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Mechanistic evaluation of plant-mediated silver nanoparticles on MDR *Bacillus cereus* through comparative proteomics

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Abstract

The proteomic response of multidrug-resistant *Bacillus cereus* to silver nanoparticles (AgNPs) made using extract from *Boerhavia diffusa* is assessed in this work. The presence of bioactive metabolites involved in the production of nanoparticles was verified by LC-MS analysis. Ten proteins were found to be significantly changed ($p < 0.05$) by proteomic profiling using 2D-GE and MALDI-TOF MS. The proteins most commonly downregulated were those involved in membrane transport (SecA, GerN), nucleotide biosynthesis (PurL), DNA repair (AddB, MutS2), and detoxification (FosB). Transmembrane transport, nucleic acid binding, and ATP binding were all disrupted, according to Gene Ontology analysis. The results show that AgNPs generated from *B. diffusa* have a multitarget antibacterial impact by inducing oxidative stress and suppressing vital cellular processes in MDR *B. cereus.*

Key words : Multidrug resistance, Nanoparticles, *Bacillus cereus*, Proteomics, MALDI-TOF, Gene Ontology.

Global public health is seriously threatened by the rising incidence of multidrug-resistant (MDR) bacterial infections[15,17]. Among these, foodborne disease and opportunistic infections are caused by the Gram-positive, spore-forming bacterium *Bacillus cereus*[3]. Treatment plans are complicated by its resistance to several antibiotics[8]. Antimicrobial drugs based on nanotechnology, especially silver nanoparticles (AgNPs), have drawn interest because of their broad-spectrum antibacterial capabilities[10,13]. Eco-friendly and biocompatible nanoparticles enhanced with bioactive phytochemicals are produced using green synthesis utilizing medicinal plants[1]. Flavonoids, alkaloids, and phenolic compounds with antibacterial and antioxidant qualities are found in *Boerhavia diffusa*, a medicinal herb that is frequently employed in traditional medicine[9,11]. The proteome-level mechanisms

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behind biogenic AgNPs' potent antibacterial action are still unknown. Thus, this study uses 2D-GE, MALDI-TOF MS, and Gene Ontology analysis to examine proteome changes brought on by *B. diffusa*–mediated AgNPs in MDR *B. cereus*.

Plant extraction and LC-MS Analysis :

Boerhavia diffusa whole plants were gathered from Tirunelveli, shade-dried, ground into powder, and then extracted in ethanol (10 g/100 mL, 24 hours). To find reducing and stabilizing metabolites, the filtrate was subjected to analysis using Waters LC-MS (SQD2) with Q-TOF in both positive and negative ESI modes (100–1200 m/z)[16,7].

MDR Screening and Proteomic Treatment:

The disc diffusion approach was used to screen bacteria recovered from pesticide-treated soil against twelve antibiotics[2]. *Bacillus cereus* (BR3), an MDR strain, was chosen for more research. For 24 hours, cells were exposed to 60 µg/mL of biogenic AgNPs. The TCA–acetone precipitation technique was used to extract proteins[5].

2D-GE, In-Gel Digestion and MALDI-TOF MS :

Proteins were separated using 13.5% SDS-PAGE after 13 cm IPG strips (pH 3–10 NL). Melanie 9.0 software was used to examine gel pictures. Spots that showed differential expression ($p < 0.05$) were removed, destained, digested with trypsin, and examined using MALDI-TOF MS in reflector mode. The Uni Prot database was used to identify proteins[14,12].

Table-1. Quantitative class analysis of the differentially expressed protein spots in Bacillus cereus under control (A1) and Boerhavia diffusa AgNP-treated (A2) conditions.

Spot ID	Fold	Anova (p)	A1	A2	Regulation Trend
1	3.29484	0.00412728	2819.75	9290.65	Up regulated
2	2.75199	0.00985007	9693.47	3522.35	Downregulated
3	1.39497	0.0131401	6174.27	4426.1	Downregulated
4	1.532	0.019523	15435.6	10075.5	Downregulated
5	3.36097	0.0287039	19786.7	5887.2	Downregulated
6	4.30893	0.0395415	25757.8	5977.78	Downregulated
7	3.35094	0.0465828	51561.7	15387.2	Downregulated
8	3.53727	0.0473549	20088.7	5679.15	Downregulated
9	3.63324	0.0562188	18807.1	5176.4	Downregulated
10	4.9322	0.0573363	72512.4	14701.8	Downregulated

**A1: Control (untreated); A2: B. diffusa-synthesized AgNPs treated.; Fold Change is the relative change in spot intensity. The values of Expression Ratio <1.0 display down-regulation; the values of Expression Ratio >1.0 display up-regulation.*

GO Annotation and Differential Proteome Analysis :

Proteins with a fold change of ≥1.5 or ≤1.5 ($p < 0.05$) were deemed significant. Using Gene Ontology (GO) annotation against the reference proteome of *B. cereus*, identified proteins were classified into molecular activities, biological processes, and cellular components[4,6].

Protein Separation and Quantitative Analysis :

Ten substantially distinct protein spots ($p < 0.05$) were found in the 2D-GE comparison of untreated (A1) and AgNP-treated (A2) MDR *Bacillus cereus* obtained from *Boerhavia diffusa*. Following AgNP exposure, nine proteins were downregulated and one (Spot 1) was increased (3.29-fold), as indicated in Table-1. A significant decrease in protein intensity in treated cells is confirmed by the normalized expression ratios, which show that oxidative stress and protein synthesis inhibition have occurred. Such widespread protein downregulation is consistent with metabolic inhibition and cellular stress responses brought on by nanoparticles[13,10].

Identification of Differentially Expressed Proteins :

The proteins indicated were identified by MALDI-TOF MS. The upregulation of polyribonucleotide nucleotidyltransferase (PNPase) indicates that RNA turnover and stress adaption are activated[1]. On the other hand, there was a downregulation of important proteins such as SecA translocase, GerN antiporter, PurL, phospholipase C, FosB, AddB, MutS2, and HisE. While reduction of PurL and HisE implies altered nucleotide biosynthesis[16], reduced SecA and GerN indicate defective membrane transport and ion homeostasis[9,11]. Reduced FosB suggests reduced thiol-mediated detoxification[5], while downregulation of AddB and MutS2 implicates

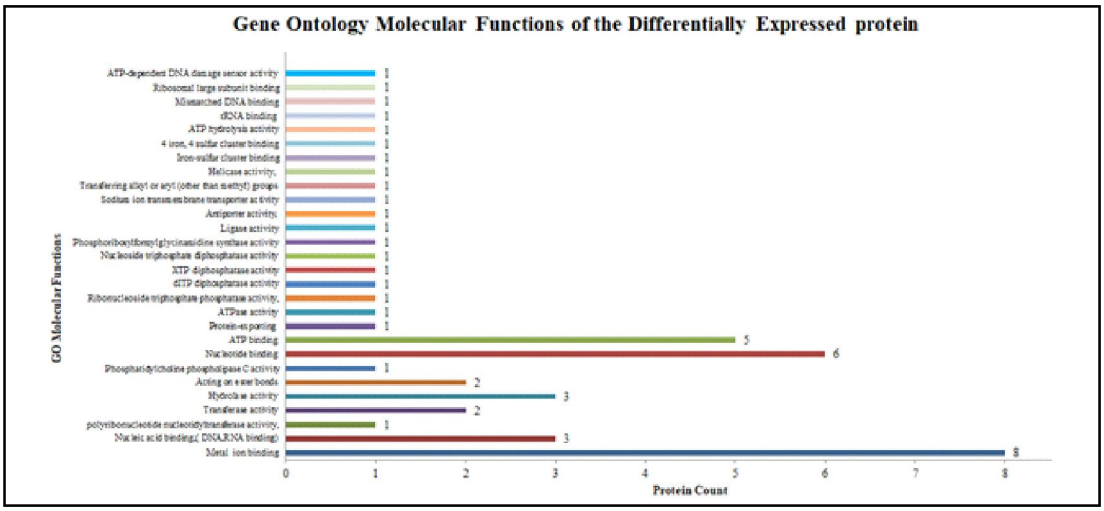

Fig. 1 Gene Ontology (GO) analysis representing the molecular functions of differentially expressed proteins

impaired DNA repair under AgNP-induced oxidative stress[7,2].

Gene Ontology Functional Classification:

As shown in Figure 1, GO analysis revealed that the differentially expressed proteins were mostly involved in ATP binding, nucleic acid binding, nucleoside metabolism, and transmembrane transport.

The majority of proteins, including the Sec translocation complex, were found in the plasma membrane and cytoplasm. The overall downregulation of DNA repair and transport proteins suggests that AgNP exposure upset critical cellular processes and overpowered bacterial defense mechanisms, even if some ATP-dependent stress proteins were preserved[12,4,6].

By causing extensive proteome changes, this study shows that silver nanoparticles mediated by *Boerhavia diffusa* have strong antibacterial efficacy against MDR *Bacillus cereus*. A multitarget inhibitory mechanism is confirmed by the downregulation of proteins involved in nucleotide production, protein translocation, DNA repair, and ion transport. Translational repression and oxidative stress are identified by proteomic analysis as key antibacterial tactics. The therapeutic promise of green-synthesized AgNPs as substitute medicines against diseases resistant to several drugs is supported by these findings.

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