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硕 士 学 位 论 文

钝顶螺旋藻对硒、碘、锌复合培养的响应与蛋白质组学研究

Proteomic analysis of *Spirulina* (*Arthrospira*) *platensis* (Cyanophyta) in response to a combination of Selenium, Iodine and Zinc treatment

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Contents

List of Figures

List of Tables

摘要

钝顶螺旋藻(*Spirulina*（*Arthrospira*） *platensis* 869S)是一种蓝藻，具有多种保健作用，而且是一种运用生物技术富集微量元素的良好材料。本文利用蛋白质组学技术研究了钝顶螺旋藻对培养基中添加复合微量元素（硒 Se，碘 I 和锌 Zn）的分子响应机制，文中采用的 Se，I 和 Zn 浓度为非致死浓度。结果表明， 20 $mg.L^{-1}$ Se, 900 $mg.L^{-1}$ I 和 10 $mg.L^{-1}$ Zn 浓度组对藻体生长没有影响，且藻体富集的 3 种微量元素浓度较高，分别为 77.25 $\mu g.g^{-1}$D.W 的硒, 18.55 $\mu g.g^{-1}$D.W 的碘和 216.6 $\mu g.g^{-1}$D.W 的锌。高浓度复合微量元素（100 $mg.L^{-1}$ Se, 1800$mg.L^{-1}$ I and 20 $mg.L^{-1}$ Zn）中只有锌对藻体具有毒性作用，而 Se 和 I 可以减缓这种毒害作用。ICP-MS 结果表明高浓度的锌实验组（100 $mg.L^{-1}$ Se, 1800$mg.L^{-1}$ I and 20 $mg.L^{-1}$ Zn）中细胞内富集的锌显著减少，但对其他金属离子影响不显著，除了 Mg。在这高浓度复合微量元素培养中，藻类总生化组分也发生了显著的改变。虽然叶绿素 a 和 PC 产量都受到了抑制影响，但叶绿素 a 更易受影响（降低了 47.33%）。同时也观察到了蓝藻对环境胁迫响应的特点，总蛋白质降低到对照组的 73%（生物量干重的比值），而碳水化合物和脂类增加，特别是脂类增加显著，比对照组增加 76%。

分别提取对照组和高浓度 Se,I 和 Zn 实验组中的总蛋白质，利用蛋白质组学技术分析了细胞在蛋白质表达水平上对高营养盐胁迫的响应。2-DE 电泳结果发现对照组和高浓度组间有 61 个差异蛋白点。利用 MALDI-TOF/TOF-MS 和 MALDI-TOF-MS 分析这 61 个差异蛋白，发现其中 44 个为上调蛋白，17 个为下调蛋白。这些蛋白质可分为几个不同的功能类群，可能与适应高浓度锌毒性的多种途径相关。其中，大多数蛋白质与细胞的翻译和光合作用系统相关。在本研究中，我们假设光合作用是持续进行的，这是通过连接肽调节的一种藻胆蛋白构象改变的结果，可以使光能传递至反应中心。硒和碘对细胞在高浓度金属毒害中的保护作用可能与多功能的氧化还原酶（解毒酶）有关。令人意外的是也观察到了 2 个延伸因子-Tu 基因的拮抗调节。同时，检测到了 2 个蓝藻中了解很少的蛋白，分别为 DNA 旋转酶肽酶 U62 调节子和带 7 蛋白。研究结果表明，高特异性基因组在钝顶螺旋藻适应外界胁迫中具有重要作用。

关键词：钝顶螺旋藻；硒；碘；锌；蛋白质组学

Abstract

Spirulina（*Arthrospira*） *platensis* strain 869S, a cyanobacterium, exhibits myriad health benefits and is a suitable matrix for biotechnological incorporation of new food trace element preparations. Proteomic analysis was carried out to gain insight into the molecular mechanisms underlying the response of this alga to a mixture of trace elements in the growth medium.

In our study, we used the culture groups differing in Se, I and Zn concentration combinations, but in no-lethal range. The results showed that at suitable concentration combination, the supplemented elements were incorporated into *Spirulina platensis* cells. At 20 $mg.L^{-1}$ Se, 900 $mg.L^{-1}$ I and 10 $mg.L^{-1}$ Zn combination treatment, the cellular accumulation of Se, I and Zn amounted up to 77.25 $\mu g.g^{-1}$D.W, 18.55 $\mu g.g^{-1}$D.W and 216.6 $\mu g.g^{-1}$D.W respectively, without any adverse effect on algal growth. Upon high concentration combination treatment (100 $mg.L^{-1}$ Se, 1800$mg.L^{-1}$ I and 20 $mg.L^{-1}$ Zn), only Zn was found to be toxic to cells while sufficient availability of Se and I was beneficial for the algal protection against damaging effects of the former. ICP-MS analysis results indicated that *S. platensis* response to the 100 $mg.L^{-1}$ Se, 1800 $mg.L^{-1}$ I and 20 $mg.L^{-1}$ Zn treatment led to drastic decrease in cellular Zn accumulation without significant effect to the other metals (Ca, Fe and K), except Mg. At this high concentration combination, the general biochemical composition of the alga was also significantly altered. Although inhibitory effect was observed to both Chl *a* and phycocyanin production, the Chl *a* was the more vulnerable reaching up 47.33% decrease. Likewise, the general features of acclimation process for cyanobacteria upon stress exposure were observed. Hence, total protein content in dried biomass dropped to about 73% compared to the control, whereas carbohydrates and lipids increased, the latter being the most enhanced up to 76% over the control.

Protein expression profile in response to this stress was examined using proteomic approach. Total proteins were extracted from both control and the treated cells with high Se, I and Zn combination treatment, and separated by two-dimensional gel electrophoresis. A total of 61 differentially abundant spots were found from the 2-DE result. The 61 differentially protein spots were then analyzed using MALDI-TOF/TOF-MS and MALDI-TOF-MS and the results showed that 44 of

which were up regulated and 17 down-regulated. The identified proteins were grouped into different functional categories and were related to diverse processes which might work cooperatively to adapt to zinc toxicity. The majority of identified proteins are associated with translation and photosynthesis system. In this work, we supposed that photosynthesis was sustained as a result of PBSs conformational adaptation via their linker peptides regulation so as to enable light energy transfer to the reaction center. The protective role of Se and I in *S.platensis* response to the heavy metal toxicity was associated with multifunctional oxidoreductases. Unexpectedly, the antagonistic modulation of two elongation factor-Tu genes was found. Also, the regulation of Peptidase U62 modulator of DNA gyrase and Band 7 protein, whose functions are poorly understood in cyanobacteria, was noted. The results from the present work suggested that the recently revealed high proportion of genome specificity in this species is very crucial in tolerance to environmental disturbances.

Keywords: *Spilurina platensis*; Selenium; Iodine; Zinc; Proteomics

List of Abbreviations

2-DE: two-dimensional electrophoresis

ABC: ATP binding cassette

ACN: acetonitrile

APC: Allophycocyanin

APS: Ammonium persulfate

APX: Ascorbate peroxidase

ATP: Adenosine triphosphate

BSA: Bovine serum albumin

CA: carrier ampholytes

Ca-SP: calcium Spirulana

CCD: charged-coupled device

CHAPS: 3-[(3-cholamidopropyl) dimethylamino]-1-propanesulfonate

Chl *a:* Chlorophyll *a*

C-PC: C-phycocyanin

DTT: dithiothreitol

DW: Dry Weight

ESI-MS: electrospray ionization-mass spectrometry

ESTs: expressed sequence tags

FT-ICR-MS: Fourier transform ion-cyclotron resonance- mass spectrometry

GPX: Glutathione peroxidase

GST: Glutathione S-transferase

GST: Glutathione S-transferase

HCCA/CHCA: α-cyano-4-hydroxycinnamic acid

HPLC: high-performance liquid chromatography

IAA: Iodoacetamide

IARC: International Agency for Research on Cancer

ICP-MS: Inductively Coupled Plasma –Mass Spectrometry

IDD: Iodine deficiency disorders

IEF: Isoelectric focusing

IPG: immobilized pH gradient

IT-MS: ion trap-mass spectrometry

LPS: Lipopolysaccharide

MALDI-TOF: Assisted Laser Desorption/Ionization- Time of Flight

Mowse: Moleculae weight search

MS: Mass Spectrometry

MW: molecular weight

NADPH: Nicotinamide Adenine Dinucleotide Phosphate

NCBI: National Centre for Biotechnology Information

OD: Optical Density

OT-MS: Orbitrap-mass spectrometry

PBSs: Phycobilisomes

pI: isoelectric point

PMF: peptide mass fingerprinting

PMSF: phenylmethanesulphonylfluoride

PS: Photosystem