell samples were determined by the incubation times required to bring about a color change. Each cell suspension was also incubated in a reaction mixture that did not contain NAD. If this control indicated a source of acid release other than by NAD hydrolysis, the sample was assayed by the cyanide-addition method

Robert E. Nelson