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A Thorough Transcriptome Reveals the Toxicity of Cadmium and A Brand-New Metallothionein in Silkworms

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Abstract

Global heavy metal contamination has grown significantly in importance. The fact that heavy metals are absorbed by soil and have an impact on practically all organisms through biological cycles contributes to its broad scope. Through the soil-mulberry-silkworm system, heavy metal poisoning of silkworms (Bombyx mori) prevents larval growth and development and reduces silk production. In the current study, we used transcriptome sequencing of larval midguts exposed to cadmium to investigate the toxicological mechanism of the heavy metal. We discovered that endocytosis, oxidative phosphorylation, and MAPK signalling are three potential pathways that may be involved in cadmium infiltration. Additionally, we discovered a new metallothionein in silkworms that can enhance heavy metal tolerance in B. mori cell lines and is blocked by cadmium exposure. We also created a transgenic silkworm strain that overexpressed metallothionein, and the results demonstrated that this protein significantly improved larval survivability when exposed to cadmium. This study found and functionally validated BmMT, providing a novel possible heavy metal-tolerant silkworm type, and revealed a mechanism for cadmium toxicity.

Keywords: Metal Pollution; Cadmium; Toxicology; Silkworm; RNA

Introduction

Due to the ongoing growth of global industrialization, particularly those sectors involved in mining, metal processing and smelting, chemical production, and factory emissions, heavy metal pollution has become a significant environmental issue in many parts of the world. Cadmium (Cd), plumbum (Pb), chromium (Cr), mercury (Hg), and copper (Cu) are examples of heavy metals. The biological environment is severely harmed by an excess of these heavy metal ions in the pedosphere because they drastically lower soil production and are rarely destroyed. And to make matters worse, ecological cycles caused by heavy metal ions injure humans, animals, and even plants. These ions, for instance, build up in agricultural soils. For instance, these ions build up in agricultural soils, where they are absorbed by plants and crops. As a result, the food and water supply can have an impact on the health of animals and people. There are numerous documented instances of heavy metal contamination. Because of mercury poisoning in the water, thousands of people perished in Japan's infamous Minamata Disease Events between the 1950s and 1970s. China saw multiple incidences between 2005 and 2010 where hundreds of individuals became lead poisoned [1].

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Cell lines and a strain of silkworm

This study examined the toxicity mechanism of Cd, the most harmful heavy metal in China, using silkworms as a model organism. Because the larval midgut is the primary organ for mulberry leaf digestion and nutritional absorption, via which heavy metal ions enter the body, we select it as the target organ for the toxicological experiment. We performed a thorough transcriptome analysis of the midgut of larvae fed either regular mulberry leaves or leaves soaked in CdCl2 solution in order to better understand Cd toxicity and the mechanism by which silkworms react to it. To study the toxicological consequences of Cd, differentially expressed genes (DEGs) were discovered. Additionally, we verified one. In addition, we verified the validity of the first new metallothionein gene in silkworms, which improved Cd tolerance and survival in silkworm cell lines and Escherichia coli (E. coli). Additionally, a transgenic variant of silkworm that overexpresses metallothionein was developed to provide defence against extremely high Cd concentrations [3, 4].

Long-term domesticated model insect, the silkworm (Bombyx mori), has gradually lost its capacity for both pathogenic microbial toleration and treatment resistance. Mulberry trees store heavy metal ions and other harmful substances in their leaves, which they collect from the soil and atmosphere. As a result, the silkworms that eat them produce less cocoon silk, which is terrible for the silk business. Therefore, it was crucial to examine detoxification mechanisms in silkworms and the toxicological effects of heavy metals and other harmful elements. Several studies have revealed the toxicity of heavy

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metals and its method of action by infecting. Several investigations have reported the toxicity of heavy metals and its mode of action through infection of larval or cell lines. It is still unclear, though, if silkworms have the metallothionein gene and how to improve the insect's tolerance to heavy metals [5, 6].

In this study, the frequently utilised silkworm strain Dazao was examined. At 27 °C, fresh mulberry was fed to the larvae. We employed the silkworm cell lines BmE and Sid1, the former of which was derived from the ovary and the later from the embryo. BmE cells were grown in a culture dish containing 10% foetal bovine serum, penicillin/streptomycin, and Grace medium at a temperature of 27 °C. In IPL-41 media with 10% foetal bovine serum, Sid1 cells were grown. The State Key Laboratory of Silkworm Genome Biology provided them both. We provided mulberry leaves steeped in 80 mg/kg $CdCl_2$ solution or ddH_2O to larvae on day two of the fifth instar to investigate the harmful mechanism of Cd on silkworms. Larvae exposed to Cd showed definite toxic effects two days later. Compared to larvae fed with leaves steeped in ddH_2O, those exposed to Cd two days later displayed evident toxic signs as developmental delay, sluggishness, decreased appetite, and epidermal shrinking. Each larval midgut was gathered in order to learn more about the underlying mechanisms of Cd poisoning [7, 8].

Discussion

Through the entire food chain, heavy metal is a significant environmental stressor that has an impact on agricultural performance. The soil-mulberry-silkworm system causes heavy metal poisoning in silkworms, an important commercial insect (Jiang et al., 2020). This toxicity prevents larval growth and development, which lowers the quantity and calibre of silkworm cocoons produced. We chose Cd, the most prevalent soil contaminant in China, for this investigation [9].

Conclusion

The transcriptome analysis utilised in this study helped researchers understand how Cd exposure affected the B. mori midgut. The findings revealed that Cd toxicity involves endocytosis, oxidative phosphorylation, and the MAPK pathways. Additionally, we discovered a brand-new metallothionein in silkworms that enhances the tolerance to heavy metals in B. mori cell lines and E. coli. In addition, transgenic silkworms overexpressing metallothionein reduced the death of larvae exposed to Cd [10].

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Conflict of interest statement

The authors affirm that they have no known financial or interpersonal conflicts that would have appeared to have an impact on the research presented in this study.

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