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Proteome and phospholipid alteration reveal metabolic network of *Bacillus thuringiensis* under triclosan stress

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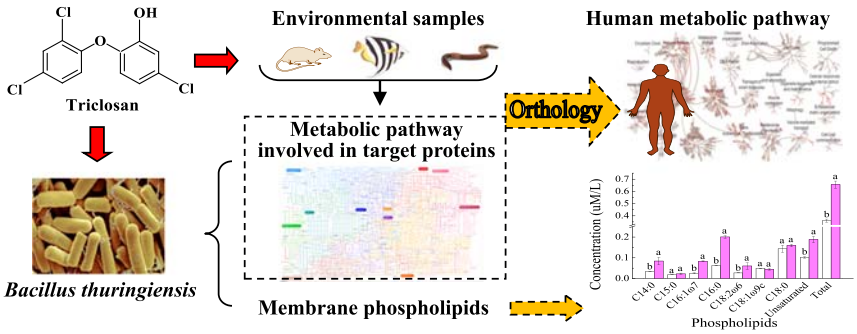
HIGHLIGHTS

- Cell metabolism under triclosan stress at pathway and network levels was explored.
- PyrH and Eno would be biomarkers to reflect triclosan stress.
- Glycolysis and pyruvate metabolism were inhibited by triclosan.
- Ten proteins responded to triclosan stress were mapped in human metabolic network.
- Omics approach was developed to evaluate the safety of target compounds.

GRAPHICAL ABSTRACT



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ABSTRACT

Triclosan is a common antibacterial agent widely applied in various household and personal care products. The molecule, cell, organ and organism-level understanding of its toxicity pose to some target organisms has been investigated, whereas, the alteration of a single metabolic reaction, gene or protein cannot reflect the impact of triclosan on metabolic network. To clarify the interaction between triclosan stress and metabolism at network and system levels, phospholipid synthesis, and cellular proteome and metabolism of *Bacillus thuringiensis* under 1 μM of triclosan stress were investigated through omics approaches. The results showed that C14:0, C16:1ω7, C16:0 and C18:2ω6 were significantly up-produced, and 19 proteins were differentially expressed. Whereas, energy supply, protein repair and the synthesis of DNA, RNA and protein were down-regulated. PyrH and Eno could be biomarkers to reflect triclosan stress. At network level, the target proteins ACOX1, AHR, CAR, CYP1A, CYP1B1, DNMT1, ENO, HSP60, HSP70, SLC5A5, TPO and UGT expressed in different species shared high sequence homology with the same function proteins found in *Homo sapiens* not only validated their role as biomarkers but also implied the potential impact of triclosan on the metabolic pathways and network of humans. These findings provided novel insights into the metabolic influence of triclosan at network levels, and developed an omics approach to evaluate the safety of target compound.

1. Introduction

As an antimicrobial agent and bactericide with a broad spectrum of antimicrobial activity, triclosan is widely used in personal care products, medical supplies and household cleaning products. Triclosan easily enters the natural environments through wastewater discharge and has been

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detected in most of the environmental samples due to its widespread use and incomplete removal from wastewater treatment plants (Amariei et al., 2017). For decades accumulation in natural ecosystems, its concentration in the aquatic environments, sediments and biosolids is up to 26.8 $\mu g \cdot L^{-1}$, 800 $\mu g \cdot kg^{-1}$ and 30 $mg \cdot kg^{-1}$, respectively (Dann and Hontela, 2011).

The biological toxicity and potential influences of triclosan on some species have been investigated. For example, in mammals, triclosan acted as an endocrine disruptor that reduced the expression of enzymes in thyroid hormone of rat and further affected brain development (Axelstad et al., 2013). For aquatic organisms, triclosan showed a potential risk against hemocytes of the marine gastropod *Haliotis tuberculata* (Gaume et al., 2012). At a concentration of 1 μM, it could fluctuate the lysosomal membrane of the mussel *Mytilus galloprovincialis* (Canesi et al., 2007). For bacterial, triclosan could disrupt the stability of bacterial membranes, leading to structural disturbances and loss of permeability barriers (Phan and Marquis, 2006). At molecular level, triclosan was found block bacterial fatty acid synthesis via inhibiting acyl carrier protein reductase (Levy et al., 1999). It was also reported that triclosan induced the transcriptional regulation of *fabI*, an efflux pump in *Pseudomonas*, and posed influences on the transport of substituted acyl-CoA reductase in *Alcaligenes xylosoxidans* (Meade et al., 2001).

However, each metabolic reaction, gene or protein is only a single node in a complicated metabolic network. The differentially expressed regulation of a single biomolecule does not imply the corresponding alteration of the related pathway or network. Omics aims at the collective characterization, function and quantification of all biomolecules produced in target cells or organisms (Mayer et al., 2011). It could be an insightful approach to reveal global metabolic response of organisms under various stresses. To clarify the interaction between triclosan stress and cellular metabolism at network and system levels, and develop an omics approach to evaluate the toxicity of target compounds, the current study would investigate the protein, phospholipid and metabolism response of target organism to triclosan stress using proteomics and lipidomics technologies. Triclosan is a chlorinated aromatic compound that has functional groups representative of both ethers and phenols. The potential findings related to its omics responses will present an insightful reference to the compounds with the same functional groups.

The Gram-positive bacterium *Bacillus thuringiensis* is famous for forming insecticidal crystal proteins, which have highly toxic effect to some susceptible insects (Treitz et al., 2015). In insect midgut, crystal proteins can activate cellular signaling thereby lead to cell death by binding the midgut cadherin-like protein (Zhang et al., 2005). Due to this insecticidal effect, *B. thuringiensis* has been widely used as a biopesticide employed in agriculture (van Frankenhuyzen, 2009). Moreover, *B. thuringiensis* owns unique gene families related to the degradation of pollutants (Tang et al., 2016) and the regulation of complicated metabolic network under a wide range of environmental fluctuations (de Been et al., 2006). Therefore, it would be a perfect modal microbe for investigating the metabolic network response of cells under triclosan stress.

The aim of this work is to explore the interaction between triclosan stress, protein interaction and cellular metabolism of *B. thuringiensis* at pathway and network levels. To this end, the membrane potential, phospholipids and proteome expression of *B. thuringiensis* under triclosan stress were investigated through flow cytometry, gas chromatography-mass spectroscopy and Sciex Triple TOF 5600 mass spectrometer analysis. The metabolic impact of triclosan on the different species and human was also studied through phylogenetic and reactomic approaches. The findings would provide novel insights into the metabolic influence of triclosan on cells at system and network levels.

2. Materials and methods

2.1. Strain and chemicals

B. thuringiensis GIMCC1.817 was an effective microbe for the degradation of multi-pollutants and was stored at the Microbiology Culture Centre of Guangdong Province, China. Triclosan (CAS registration number 3380-34-5) was purchased from Sigma–Aldrich (St. Louis, MO, USA), and was dissolved in the chromatographic grade of methanol to prepare a stock solution at 1 $g \cdot L^{-1}$. Lysogeny broth used for cell culture contained (in $g \cdot L^{-1}$) 5 beef extract, 5 NaCl and 10 peptone. The treatment medium for cellular exposure to triclosan contained (in $g \cdot L^{-1}$) 0.03 beef extract, 0.1 peptone, 0.07 NaCl, 0.03 KH_2PO_4, 0.03 NH_4Cl and 0.01 $MgSO_4$, respectively.

2.2. Microbial culture and triclosan treatment

The cells of *B. thuringiensis* GIMCC1.817 were inoculated into lysogeny broth medium and cultured at 100 $r \cdot min^{-1}$ on a rotary shaker at 30 °C for 12 h. After separation from the medium by centrifugation at 1300 × *g*, the cells were washed three times by sterile phosphate buffer (pH 7.4).

The washed cells were then used to prepare a cellular suspension by adding sterile phosphate buffer. Immediately, the bacterial suspension was added to the treatment medium at the final biomass of 1.5×10^{12} colony-forming unit. Subsequently, the triclosan stock solution at 1 $g \cdot L^{-1}$ was pipetted into the treatment medium, in which the initial concentration of triclosan was 1 μM. All samples were cultured in the dark at 30 °C on a rotary shaker at 100 $r \cdot min^{-1}$. The cells in two groups of samples were collected in 0, 5, 12, 18 and 24 h, respectively, and were utilized to measure membrane potential. In addition, the cells in two groups of samples collected in 24 h were used to extract phospholipids, proteins and RNA.

2.3. Analysis of membrane potential

The BD™ MitoScreen kit (BD, San Jose, US), which consists of lyophilized JC-1 reagent, dimethyl sulfoxide (DMSO) and 10-fold assay buffer, was designed for use in flow cytometry. Briefly, the lyophilized JC-1 reagent was dissolved at room temperature with 125 μL DMSO per vial to yield a JC-1 stock solution. The 1-fold assay buffer was prepared by diluting the 10-fold assay buffer in deionized water. Next, the JC-1 dye solution was prepared by diluting the JC-1 stock solution 1:100 with 1fold assay buffer.

After the collected cells were suspended in 200 μL of JC-1 dye solution, all the samples were incubated at 25 °C for 15 min in a dark place. Subsequently, the stained cells were pipetted to tubes with BD CaliBRITE™ beads and analyzed by FACSAria flow cytometer (BD, USA). Briefly, 10,000 cells of each sample were analyzed by the laser beam at the excitation wavelength of 488 nm individually. The detector measured emission intensity was set at 605 to 625 nm. For each cell, the scattered light was detected by a photo diode at 2 different positions (forward and side scattered light) and converted into electric signals and fluorescence intensity. The JC-1 monomers emitted green fluorescence, whereas, the aggregates emitted red fluorescence. These two kinds of fluorescence were captured through 527 and 590 nm long-pass filters, respectively. The mean values of three parallel samples were statistically analyzed by SPSS version 17.0 using the one-way ANOVA method.

2.4. Extraction and determination of membrane phospholipids

The extraction method of phospholipids was referred to reference (Yang et al., 2017). After extraction, phospholipids were analyzed using gas chromatograph tandem mass spectrometer (GC-MS) (SHIMADZU GCMS-QP 2010 Ultra) equipped with a DB-5MS (30 m × 0.25 mm × 0.25 μm) quartz capillary column. The condition of GC-MS analysis was as follows: the column temperature was 140 °C, remained 2 min and heated to 260 °C at a rate of 3 $°C \cdot min^{-1}$. The carrier gas was He. The sample inlet temperature and ion source temperature were set to 250 °C and 230 °C, respectively. The mass spectrometry scanning range was 50–500 $m \cdot z^{-1}$. Quantification of phospholipids was used