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Biocontrol potential of Hydrogen cyanide producing microbes against root knot nematode

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Introduction
Root knot nematodes (RKNs) pose a significant threat to agricultural productivity worldwide by causing substantial yield losses in various crops. Traditional chemical nematicides are effective but have associated environmental and health concerns. Biological control using hydrogen cyanide (HCN)-producing microbes presents a promising alternative. This review explores the biocontrol potential of HCN-producing microbes against RKNs, focusing on their mode of action, efficacy under different environmental conditions, and potential for integration into sustainable agricultural practices. Key microbial genera such as Pseudomonas, Bacillus, and others are discussed for their ability to produce HCN and their antagonistic effects on RKNs through mechanisms such as direct toxicity and induced systemic resistance in host plants. Furthermore, challenges and future research directions in optimizing the application of HCN-producing microbes for RKN management are highlighted to enhance their practical implementation in agriculture.

Keywords: Biocontrol; Growth promotion; Hydrogen cyanide; Root knot nematode

Introduction

The primary factors that restrict agricultural yields include plant diseases, insect infestations, weeds, and abiotic stressors. These issues significantly hinder plant growth and can lead to substantial losses in biomass. Diseases caused by plant pathogens and insect pests alone contribute to annual yield reductions ranging from 20% to 40% across different cereal and legume crops worldwide.

Root-knot nematodes, specifically Meloidogyne spp., are significant pests that severely reduce the productivity of various economic crops. Finding biological methods to manage these nematodes is crucial because plants often lack natural resistance against them. Moreover, the use of chemical controls is controversial due to environmental risks and potential harm to human health.

The advancement of environmentally friendly control methods could involve utilizing nematicidal compounds derived from plants or microorganisms. These compounds, such as oxalic acid from *Aspergillus niger* F22 and 2,4-diacetylphloroglucinol (DAPG) from

Pseudomonas fluorescens, exhibit diverse chemical properties and have been identified as effective against nematodes. Additionally, microbial metabolites like pyrrolnitrin and hydrogen cyanide (HCN) contribute to the antagonistic activity of *Pseudomonas chlororaphis* PA23 against nematodes such as Caenorhabditis elegans. Consequently, strategies for nematode control may include formulations incorporating these microbes.

Certain bacterial strains exhibit nematode control capabilities due to their capacity to produce hydrogen cyanide (HCN). The interaction between HCN-producing bacteria and nematodes directly influences the efficacy of nematode management strategies. Additionally, HCN has the potential to influence plant establishment and inhibit the development of bacterial diseases in plants, highlighting its significant role in plant disease control.

Role of Hydrogen cyanide

Cyanide forms stable complexes with essential elements such as Cu^{2+}, Fe^{2+}, and Mn^{2+}, crucial for protein function, rendering it highly toxic to most organisms. Hydrogen cyanide (HCN) is a volatile secondary metabolite produced by numerous rhizobacteria and exerts potent effects on various organisms. HCN disrupts electron transport and compromises cellular energy supply, ultimately leading to organismal death. Several bacterial genera capable of HCN production include *Alcaligenes*, *Aeromonas*, *Bacillus*, *Pseudomonas*, and *Rhizobium*

HCN acts as a potent inhibitor of cytochrome c oxidase and other metalloenzymes, making it toxic to aerobic organisms. Despite this, cyanogenesis has been observed in numerous organisms including plants, fungi, myriapods, beetles, butterflies, and bacteria. Many organisms have evolved various mechanisms to prevent cyanide toxicity, such as converting HCN to thiocyanate using the rhodanese enzyme or utilizing cyanide-insensitive oxidases (Sehrawat et al., 2022).

Estimation of hydrogen cyanide (HCN)

To assess hydrogen cyanide (HCN) production by bacterial isolates, a qualitative method described by Alström and Burns (1989) is commonly employed. Bacterial cultures are grown in King's B medium broth supplemented with 4.4 g L^{-1} glycine. Sterilized 3-mm strips of Whatman no. 42 filter paper are immersed in a solution containing 0.5% picric acid and 2.0% sodium carbonate. After drying, these filter paper strips are placed into test tubes (15 mm × 125 mm) inoculated with 5.0 mL of bacterial cultures. The tubes are sealed with cotton plugs and then incubated at 28 ± 2°C for 3–5 days. A color change from yellow to orange-red on the filter paper strips indicates the production of hydrogen cyanide (HCN).

To quantitatively determine the amount of hydrogen cyanide (HCN) produced by rhizobacterial isolates or present in plant tissue, the picric acid method is commonly used. In this method, filter paper strips, which change colour in the presence of HCN, are eluted by placing them in a test tube containing 10 mL of distilled water. The strips are mixed using a vortex mixer to ensure the reddish-brown colour transfers completely into the water. The optical densities of the resulting solutions are then measured at 515 nm. The quantity of HCN produced or present is determined using a standard curve prepared with known concentrations of potassium cyanide (KCN).

Nematicidal potential of HCN producing microorganisms

Various rhizobacterial strains have been documented for their ability to manage nematode populations through diverse mechanisms, leading to enhanced plant health and increased yields. These mechanisms include the production of siderophores, antibiotics, hydrogen cyanide (HCN), and ammonia, all of which contribute to suppressing interactions between plant-parasitic nematodes. Promising biocontrol candidates encompass Pseudomonas species *Bacillus* strains and Streptomyces isolates.

Likewise, studies have shown that HCN-producing Pseudomonas aeruginosa can be lethal to nematodes. Another example involves *Pseudomonas chlororaphis* strain O6, which colonizes roots and demonstrates effectiveness against root-knot nematodes in tomatoes. The production of HCN by this strain is thought to significantly contribute to its nematicidal activity. six rhizobacterial isolates capable of producing hydrogen cyanide (HCN) — specifically Pseudomonas japonica strain NBRC 103040, *Bacillus megaterium* strain CtST3.5, *Pseudomonas* sp. strain Gamma-81, Pseudomonas tolaasii strain, *Pseudomonas chlororaphis* strain, and *Pseudomonas mosselii* strain demonstrated an impact on the viability of *Meloidogyne incognita* juveniles in laboratory conditions (Abd El-Rahman et al., 2019)

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