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SHORT REPORTS

BACTERIAL CONTAMINATION AS A CAUSE OF SPURIOUS CYANIDE TESTS

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Key Word Index—*Pseudomonas chlororaphis*; Pseudomonadaceae; bacterial contamination; cyanogenesis in fungi; hydrogen cyanide.

Abstract—Cyanogenic bacteria were common contaminants in cyanide tests of fungi. This observation demonstrated the need to ensure that the origin of hydrogen cyanide in a test is from the sample and is not microbial. This can be accomplished by recording cyanide test results after short incubation periods or by adding antibiotic solutions.

Cyanogenesis, the ability of an organism to synthesize hydrogen cyanide, is typically detected by noting the characteristic color change of a reagent-impregnated filter paper strip suspended over a macerated specimen in a small vial[1]. Although this method forms the basis for most literature reports of cyanogenesis in higher plants, insects, fungi and cultures of micro-organisms, it is not without criticism. The test reagents most commonly used may lack absolute specificity for cyanide ion, be subject to interference by other compounds and/or have limited sensitivity[1, 2].

Another problem not previously documented is the occurrence of false-positive cyanide tests due to the contamination of test materials with cyanogenic bacteria. In a recent survey of cyanide production in the Agaricales [Saupe, S. G., in preparation], *ca* 50% of the species tested gave a positive reaction for hydrogen cyanide after 18–24 hr by the Feigl–Anger [2] method described above. This cyanogenic response was delayed or completely inhibited by the addition of 1–3 drops of an antibiotic solution (1.34 mM penicillin G potassium + 0.34 mM streptomycin sulfate). These observations and the fact that the Feigl–Anger assay can detect minute quantities of cyanide (60 nmol CN/g fr.wt for a 200 mg or larger sample) within 3 hr suggested that the origin of cyanide might be due to microbial contamination of the basidiocarps employed in the assays.

To isolate a possible causal organism(s), sterile water (2 ml) was added to a vial containing a macerate (HCN+ after 24 hr) of *Russula emetica* (Schaeff. ex Fr.) S. F. Gray (ILLS-42453). An aliquot of this solution (0.1 ml) was aseptically removed, serially diluted, plated on TGE agar (Difco) and incubated at 25°. Individual colonies, which developed at high dilution, were transferred to fresh media and tested for cyanide. Two bacterial isolates yielded strongly positive hydrogen cyanide tests (Feigl–Anger) within 15 hr of inoculation. Hydrogen cyanide production was confirmed by the formation of Prussian blue[3] on a reagent-impregnated filter paper strip which was swept with air that had been passed over a culture (TGE broth, 1.0 l.) in a Fernbach flask.

Both isolates were motile rods that synthesized and excreted a green diffusible pigment, especially during growth in iron-deficient media[4]. This pigment, which fluoresced blue in UV light, eventually crystallized around the colonies. DNA from one isolate was extracted and purified by the method described by Marmur[5]. The G+C content of the DNA, which was determined by its buoyant density in caesium chloride[6], was 63.3 mol%. This bacterium used ethanol as a carbon source but could not metabolize DL-arabinose. These characteristics confirm the identification of this micro-organism as a strain of *Pseudomonas chlororaphis* (Guignard and Sauvageau) Bergey *et al.* (Pseudomonadaceae)[7].

Pseudomonas chlororaphis is one of five bacteria known to be cyanogenic[8]. Cyanogenic bacteria are primarily saprophytic and they have a widespread distribution[9, 10]. The frequent association of cyanogenic bacteria with plants, soil and other organic substrates suggests that the origin of hydrogen cyanide in a test may be due to bacterial contamination unless adequate precautions are observed. This is especially true for those materials (e.g. decaying plants, aquatic plants, fungi) that would serve as substrates for bacterial colonization. This problem can be minimized by recording cyanide test results within 3 hr and/or by adding antibiotic solutions for longer term studies of weakly cyanogenic organisms.

Clawson and Young[11] published the first paper concerning the classification and physiology of cyanogenic bacteria. In this publication they speculated that the cyanide liberated from linseed meal, beans, grains, and cassava was microbial in origin. Although they erroneously attributed cyanide production in these cyanogenic plants to bacterial contamination, their basic hypothesis that contamination by cyanogenic microbes could be responsible for positive tests in non-cyanogenic

organisms is correct. It should be noted that in an analogous study, Nartey[12] demonstrated that the cyanide produced by fermenting cassava products was due, in part, to contamination by cyanogenic bacteria (and/or the catabolism of endogenous cyanogenic glycosides by extracellular enzymes excreted by non-cyanogenic fungi and bacteria).

A survey of the literature of fungal cyanogenesis suggested that the cyanide reported for several fungi may be bacterial in origin. Fungi that were possibly contaminated with cyanogenic bacteria include *Grifola frondosa* (Dickson ex Fr.) S. F. Gray (=*Polyporus*; Polyporaceae)[13], *Grifola umbellata* (Pers. ex Fr.) Pilát (=*Polypilus*; Polyporaceae)[13], *Melanoleuca cognata* (Fr.) Konr. and *Omphalina griseopallida* (Desm.) Quel (Tricholomataceae)[14]. According to the original reports, these species failed to liberate detectable quantities of cyanide for at least 24 hr and most generally required longer incubation periods. When positive, these species gave a weak cyanogenic response. We were unable to detect cyanide in fresh basidiocarps of *G. frondosa* with Feigl–Anger or picrate reagent. This observation confirmed Bach's[15] earlier work with the same fungus. Pending further investigation, the cyanogenic ability of the fungi listed above is questionable. It is likely that cyanogenic bacteria are responsible for other reports of cyanogenesis in fungi and/or plants. Unfortunately, few investigators cite the period of incubation required for a positive cyanide test, information that is critical for determining the potential for bacterial contamination.

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AN ARABINOXYLOGLUCAN FROM EXTRACELLULAR POLYSACCHARIDES OF SUSPENSION-CULTURED TOBACCO CELLS

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Key Word Index—*Nicotiana tabacum*; Solanaceae; tobacco; cell culture; extracellular polysaccharide; arabinoxyloglucan.

Abstract—An arabinoxyloglucan (AXG) isolated from extracellular polysaccharide of suspension-cultured tobacco cells was investigated by methylation analysis, partial acid hydrolysis and ^{13}C NMR spectroscopy. It was found that the AXG is structurally similar to that isolated from the midrib of tobacco leaves.

INTRODUCTION

Xyloglucans have been known as one of the major polysaccharides of the cell walls of dicotyledonous plants [1]. Tobacco cell walls, however, contain arabinoxyloglucan (AXG), not xyloglucan, as one component of the hemicellulosic polysaccharides. Tobacco AXG has been isolated from the midrib of the leaves and structurally characterized [2–4].

Suspension-cultured cells of tobacco secrete into their growth medium extracellular polysaccharides (ECP). Methylation studies of the ECP [5] have suggested that AXG may also be present in ECP. This