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生物实验室

基于非数据依赖的鞘脂菌蛋白质组学分析方法的建立

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摘 要:【背景】鞘脂菌是一类可高效降解以菲为代表的多环芳烃有机污染物的菌株，其在环境污染治理及生物技术领域具有广阔的应用前景。【目的】为了优化测试方法，获得更完整的鞘脂菌 *Sphingobium yanoikuyae* SJTF-8 在菲胁迫下表达差异的蛋白。【方法】利用数据依赖型及数据非依赖型两种蛋白质组学数据采集方法，比较了鞘脂菌 SJTF-8 在菲胁迫下蛋白质水平的表达变化。【结果】两种技术方法下共得到 580 个表达差异蛋白，这些蛋白在细胞代谢、转运和调控等方面发挥一定功能。【结论】数据非依赖性采集(data-independent acquisition，DIA)技术在重复性以及低丰度蛋白的检测上明显好于数据依赖型采集(data dependent acquisition，DDA)技术，因此，DIA 在实际可用的表达差异蛋白检出方面具备明显优势，为发现菲胁迫下细胞诱导表达的低丰度调控蛋白提供帮助。

关键词: 数据非依赖性质谱采集方法，数据依赖型质谱采集方法，菲，鞘脂菌

Establishment of a data-independent acquisition proteomic analysis method for *Sphingobium yanoikuyae*

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Abstract: [Background] *Sphingobium* is a kind of bacteria which can effectively degrade polycyclic aromatic hydrocarbons (PAHs) represented by phenanthrene. This characteristic makes *sphingobium* have broad application prospects in the field of environmental pollution control and biotechnology. **[Objective]** The aim of this study is to optimize the quantification method in order to acquire the complete differentially expressed proteins of *Sphingobium yanoikuyae* SJTF8 under phenanthrene stress. **[Methods]** Data dependent acquisition (DDA) and data-independent acquisition (DIA) proteomics analysis methods were used in this experiment. **[Results]** These two methods had commonly quantified 580 differentially expressed proteins, which played important roles in cell metabolism, transport and regulation. **[Conclusion]** DIA has a significant advantage in the quantification of differentially expressed proteins, which is helpful for the discovery of low abundance regulatory proteins induced by phenanthrene stress.

Keywords: Data-independent acquisition, Data dependent acquisition, Phenanthrene, *Sphingobium yanoikuyae*

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多环芳烃(PAHs)是一种普遍存在于生态系统中的全球性污染物，由于其具有潜在的毒性、致癌性、致畸性和致突变性，因而受到人们的广泛关注[1]。微生物法修复环境中多环芳烃污染是一种被广泛研究的手段[2]。菲作为美国环境保护署重点关注的污染物之一，已被广泛用作研究这些微生物降解多环芳烃的模型物质[3]。菲是一种低分子量、具有 3 个苯环结构的疏水性多环芳烃。菲的化学性质非常稳定，易在环境中沉积，并通过食物链向人体或者动物富集使细胞产生癌变[4]。

数据非依赖性采集(data-independent acquisition，DIA)技术是近几年新出现的蛋白质组学非标记定量技术，最早由 Venable 在 2004 年提出[5]。不同于传统的需要选择特定母离子进行碎裂的数据依赖型采集(data dependent acquisition，DDA)技术，DIA 可采集所有的碎片离子信息，从而具有全景式扫描、数据可回溯等优势[6]。得益于数据分析和质谱采集速度、质量精度和分辨率的显著进步，近几年来 DIA 技术发展迅速，涌现出大量新的 DIA 优化改进技术和多个自动化 DIA 数据分析工具[7]。高质量的 DIA 定量技术作为蛋白质组定量新技术将会被逐渐推广并应用于生命科学和医学等不同领域的研究中[8-9]。

本实验室长期从事微生物环境修复菌的筛选和降解调控机制的研究。我们在土壤中筛选得到一株可高效降解菲的鞘脂菌(*Sphingobium yanoikuyae* strain SJTF-8)，为了进一步比较 DDA 和 DIA 定量技术的特点和在蛋白质组学中的应用范围，本研究以这株可高效降解菲的鞘脂菌为研究对象，利用 DDA 和 DIA 两种蛋白质组学定量技术比较鞘脂菌 SJTF-8 在菲胁迫下蛋白质水平的表达变化，以期发现更多参于多环芳烃降解和调控的蛋白，为微生物降解多环芳烃机制的研究提供理论依据。

1 材料与方法

1.1 样品

实验菌株鞘脂菌 SJTF-8 由上海交通大学梁如冰实验室分离得到，在菲中生长良好。

1.2 培养基

基础盐培养基(g/L)：KH_2PO_4 4.500，K_2HPO_4 13.750，$(NH_4)_2SO_4$ 2.000，$MgSO_4·7H_2O$ 0.160，$FeSO_4·7H_2O$ 0.005，$CaCl_2$ 0.011，$MnCl_2·4H_2O$ 0.002。

1.3 主要试剂和仪器

菲，Sigma 公司；测序用胰酶，Promega 公司。非接触式超声破碎仪，Scientz 公司；XBridge Peptide BEH C_{18} 色谱柱，Waters 公司；Ultimate3000 液相色谱仪、EASY-nLC 1200 纳升液相色谱仪、Q-Exactive Plus 质谱仪、离心浓缩仪，Thermo Fisher 公司。

1.4 实验方法

实验方法设计流程图详见图 1。

1.4.1 细菌培养

采用 LB 培养基和含 250 μg/mL 菲的基础盐培养基培养细胞。鞘脂菌 SJTF-8 在 30 °C、220 r/min 振荡培养，12 000 r/min 离心 20 min 收集 LB 培养的鞘脂菌 SJTF-8，用 1×PBS 缓冲液洗涤 3 次，将部分预培养细菌接种到含有菲的培养基中，并按上述方法培养，每种条件下培养 3 份样品。

1.4.2 菌全蛋白的提取及方法优化

12 000 r/min 离心 20 min 收集培养细胞，并用 1×PBS 缓冲液洗涤 3 次。加入 600 μL 细胞裂解液(50 mmol/L NH_4HCO_3，8 mol/L 尿素)充分悬浮细胞，置于非接触式超声破碎仪中超声破碎。超声程序为：效率 99%，超声 10 s，停止 5 s。为了测试非接触式超声破碎仪破碎鞘脂菌的效率，优化鞘脂菌的提取方法，细菌的超声时间分别设置为 15、30、45、60 min。不同时间超声获得的细胞裂解液分别于 4 ℃、12 000 r/min 离心 30 min 去除细胞碎片。用 BCA 法测定蛋白浓度。

1.4.3 蛋白的酶解

在 37 °C 下使用 1 mol/L DTT 将每个样品中的 50 μg 蛋白质还原 1 h。使用 1 mol/L 碘乙酰胺(IAA)在避光处室温下处理半胱氨酸残基 40 min。参考 FASP 方法，使用测序级胰蛋白酶(Promega，USA)酶解蛋白过夜，胰蛋白酶与蛋白质的质量比为 1:50[10]。12 000 r/min 离心 20 min 收集酶解后产生的多肽混合物，用离心浓缩仪干燥样品，溶解在 0.1%甲酸溶液中待测。

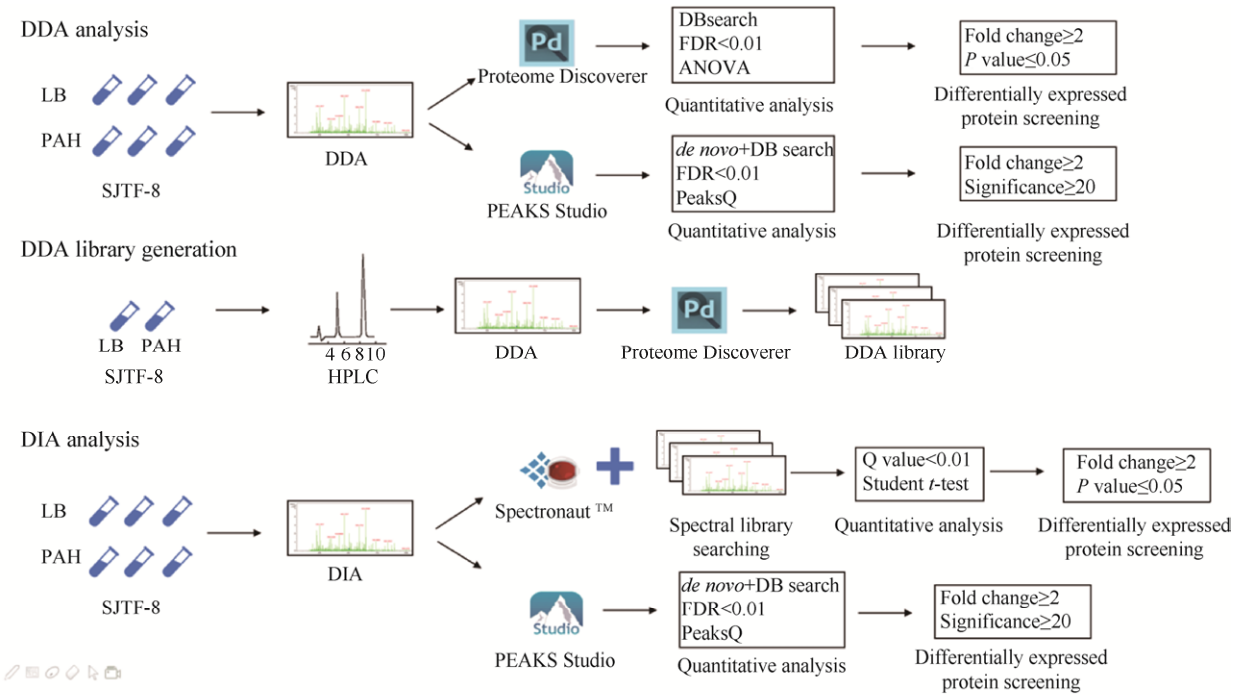

图 1　质谱数据处理流程图

Figure 1　Mass spectrum data processing flow

1.4.4　DDA 分析

酶解后的蛋白通过 Easy-nLC 1200 纳升液相色谱仪和含有纳米喷雾源的 Q-Exactive Plus 质谱仪进行分析。每个多肽样品用 0.1%甲酸重新溶解，并在 C_{18} 色谱柱(50 mm×15 cm，1.7 μm)中分离。采用溶剂体系(流动相 A：99.9%水和 0.1%甲酸；流动相 B：80%乙腈和 0.1%甲酸)分离多肽。以 200 nL/min 的流速使用 120 min 梯度洗脱肽，多肽洗脱梯度为：0−2 min，2%−6% B；2−97 min，6%−22% B；97−107 min，22%−32% B；107−109 min，32%−95% B；109−119 min，95% B；119−120 min，95%−2% B。质谱仪在数据依赖(DDA)模式下操作，在分辨率为 70 000 (AGC 3e6)的轨道阱中进行全扫描采集(*m/z* 350−1 500)。将分离出的前 20 个肽信号(电荷态≥+2)母离子通过高能碰撞(HCD)破碎，标准化碰撞能(normalized collision energy，NCE)为 28.0。毛细管的温度是 275 °C，喷雾电压是 1 900 V。子离子在分辨率为 17 500 (AGC 1e5)的轨道上测量。全扫描和 MS-MS 扫描的最大填充时间分别设置为 50 ms 和 45 ms，动态排除时间设置为 30 s。

1.4.5　DDA 谱图库的建立

取 200 μg 鞘脂菌蛋白的酶解产物通过高 pH 反相柱分离成 8 个组分。采用 XBridge Peptide BEH C18 色谱柱(250×4.6 mm，5 μm，Waters)，流动相 A 为 5 mmol/L 甲酸铵(pH 10.0)，流动相 B 为 5 mmol/L 甲酸铵和 95%乙腈混合液(pH 10.0)，流速 0.8 mL/min，进样量 25 μL，柱温 40 °C，分离梯度为 0−5 min，2% B；5−6 min，2%−5% B；6−8 min，5%−8% B；8−43 min，8%−25% B；43−54 min，25−40% B；54−54.5 min，40%−95% B；54.5−59.5 min，95% B；59.5−60 min，95%−2% B；60−65 min，2% B。将收集的溶液进行干燥，并重新溶解在 0.1%甲酸中，用 nano-LC-MS/MS 进行分析，分析仪器及分析方法同 1.4.4。

将质谱产生的原始数据用 Proteome Discovery (PD，2.3 版本)进行分析，生成谱图库。消化酶设置为 Trypsin，最大漏切设置为 2。母离子和子离子质量偏差分别设置成 1×10^{-5} 和 0.02 Da。固定修饰

为 Carbamidomethyl (C),可变修饰为 Oxidation (O), Deamidated (NQ)。使用 Target/Decoy 验证方法，多肽和蛋白的 FDR 均设置为 1%。用 Spectronaut 11.0 (Biognosys AG 2013，后文及表格中简写为 SPE)打开 PD 生成的.pdresult 搜库结果文件，除不选择 iRT 校正外，谱图库参数均按照软件默认设置。

1.4.6 DIA 分析

酶解后的多肽样品色谱分析方法同 1.4.4 DDA 分析。质谱仪在数据非依赖(DIA)模式下操作，在分辨率为 70 000 (AGC 3e6)的轨道阱中进行全扫描采集(*m/z* 350−1 405)，见表 1 设置 30 个可变窗口，全扫描 IT 为 50 ms。子离子在分辨率为 17 500 (AGC 1e6，IT 自动化，默认带电量为 2)的轨道上测量。

表 1 DIA 隔离窗口设置

Table 1 DIA isolation window settings

窗口序号 Window No.	开始质核比 Start m/z	宽度 Width
1	372.5	34.5
2	407	25
3	432	21
4	453	18
5	471	18
6	489	18
7	507	18
8	525	18
9	543	18
10	561	18
11	579	18
12	597	18
13	615	18
14	633	18
15	651	18
16	669	18
17	687	18
18	705	18
19	723	18
20	741	18
21	759	18
22	777	18
23	795	22
24	817	25
25	842	25
26	867	25
27	892	42
28	934	140
29	1 074	220
30	1 294	No data

1.4.7 数据处理

(1) PD 中 DDA 数据处理(PD-DDA)

将 DDA 质谱原始数据导入 PD 搜索工具，在峰识别后对每个样品进行 Label-free 定量分析，使用 Uniprot_SJTF-8 蛋白数据库(5 343 条目)进行搜库处理。设置如下：消化酶设置为 Trypsin，最大漏切设置为 2。母离子和子离子质量偏差分别设置成 1×10^{-5} 和 0.02 Da。固定修饰为 Carbamidomethyl (C)，可变修饰为 Oxidation (O)，Deamidated (NQ)。多肽和蛋白定性的 FDR 设置为 1%，蛋白质的定量基于前 3 个肽段母离子峰面积。以 LB 培养细胞中的蛋白质为对照组进行无标记分析，将蛋白质表达的倍数变化 2 或 0.5 和 $P\leq0.05$ 作为生物学效应的阈值。

(2) PEAKS 中 DDA 与 DIA 数据处理(PEAKS-DDA，PEAKS-DIA)

将采集到的质谱数据导入 PEAKS Studio10.0 (Bioinfomatics Solutions Inc.)中，使用 Uniprot_SJTF-8 蛋白数据库(5 343 条目)进行数据处理。将数据采集模式分别设置为 DDA 模式和 DIA 模式，其余参数设置相同：母离子和二级谱图的质量偏差分别设置成 1×10^{-5} 和 0.02 Da；胰酶的最大漏切位点数为 2；固定修饰为 CarbamiDomethyl (C)，可变修饰为 Oxidation (O)、Deamidated (NQ)；多肽和蛋白定性的 FDR 设置为 1%。以 LB 培养细胞中的蛋白质为对照组进行无标记分析，将蛋白质表达变化倍数≥2 或≤0.5 和 Significance≥20 作为生物学效应的阈值。

(3) Spectronaut 中 DIA 数据处理(SPE-DIA)

将经 DIA 分析后的质谱数据导入 Spectronaut 11.0 (Biognosys AG 2013)中，使用 PD 生成的谱图库进行数据处理。分析参数使用软件默认设置，所有蛋白和肽定性分析在 *Q* value 为 0.01 的水平下进行(相当于 FDR 为 1%)，在 MS2 水平下通过峰面积进行定量分析，肽丰度通过对其各自的 MS2 片段离子的峰面积求和计算，蛋白质丰度是通过对其各自的肽丰度求和计算。以 LB 培养细胞中的蛋白质为对照组进行无标记分析，将蛋白质表达变化倍

数≥2 或≤0.5 和 P≤0.05 作为生物学效应的阈值。

1.4.8 生物信息学分析

得到差异蛋白后，使用 Uniprot (https://www.uniprot.org/uploadlists/)和 String (version 11.0 http://string-db.org/)，分别对差异蛋白进行 GO 分析和 KEGG 信号通路分析[11]。

2 结果与分析

2.1 鞘脂菌 SJTF-8 全蛋白提取效率优化

实验结果发现在含菲培养基中生长的鞘脂菌(菲细胞)生长过程中会产生大量胶状物质，与 LB 条件下培养获得的细胞(LB 细胞)相比，在相同的超声破碎条件下，菲细胞的破碎效率低于 LB 细胞。据此推测可能是因为鞘脂菌会分泌一种名叫结冷胶的胞外物质，导致了菲细胞破碎效率的降低[12-13]。因此专门对菲细胞破碎效率做了优化，如图 2 所示，破碎时间为 45 min 及以上的样品蛋白浓度是 30 min 及以下的近 2 倍，说明破碎时间对鞘脂菌 SJTF-8 细胞中的蛋白提取效率有影响，更长的超声时间有助于提高细胞破碎效率，超声时间超过 45 min 时，破碎效率没有明显变化。因此，选择使用 45 min 的超声时间来裂解细胞。

2.2 DDA 与 DIA 数据采集模式结果比较

2.2.1 定性定量结果比较

分别整合了不同软件对 DDA 和 DIA 两种模式下所得数据的分析结果并进行比较。如图 3 所示，在 DDA 模式下，共定性和定量到 2 894 个和 2 887 个蛋白；在 DIA 模式下，共定性和定量到 2 661 个和 2 623 个蛋白。以上结果表明 DIA 和 DDA 模式得到的定性定量蛋白数量相当，DIA 对于 DDA 没有明显优势。然而在高等动物的蛋白质组学研究中，DIA 技术的定量能力优于 DDA[14]，推测可能是由于本实验以细菌作为研究样本，较为简单，蛋白数目比高等生物少，现有的仪器在 DDA 模式下就可以采集到较为全面的数据。另外，比较了各个软件对 DDA 及 DIA 数据的分析结果。SPE 中得到的 DIA 蛋白数量略少于 DDA 所得结果，这可能是因为 SPE 的分析方法依赖于事先构建好的 DDA 谱图库，而已经构建的 DDA 谱图库不能包含所有肽段信息，导致部分蛋白分析结果丢失[15]。因此尝试在 PEAKS 软件中使用搜库的方法对 DDA 和 DIA 的数据同时进行分析，结果表明 DIA 的蛋白鉴定

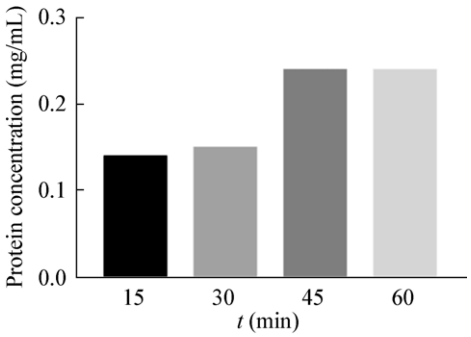

图 2 不同超声时间下鞘脂菌 SJTF-8 全蛋白提取效率比较

Figure 2 Comparison of total protein extraction efficiency of *Sphingobium yanoikuyae* strain SJTF-8 under different breaking time

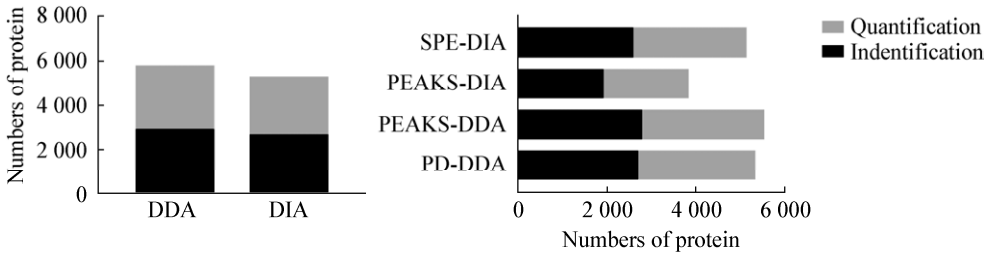

图 3 DDA 与 DIA 定性与定量蛋白数目比较

Figure 3 Comparison of identity and quantity protein numbers between DDA and DIA

注：A：DDA 和 DIA 两种质谱模式下采集数据在不同软件中分析结果的整合；B：4 种软件对 2 种质谱模式下所得数据分别分析的结果.

Note: A: The integration of data collected in different software analysis results under DDA and DIA; B: The respectively analyzing result by four software under two mass spectrometry modes.

数目仍然小于 DDA 中蛋白鉴定数目，这可能是由于 DIA 二级谱图包含采集窗口内所有子离子信息，数据量远大于 DDA 的数据量，PEAKS 软件使用 *de novo* 算法结合直接搜库的功能对 DIA 数据进行图谱解析较困难，从而使得 DIA 谱图解析率达不到 DDA 的水平。

2.2.2 缺失值分析

在对所有数据进行整体分析后，根据每组中样品的检出度，将所有定量到的蛋白分成A和B两组，如表 2 所示，A 组为菲诱导表达而 LB 完全不表达蛋白以及 LB 诱导表达而菲完全不表达蛋白，B 组为剔除 A 组蛋白后剩余定量蛋白。A 组蛋白部分样品定性定量的缺失是由于样品本身蛋白表达差异造成，不适合用来做软件缺失值分析。因此，仅筛选了 B 组蛋白完成缺失值分析。如表 2 所示，在 DIA 质谱方法下，样品的缺失率为 2.38%和 1.95%，明显小于 DDA 方法。推测可能是由于在 DDA 模式下，实时信号采集会根据母离子信号强度歧视性地选择碎裂离子，对于丰度较低的肽段和相应蛋白很难保证每次实验信号采集的可重复性[16-17]。所以，相比于 DDA 技术，DIA 质谱采集方法得到的数据稳定性更好、可重复性更强、定量的准确性更高。

2.2.3 重现性分析

利用 Spectronaut 软件分析 DIA 数据获得定量蛋白火山图。如图 4 所示，软件根据蛋白丰度从上到下进行排序后发现，A 组中大多数蛋白为低丰度蛋白，并且其中有相当一部分低丰度蛋白仅在菲中表达，说明 DIA 模式对发现鞘脂菌在多环芳烃中低丰度表达的差异蛋白具有一定意义。

为了判断 A 组中低丰度蛋白在 DDA 和 DIA 中的重现性，我们选取了 PEAKS-DDA 与 SPE-DIA 作为 DDA 和 DIA 结果的代表，将 SPE-DIA 和 PEAKS-DDA 的 A 组蛋白和对方的所有定量蛋白以及经 CV≤20%筛选后的定量蛋白进行比较。如图 5 所示，SPE-DIA 的 A 组蛋白中有 260 个蛋白在 PEAKS-DDA 中也被鉴定到，占总蛋白数目的 71.8%。但是经 CV≤20%筛选后重叠蛋白数显著降低，只有 22 个蛋白在 PEAKS-DDA 中被鉴定到，约 90% (238 个)的蛋白在 PEAKS-DDA 定量方式中因为重现性差而被过滤弃用。用同样的方法分析 PEAKS-DDA 的 A 组数据，在 SPE-DIA 中鉴定到的蛋白数目过滤前后分别为 44 和 30 个，重叠蛋白数目变化不大。DIA 采集模式的原理是对于所有落在隔离窗口范围内的多肽离子都将被同时碎裂和检测，因而许多低丰度的蛋白也被系统化、无歧视地一并记录下来，从而表现出组内蛋白定量变异系数小的特点[18-19]。然而在 DDA 中，低丰度蛋白由于歧视效应较难被可重复性检测，因而其组内蛋白定量变异系数大，导致定量准确性差。

2.2.4 DDA 与 DIA 技术方法比较

如表 3 所示，我们从样品前处理到数据分析及技术性能等多方面比较了 DDA 与 DIA 技术的相同点与不同点。可以发现，对于 DDA 和 DIA 方法

表 2 两种质谱模式下定量结果缺失值比较

Table 2 Comparison of missing values of quantitative results from DDA and DIA in three different software

分析软件 Analysis software	定量蛋白 Total quantitative protein	A Protein number	B		
			蛋白数量 Protein number	缺失值* Missing value	缺失率 Missing rate (%)
PD-DDA	2 645	77	2 568	603	3.91
PEAKS-DDA	2 757	106	2 652	508	3.19
PEAKS-DIA	1 919	34	1 885	221	1.95
SPE-DIA	2 555	362	2 194	313	2.38

注：A：菲诱导表达而 LB 完全不表达蛋白，以及 LB 诱导表达而菲完全不表达蛋白；B：剔除 A 组的蛋白后剩余定量蛋白；*：缺失值根据所有样品中未检出数确定,缺失率为缺失值与所有样品中蛋白定量总数的比值.

Note: A: Proteins which are expressed only in phenanthrene condition or only in LB condition; B: Residual quantitative proteins after removing proteins from group A; *: The missing value is determined according to the undetected protein number in all samples, and the missing rate is the ratio of the missing value to the total quantitative amount of protein in all samples.

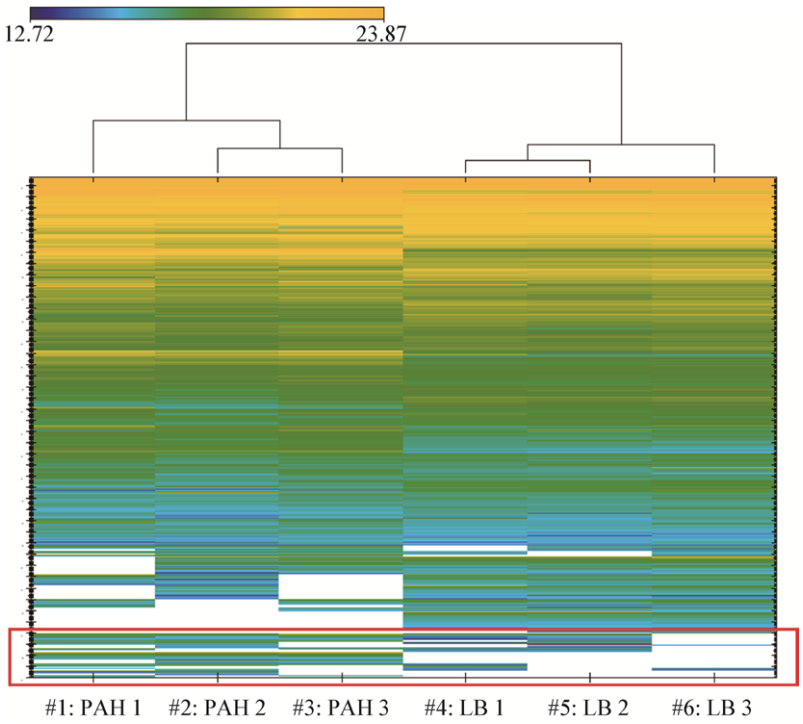

图 4　Spectronaut 中所有定量蛋白火山图

Figure 4　Volcanic map of all quantitative proteins from Spectronaut

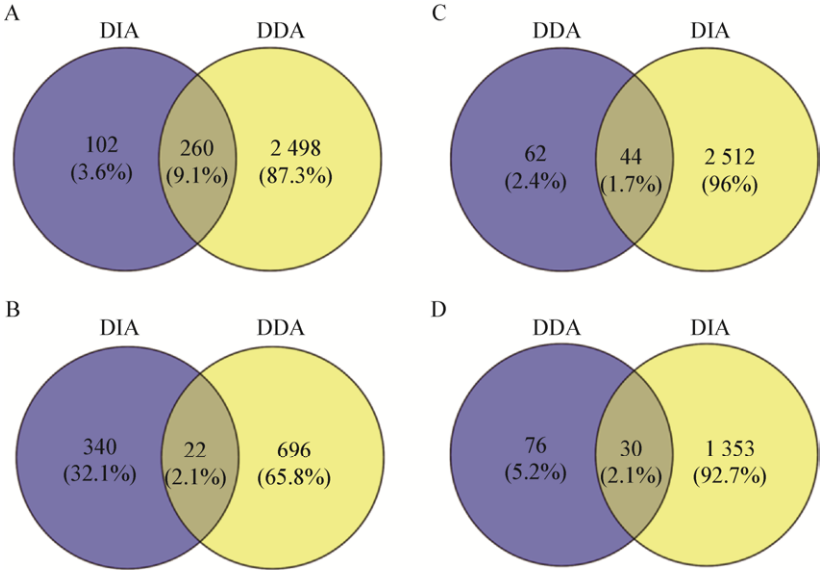

图 5　PEAKS-DDA 和 SPE-DIA 的 A 组蛋白定量结果差异比较

Figure 5　Difference comparison of quantitative proteins from group A between PEAKS-DDA and SPE-DIA

注：A：SPE-DIA 中 A 组蛋白相对于 PEAKS-DDA 全部定量蛋白的覆盖情况，B：SPE-DIA 中 A 组蛋白相对于 CV≤20%的 PEAKS-DDA 定量蛋白的覆盖情况，C：PEAKS-DDA 中 A 组蛋白相对于 SPE-DIA 全部定量蛋白的覆盖情况，D：PEAKS-DDA 中 A 组蛋白相对于 CV≤20%的 SPE-DIA 定量蛋白的覆盖情况.

Note: A: The coverage of proteins from group A in SPE-DIA relative to all quantitative proteins in PEAKS-DDA; B: The coverage of proteins from group A in SPE-DIA relative to quantitative proteins with CV lower than 20% in PEAKS-DDA; C: The coverage of proteins from group A in PEAKS-DDA relative to all quantitative proteins in SPE-DIA; D: The coverage of proteins from group A in PEAKS-DDA relative to quantitative proteins with CV lower than 20% in SPE-DIA.

而言，样品前处理与液相色谱方法没有什么差别，本实验中两种技术也都可在 Q-Exactive Plus 仪器上实现。然而根据两种技术的原理不同，DIA 在质谱采集过程中会引入大量肽段并将它们同时碎裂，从而产生高度混合的 MS2 谱图[20]。如此复杂的 MS2 谱图虽然也可以通过传统的基于在线数据库匹配的方法直接分析，但效果不佳，因此人们开发了称之为“肽段中心打分”的分析方法。在这项策略中，采集到的数据集会和一套已知肽段各类参数的数据集进行比对搜索，最常用的方法就是预先采集一套谱图数据库[21]。另外，由于 DDA 数据采集的随机性和限制性，其肽段检测的可重复性及定量一致性较差，而 DIA 模式不涉及到对肽段离子的限制性筛选而获得更完整的信息，且相对于母离子检测来说，碎片离子检测的灵敏度更高，包含的信息也更丰富，因此 DIA 相较于 DDA 来说更稳健、更灵敏。

2.3 PEAKS 与 Spectronaut 软件对 DIA 数据分析方法比较

对同一批 DIA 数据利用 PEAKS Studio10.0 (Bioinfomatics Solutions Inc.)和 Spectronaut 11.0 (Biognosys AG 2013)两种搜索工具分别进行分析，从软件对服务器的要求、处理步骤和效率、定性定量结果等方面比较两种软件的性能。从表 4 分析结果可以看出，两种软件在分析性能方面各有优缺点。PEAKS 可通过直接搜库的方法对 DIA 数据进行处理，因此操作步骤较为简单，省去构建 DDA 实体库的步骤，前期准备工作较少；然而由于其融合 *de novo* 的算法对肽段进行定性分析，在分析时间上要远长于 SPE 的数据处理时间。同时，PEAKS 软件定量的肽段数目有 23 891，高于 SPE 软件的 14 641，但是蛋白定量数目明显低于 SPE 软件，说明 *de novo* 的方法对肽段的鉴定数量有了较大的提升，然而对蛋白的定量结果并没有明显帮助。分析其中原因，可能是由于 DIA 数据较为复杂，直接进行线上搜库匹配的方法使得谱图匹配效率降低。SPE 通过构建实体库的方法进行 DIA 数据分析，大大降低了谱图解析难度，增强了分析效果。除此之外，PEAKS 是利用一级谱图母离子峰面积或者峰强度对蛋白定量，为了达到定量要求，一级谱图信噪比要求较高，增大了低丰度蛋白的定量难度，导致很多低丰度不能被有效定量[22]；而 SPE 在二级谱图的基础上根据子离子峰面积或者峰强度进行定量，有利于信噪比的提高和低丰度蛋白的检出，增强了定量能力[8]。

2.4 差异蛋白及生物信息学分析

2.4.1 差异蛋白的筛选

根据图 1 中不同软件对差异蛋白的筛选条件，分别对 DDA 和 DIA 模式下获得的质谱数据进行差异蛋白分析，然后将 DDA 在 PD 和 PEAKS 中所得差异蛋白合并以及将 DIA 在 SPE 和 PEKAS 中所得差异蛋白合并；为了更准确地得到表达差异蛋白，筛选获得在 DDA 与 DIA 共得到的 580 个差异蛋白进行进一步的生物信息学分析，差异蛋白列表详见表 5，其中菲诱导的表达下调蛋白有 327 个，表达上调蛋白有 253 个。

表 3 DDA 与 DIA 技术方法比较

Table 3 Comparison of DDA and DIA method

Feature	Category	DDA	DIA
Differences	Principle	Chooses the largest peaks for acquisition of MS2 spectra and peptide identification	All ionized compounds are fragmented in a systematic and unbiased fashion
	Data acquisition	Requires definition of TopN methos, MS2 trigger threshold and dynamic exclusion time	Requires definition of mass range to cover, precursor isolation window width and number of MS2 scans per cycle
	Spectrum library establishment	No requirement	Generally requires
	Software	PD, PEAKS, Mascot, Maxquant	PEAKS, Spectronaut, OpenSWATH, Skyline
	Reproducibility	Low, due to stochastic sampling in DDA	High, due to peptide-centric scoring analysis
Similarities	Sample treatment method, chromatographic method, mass spectrometer		

表 4 PEAKS 与 Spectronaut 软件处理 DIA 数据性能比较

Table 4 Performance comparison of PEAKS and Spectronaut software in processing DIA data

Software	肽段	蛋白	定性方法	定量方法	软件安装要求	处理步骤	分析效率
	Peptide	Protein	Identification	Quantification	Software installation	Data processing steps	Analysis efficiency
PEAKS	23 891	1 921	*de novo*+DB search	MS1	16thread	Less	Slower
SPE	14 641	2 595	Spectral library search	MS2	16thread	More	Faster

表 5 在 DDA 与 DIA 共同所得差异蛋白

Table 5 Differentially expressed protein commonly obtained in DDA and DIA

Down-regulated protein			Up-regulated protein		
No.	Protein accessions	Protein names	No.	Protein accessions	Protein names
1	A0A085K0S9	L-ectoine synthase	1	A0A085K9P4	Formyltetrahydrofolate deformylase
2	A0A084ENS0	50S ribosomal protein L32	2	A0A085KA57	Glucoamylase (Glycoside hydrolase family 15 protein)
3	A0A085KB35	30S ribosomal protein S20	3	A0A3G2UT35	Organic hydroperoxide resistance protein
4	A0A085K2P5	Sec-independent protein translocase protein TatC	4	A0A085KAK8	UPF0301 protein AX777_21150
5	A0A085K6N1	Aromatic amino acid aminotransferase (Aspartate/tyrosine/aromatic aminotransferase)	5	A0A085JYY1	Tetratricopeptide repeat protein
6	A0A084EPD8	50S ribosomal protein L15	6	A0A085K5K6	TonB-dependent receptor (TonB-dependent siderophore receptor)
7	A0A084EUR9	Nucleoside diphosphate kinase (NDK) (NDP kinase)	7	A0A3G2UKT1	IS6 family transposase
8	A0A084ECG9	50S ribosomal protein L35	8	A0A085K649	Signal peptidase I
9	A0A3G2UTA2	Adenine phosphoribosyltransferase (APRT)	9	A0A3G2UTD7	Polysaccharide export protein
10	A0A085K7P9	Alanine dehydrogenase	10	A0A085K5I6	NAD-dependent succinate-semialdehyde dehydrogenase (Succinate-semialdehyde dehydrogenase)
11	A0A3G2UQQ0	Peptide MFS transporter	11	A0A085K062	TonB-dependent receptor
12	A0A084EHR1	30S ribosomal protein S21	12	A0A084EUH6	Chemotaxis protein CheY (DNA-binding response regulator) (Response regulator of the LytR/AlgR family protein) (Two-component system response regulator)
13	A0A085K0T0	Ectoine hydroxylase	13	A0A085JZ02	Uncharacterized protein
14	A0A3G2V2E7	Acylase	14	A0A085K9E4	Histidine kinase (Response regulator)
15	A0A084ELD5	Lipoyl synthase	15	A0A085KA86	Alpha-D-glucose phosphate-specific phosphoglucomutase (Phosphoglucomutase)
16	A0A085K013	50S ribosomal protein L21	16	A0A085K729	TonB-dependent receptor (TonB-dependent siderophore receptor)
17	A0A085K2A3	30S ribosomal protein S10	17	A0A085K912	Class I SAM-dependent methyltransferase (Cyclopropane-fatty-acyl-phospholipid synthase) (Methyltransferase, cyclopropane fatty acid synthase) (SAM-dependent methyltransferase)
18	A0A085K299	30S ribosomal protein S12	18	A0A085K7B1	Nitrite/sulfite reductase (Sulfite reductase)
19	A0A3G2UPG2	Oleate hydratase	19	A0A085KB08	3-dehydroquinate dehydratase (3-dehydroquinase)
20	A0A084ET38	30S ribosomal protein S4	20	A0A085JZI0	Membrane protein (SPFH/Band 7/PHB domain protein)
21	A0A085K1S4	30S ribosomal protein S15	21	A0A085K679	DUF853 family protein
22	A0A085K2G0	Peptidase S10	22	A0A3G2US14	Copper resistance protein B
23	A0A084E3I3	Arsenate reductase	23	A0A085K6I1	Uncharacterized protein
24	A0A085K6N3	Homogentisate 1,2-dioxygenase (HGDO)	24	A0A3G2UPU6	Polysaccharide biosynthesis tyrosine autokinase

(待续)

(续表 5)

25	A0A085K6X3	Phosphogluconate dehydratase	25	A0A085K7F0	ABC transporter substrate-binding protein (Lipoprotein-releasing ABC transporter permease subunit)
26	A0A084EBB1	HAD family hydrolase	26	A0A084ELN5	Peptidoglycan-associated protein
27	A0A084EFC1	HU family DNA-binding protein (Integration host factor) (Transcriptional regulator)	27	A0A085K6Y5	3-hydroxyacyl-CoA dehydrogenase
28	I0IW05	30S ribosomal protein S8	28	A0A3G2UPQ0	NADH:flavin oxidoreductase/NADH oxidase
29	A0A085K012	50S ribosomal protein L27	29	A0A3G2URM5	Aldehyde dehydrogenase family protein
30	A0A085KA25	Alpha-L-fucosidase	30	A0A085KB61	Beta-glucosidase (Beta-glucosidase BglX)
31	A0A085K4Y1	Amino acid dehydrogenase (Glu/Leu/Phe/Val dehydrogenase)	31	A0A085KA56	Trehalose-6-phosphate synthase
32	A0A085KAT1	50S ribosomal protein L28	32	A0A085K311	Cytochrome C (Cytochrome c family protein)
33	A0A084EUN9	Dihydroxy-acid dehydratase	33	A0A085K7P8	AsnC family transcriptional regulator (Lrp/AsnC family transcriptional regulator)
34	A0A085K8U7	Helix-turn-helix transcriptional regulator (Peptidase S24)	34	A0A3G2UTB7	UDP-glucose 6-dehydrogenase
35	A0A085K8D1	TIGR02300 family protein	35	A0A085K1H2	TonB-dependent receptor
36	A0A085K9X4	Urocanate hydratase (Urocanase)	36	A0A085KAR7	Membrane protein
37	A0A084E4U8	DNA-binding protein (Heat shock protein HspQ) (Hemimethylated DNA binding domain-containing protein)	37	A0A084ELS5	AAA family ATPase (Cell division protein FtsH)
38	A0A084EL94	Biotin synthase	38	A0A085K801	Cytochrome C (Cytochrome c1)
39	A0A085K2A6	50S ribosomal protein L23	39	A0A3G2UMU2	IclR family transcriptional regulator
40	A0A085K6N2	4-hydroxyphenylpyruvate dioxygenase	40	A0A085K5D3	Outer-membrane lipoprotein carrier protein
41	A0A085K7W6	Flagellin	41	A0A085KBB1	DUF4136 domain-containing protein
42	A0A085K5E1	Uncharacterized protein	42	A0A085K671	Opacity protein
43	A0A085KA36	Glycerol kinase	43	A0A3G2UQ29	Glycosyltransferase family 2 protein
44	A0A3G2ULM1	Aromatic ring-hydroxylating dioxygenase subunit alpha	44	A0A085K1M7	Glutathione S-transferase (Glutathione S-transferase family protein)
45	A0A085K6N9	Corrinoid adenosyltransferase (Cob(II)alamin adenosyltransferase)	45	A0A3G2UQ32	TrbI/VirB10 family protein
46	A0A085K2T8	Methyltransferase	46	A0A084ES41	DUF1508 domain-containing protein
47	A0A3G2UPV1	L-2,4-diaminobutyric acid acetyltransferase (DABA acetyltransferase)	47	A0A3G2UQM6	NAD(P)-dependent oxidoreductase
48	A0A3G2UX96	Phosphofructokinase	48	A0A085K603	Fumarate hydratase class II (Fumarase C)
49	A0A085K4Q1	ArsR family transcriptional regulator (Putative ArsR family transcriptional regulator) (Transcriptional regulator)	49	A0A085K235	Membrane protein
50	A0A085K526	Cytochrome P450	50	A0A3G2UM71	DUF4142 domain-containing protein
51	A0A085K6V0	Glycoside hydrolase family 105 protein (Glycosyl hydrolase family 88)	51	A0A0J9D3E5	Uncharacterized protein
52	A0A085K9A8	Beta-lactamase (Serine hydrolase)	52	A0A085K681	Cysteine synthase
53	A0A084EDE6	Acetolactate synthase (Thiamine pyrophosphate-binding protein) (Thiamine pyrophosphate-dependent enzyme, possible carboligase or decarboxylase)	53	A0A3G2UR44	Dicarboxylate/amino acid:cation symporter
54	A0A085KB48	Aminobenzoate synthetase (Aminotransferase) (Para-aminobenzoate synthase, component I)	54	A0A085K478	Opacity protein
55	A0A084ECJ4	FAD-dependent oxidoreductase (NAD(P)/FAD-dependent oxidoreductase)	55	A0A084ECW6	CoA-binding protein (Putative CoA-binding protein)
56	A0A085JZ36	PspC domain-containing protein (Stress-responsive transcriptional regulator)	56	A0A085K047	Uncharacterized protein
57	A0A085K239	Uncharacterized protein	57	A0A085K8X5	Glutamate synthase (Glutamate synthase large subunit)

(待续)

(续表 5)

58	A0A085K242	Pirin (Pirin family protein)	58	A0A085KAH0	Acetyl-CoA C-acetyltransferase (Acetyl-CoA acetyltransferase)
59	A0A085K5A7	UPF0260 protein EBF16_22055	59	A0A3G2UMR2	Uncharacterized protein
60	A0A085K5L0	Thioesterase (Thioesterase family protein)	60	A0A085K4Y8	Signal protein (TonB-dependent receptor)
61	A0A085K5Y5	Uncharacterized protein	61	A0A3G2UPP1	4-oxalocrotonate decarboxylase
62	A0A085K6F8	Diguanylate cyclase (GGDEF domain-containing protein)	62	A0A3G2ULZ9	Uncharacterized protein
63	A0A085K6P7	DUF721 domain-containing protein	63	A0A3G2V2Q8	PadR family transcriptional regulator
64	A0A085K6X7	Uncharacterized protein	64	A0A085K2K3	Metal/formaldehyde-sensitive transcriptional repressor (Transcriptional regulator)
65	A0A085K809	DUF3240 domain-containing protein	65	A0A085K8S2	Glycerol kinase
66	A0A085KA91	Polysaccharide biosynthesis protein GumN (TraB/GumN family protein)	66	A0A085K494	Uncharacterized protein
67	A0A085KBB9	DUF2188 domain-containing protein	67	A0A085K7I7	EmrA/EmrK family multidrug efflux transporter periplasmic adaptor subunit (Hemolysin D)
68	A0A3G2UKF6	Polyisoprenoid-binding protein	68	A0A085JZ61	ABC transporter permease (FtsX-like permease family protein)
69	A0A3G2UNN8	Antitoxin	69	A0A3G2UN54	AarF/ABC1/UbiB kinase family protein
70	A0A3G2UTE3	DUF4880 domain-containing protein	70	A0A085K253	Penicillin-binding protein activator LpoB (Protein involved in formation of curli polymers)
71	A0A3G2V109	Uncharacterized protein	71	A0A3G2UTG9	SGNH_hydro domain-containing protein
72	A0A3G2V1E7	DUF2779 domain-containing protein	72	A0A085K800	Cytochrome b
73	A0A3G2V1F3	Uncharacterized protein	73	A0A3G2UZ77	LLM class flavin-dependent oxidoreductase
74	A0A085K7V9	Flagellar biosynthesis anti-sigma factor FlgM	74	A0A085KAY9	Membrane protein
75	A0A085KAY2	Chemotaxis protein CheY (Response regulator) (Two-component system response regulator)	75	A0A085K766	Glycosyltransferase family 2 protein (Histidine kinase)
76	A0A085K5C1	Putative pyruvate, phosphate dikinase regulatory protein (PPDK regulatory protein)	76	A0A085K485	Catalase
77	A0A3G2UUG6	Coproporphyrinogen-III oxidase	77	A0A177JY28	Uncharacterized protein
78	A0A3G2UTF0	Type II toxin-antitoxin system ParD family antitoxin	78	A0A085K1E8	OsmC family peroxiredoxin (Peroxiredoxin)
79	A0A3G2UKT7	Cytochrome b	79	A0A085KA00	Uncharacterized protein
80	A0A084EII0	Uncharacterized protein	80	A0A085K8S3	Catalase
81	A0A3G2UXD4	ROK family protein	81	A0A2D1R5E7	Uncharacterized protein
82	A0A3G2UKI7	Pirin family protein	82	A0A084EJW0	DedA family protein (Membrane protein) (Putative membrane-associated protein)
83	A0A085K7A8	DUF885 domain-containing protein (Tat pathway signal protein)	83	A0A085K3D5	TonB-dependent receptor
84	A0A084EI05	DUF4170 domain-containing protein	84	A0A085K8R8	Phasin (Phasin family protein)
85	A0A085JZY0	Probable glycine dehydrogenase (decarboxylating) subunit 1	85	A0A084EUC7	LemA family protein (Membrane protein)
86	A0A3G2US79	Uncharacterized protein	86	A0A3G2V0F0	Uncharacterized protein
87	A0A085K2U3	Pilus assembly protein CpaE	87	A0A3G2UL12	Uncharacterized protein
88	A0A085K9S7	Peptidase M28 (Peptidase M28 family protein)	88	A0A085K0K3	Copper resistance system multicopper oxidase (Copper-binding protein)
89	A0A085K7J2	30S ribosomal protein S16	89	A0A3G2UMU8	Murein L,D-transpeptidase
90	A0A085KAU4	50S ribosomal protein L10	90	A0A3G2UTB5	Efflux transporter outer membrane subunit
91	I0IW02	30S ribosomal protein S17	91	A0A085K314	Membrane protein (OmpA family protein)
92	I0IW03	50S ribosomal protein L24	92	A0A085K9N5	Dihydroxy-acid dehydratase (DAD)
93	A0A084ERX6	GTPase Obg	93	A0A084EMQ0	DUF1134 domain-containing protein
94	A0A085K558	Peptidase M61	94	A0A3G2UV24	Autotransporter domain-containing protein
95	A0A3G2ULC1	TIGR01244 family phosphatase	95	A0A085K8X6	Dihydropyrimidine dehydrogenase (NAD(P)-dependent oxidoreductase)

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96	I0IVZ9	30S ribosomal protein S19	96	A0A3G2USP8	SDR family oxidoreductase
97	A0A085K2B2	50S ribosomal protein L29	97	A0A085K651	Uncharacterized protein
98	A0A085K983	Aminotransferase	98	A0A084EU87	DUF4136 domain-containing protein (Lipoprotein transmembrane)
99	A0A085K918	Dual-specificity RNA methyltransferase RlmN	99	A0A3G2USM1	Quinone oxidoreductase
100	A0A085K375	50S ribosomal protein L31	100	A0A3G2UM34	Uncharacterized protein
101	A0A177JXN1	2-oxoisovalerate dehydrogenase (Alpha-ketoacid dehydrogenase subunit beta)	101	A0A085K553	DUF4139 domain-containing protein
102	A0A085K2E4	Heme chaperone HemW	102	A0A3G2UTA9	RraA family protein
103	A0A085JZ30	M13 family peptidase (Peptidase M13)	103	A0A085K655	Aldolase (Class II aldolase/adducin family protein)
104	A0A085K5Z7	Crp/Fnr family transcriptional regulator (cAMP-binding protein-catabolite transcription activator)	104	A0A085KBB6	UPF0145 protein A6768_25245
105	A0A085K017	50S ribosomal protein L1	105	A0A085K3P1	DNA starvation/stationary phase protection protein (DNA-binding protein) (Putative low temperature-induced protein)
106	A0A085K960	Zinc-finger domain-containing protein	106	A0A3G2UPK4	2-oxo-hepta-3-ene-1,7-dioic acid hydratase
107	I0IW00	50S ribosomal protein L22	107	A0A3G2URD7	Polyisoprenoid-binding protein
108	A0A3G2UPQ4	Aromatic ring-hydroxylating dioxygenase subunit alpha	108	A0A085K8R3	Aromatic ring-opening dioxygenase LigA (Dioxygenase)
109	A0A085K9P9	2-nitropropane dioxygenase (Nitronate monooxygenase)	109	A0A3G2UYK9	Antibiotic biosynthesis monooxygenase
110	A0A085K9G3	Dihydrofolate reductase	110	A0A3G2UK76	Alcohol dehydrogenase AdhP
111	A0A085K949	TonB-dependent receptor	111	A0A085K790	NAD(P)H dehydrogenase (quinone)
112	A0A085K2A5	50S ribosomal protein L4	112	A0A3G2UQL4	TonB-dependent receptor
113	A0A085K2G6	Oxidoreductase (SDR family oxidoreductase)	113	A0A3G2UMG6	3-phenylpropionate/cinnamic acid dioxygenase subunit beta
114	A0A085K2A7	50S ribosomal protein L2	114	A0A085K9E7	Aldehyde oxidase (Aldo/keto reductase)
115	A0A084EPD6	Adenylate kinase (AK)	115	A0A085K991	D-3-phosphoglycerate dehydrogenase
116	A0A084EHB6	Transcription antitermination protein NusB (Antitermination factor NusB)	116	A0A3G2ULT9	Benzene 1,2-dioxygenase
117	A0A085K6Z9	Uncharacterized protein	117	A0A084ENV1	DUF3617 domain-containing protein
118	A0A085K705	50S ribosomal protein L9	118	A0A085K015	Cation:proton antiport protein (Kef family K(+) transporter)
119	A0A085K5B5	PTS IIA-like nitrogen-regulatory protein PtsN (PTS lactose transporter subunit IIC)	119	A0A3G2ULR1	NAD(P)-dependent alcohol dehydrogenase
120	A0A085K5C2	Uroporphyrinogen decarboxylase (UPD)	120	A0A3G2UMC1	Aromatic ring-hydroxylating dioxygenase subunit alpha
121	A0A085K139	DEAD/DEAH box helicase	121	A0A3G2ULV0	Acetaldehyde dehydrogenase
122	A0A084EQ82	CarD family transcriptional regulator	122	A0A3G2UPW6	Type 1 glutamine amidotransferase
123	A0A085K9A1	Pyridoxine/pyridoxamine 5'-phosphate oxidase	123	A0A3G2ULX1	Aromatic-ring-hydroxylating dioxygenase subunit beta
124	A0A085K719	Acyl carrier protein (ACP)	124	A0A3G2UPR4	DUF3597 family protein
125	A0A085K2A0	30S ribosomal protein S7	125	A0A3G2UNJ0	Tartrate dehydrogenase
126	A0A085K6R7	M20/M25/M40 family metallo-hydrolase (Peptidase M28)	126	A0A3G2ULK5	Amidohydrolase
127	A0A3G2UN82	Acetyl/propionyl/methylcrotonyl-CoA carboxylase subunit alpha	127	A0A084EII2	Pirin (Pirin family protein) (Pirin-related protein)
128	A0A085K2G3	Amidohydrolase (Peptidase M20)	128	A0A3G2UM30	FAD-binding oxidoreductase
129	A0A085KAK0	Peptidase S9 (S9 family peptidase)	129	A0A3G2UTT5	Aldehyde dehydrogenase family protein
130	A0A085K0V3	Aconitate hydratase (Bifunctional aconitate hydratase	130	A0A085KB57	Cell envelope biogenesis protein OmpA (OmpA family protein)
131	A0A085K7Y6	Acetylornithine aminotransferase (ACOAT)	131	A0A3G2UMI7	Aldehyde dehydrogenase family protein

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(续表 5)

132	I0IW06	50S ribosomal protein L18	132	A0A3G2UPW2	3-(Cis-5,6-dihydroxycyclohexa-1, 3-dien-1-yl)propanoate dehydrogenase
133	A0A085K2C2	50S ribosomal protein L30	133	A0A3G2UTS5	Alpha/beta fold hydrolase
134	A0A084ELC0	Adenosylhomocysteinase (S-adenosyl-L-homocysteine hydrolase) (AdoHcyase)	134	A0A3G2UQK3	Uncharacterized protein
135	A0A085K980	Nicotinate-nucleotide-dimethylbenzimidazole phosphoribosyltransferase	135	A0A3G2UM69	1,6-dihydroxycyclohexa-2,4-diene-1-carboxylate dehydrogenase
136	I0IW04	30S ribosomal protein S14	136	A0A3G2V6Z5	Hydrolase
137	A0A3G2UL71	Sulfonamide-resistant dihydropteroate synthase Sul4	137	A0A3G2UJT5	Aldehyde dehydrogenase family protein
138	A0A085JZ31	ATP-dependent dethiobiotin synthetase BioD	138	A0A085K4C7	DUF4142 domain-containing protein (Membrane protein)
139	A0A177JYJ0	Methionine aminopeptidase (MAP) (MetAP)	139	A0A3G2ULQ7	Flavin prenyltransferase UbiX
140	A0A084ELS6	Methyltransferase (Methyltransferase domain-containing protein) (Phospholipid N-methyltransferase)	140	A0A3G2UN96	UbiD family decarboxylase
141	A0A085K647	Pyridoxine 5'-phosphate synthase (PNP synthase)	141	A0A3G2ULI3	4-hydroxy-2-oxovalerate aldolase (HOA) (EC 4.1.3.39) (4-hydroxy-2-keto-pentanoic acid aldolase) (4-hydroxy-2-oxopentanoate aldolase)
142	A0A3G2URH6	Nucleoid-associated protein EBF16_06280	142	A0A3G2ULF3	2-dehydro-3-deoxyglucarate aldolase
143	A0A085K8V5	Alkylphosphonate utilization protein (PhnA protein) (Putative Zn-ribbon-containing protein involved in phosphonate metabolism)	143	A0A3G2UNE0	Aldolase
144	A0A084EPP2	30S ribosomal protein S11	144	A0A3G2UPT0	FAD-binding oxidoreductase
145	A0A3G2URN1	Peptidase	145	A0A3G2USX6	2-dehydropantoate 2-reductase
146	A0A085K5C5	ATPase	146	A0A3G2UMN4	Aromatic ring-hydroxylating dioxygenase subunit alpha
147	A0A085K952	Ribonucleoside-diphosphate reductase	147	A0A084EKV2	DUF541 domain-containing protein (Membrane protein)
148	A0A085K5E7	HU family DNA-binding protein (Integration host factor)	148	A0A3G2UNB8	2-hydroxymuconic semialdehyde dehydrogenase
149	A0A085K3P6	Hsp70 family protein (Molecular chaperone Hsp70)	149	A0A3G2UQV1	DUF2312 domain-containing protein
150	A0A085KBT0	Aminopeptidase	150	A0A3G2UTQ5	Cytochrome c
151	A0A0J9CTP8	Fructose-1,6-bisphosphatase class 1 (FBPase class 1)	151	A0A3G2UMK8	2-keto-4-pentenoate hydratase
152	A0A085K244	Pirin (Pirin family protein)	152	A0A3G2UPJ2	Dihydrodipicolinate synthase family protein
153	A0A085K7F4	DNA polymerase III subunit alpha	153	A0A3G2ULE1	Pyruvate, phosphate dikinase
154	A0A085K9W1	DUF885 domain-containing protein	154	A0A3G2UU56	Uncharacterized protein
155	A0A3G2V0D2	LacI family DNA-binding transcriptional regulator	155	A0A084ENF4	Arabinose ABC transporter permease (MFS transporter)
156	A0A085K5G2	CAP10 domain-containing protein	156	A0A3G2ULK2	Glutathione transferase GstA
157	A0A291N6W4	Chemotaxis protein	157	A0A3G2UT02	Anthranilate 1,2-dioxygenase
158	A0A085K3L0	tRNA-specific 2-thiouridylase MnmA	158	A0A3G2UTR6	Rieske (2Fe-2S) protein
159	A0A3G2UND1	tRNA-2-methylthio-N(6)-dimethylallyladenosine synthase	159	A0A085K990	Phosphoserine transaminase
160	A0A084ERW5	50S ribosomal protein L11	160	A0A3G2UM44	Non-heme iron oxygenase ferredoxin subunit
161	A0A085K530	Methylmalonate-semialdehyde dehydrogenase (Methylmalonate-semialdehyde dehydrogenase (CoA acylating))	161	A0A3G2UM20	LLM class flavin-dependent oxidoreductase
162	A0A085KB40	Deoxyuridine 5'-triphosphate nucleotidohydrolase (dUTPase)	162	A0A3G2V0M7	Uncharacterized protein

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163	A0A3G2UN00	Methylenetetrahydrofolate-tRNA-(uracil-5-)-methyltransferase TrmFO	163	A0A3G2UPL7	TonB-dependent receptor
164	A0A085K328	Alkaline phosphatase	164	A0A3G2ULG5	NAD-dependent succinate-semialdehyde dehydrogenase
165	A0A085K405	30S ribosomal protein S9	165	A0A3G2UPM8	Rieske (2Fe-2S) protein
166	A0A3G2V0E0	Imidazolonepropionase	166	A0A3G2UPU4	Anthranilate 1,2-dioxygenase
167	A0A3G2V1D5	SAM-dependent methyltransferase	167	A0A3G2USY5	Alpha/beta fold hydrolase
168	A0A3G2UM87	MBL fold metallo-hydrolase	168	A0A085K4U8	Aldehyde dehydrogenase (Aldehyde dehydrogenase family protein)
169	A0A085K6Q5	Phosphoribosylamine-glycine ligase	169	A0A3G2UMJ7	Benzoate 1,2-dioxygenase large subunit
170	A0A085K3G9	Adenosine kinase (Carbohydrate kinase)	170	A0A3G2UPV3	Catechol 2,3-dioxygenase
171	A0A085K2B9	50S ribosomal protein L6	171	A0A3G2ULH1	4-hydroxythreonine-4-phosphate dehydrogenase
172	A0A3G2UWN2	Glycerol-3-phosphate dehydrogenase	172	A0A3G2USZ4	TonB-dependent receptor
173	A0A085K2A2	Elongation factor Tu (EF-Tu)	173	A0A3G2UNA7	Biphenyl-2,3-diol 1,2-dioxygenase
174	A0A085K7E0	M28 family peptidase (Peptidase M28)	174	A0A085K8Q8	Membrane protein
175	A0A085K6R8	Insulinase family protein (Peptidase M16)	175	A0A0J9D4V2	Cytochrome c oxidase subunit 1
176	A0A085K9X2	Histidine ammonia-lyase (Histidase)	176	A0A085K9C3	Glycosyltransferase
177	A0A085K539	Putative Zn-dependent protease-like protein (TldD protein) (TldD/PmbA family protein)	177	A0A085K2F7	Isocitrate lyase
178	A0A085KBE2	Peptidase S10	178	A0A085JZZ6	Heme exporter protein B
179	A0A085K6C8	3-hydroxybutyryl-CoA dehydrogenase	179	A0A3G2V2U1	Acyl-CoA dehydrogenase
180	A0A085K8P2	ArsC family reductase (ArsC family transcriptional regulator) (Arsenate reductase) (Spx/MgsR family transcriptional regulator)	180	A0A3G2UT58	Enoyl-[acyl-carrier-protein] reductase [NADH]
181	A0A3G2V5X6	Signal recognition particle receptor FtsY (SRP receptor)	181	A0A3G2UT82	Type 1 glutamine amidotransferase domain-containing protein
182	A0A085K2B0	30S ribosomal protein S3	182	A0A3G2UP32	1,4-alpha-glucan branching enzyme GlgB
183	A0A085KBB7	Probable transcriptional regulatory protein A6768_25240	183	A0A085K956	Ammonium transporter
184	A0A084EU33	Translation initiation factor IF-3	184	A0A3G2USV3	Acyl-CoA synthetase
185	A0A085K950	Fumarate hydratase class I	185	A0A3G2URQ1	Xanthine dehydrogenase family protein subunit M
186	A0A084ENX3	Beta sliding clamp	186	A0A085K8R0	ATP-dependent RNA helicase
187	A0A085K236	Recombinase RecJ (Single-stranded-DNA-specific exonuclease RecJ)	187	A0A085K4Z3	Glycoside hydrolase family 43 (HlyD family efflux transporter periplasmic adaptor subunit)
188	A0A084ETU1	UPF0335 protein AX777_20220	188	A0A085K3L6	K(+)-insensitive pyrophosphate-energized proton pump
189	A0A085K0S8	Diaminobutyrate-2-oxoglutarate transaminase	189	A0A085KAF0	AarF/ABC1/UbiB kinase family protein (Ubiquinone biosynthesis protein)
190	A0A084EHA2	Translation initiation factor IF-1	190	A0A3G2UYC2	Lactoylglutathione lyase
191	A0A085K3N8	50S ribosomal protein L33	191	A0A085K6P9	NAD(+) diphosphatase
192	A0A085K793	Chemotaxis protein CheY (DNA-binding response regulator) (DNA-binding response regulator CtrA) (Two-component system response regulator)	192	A0A3G2UY88	SDR family oxidoreductase
193	A0A085K2N6	4-hydroxy-3-methylbut-2-enyl diphosphate reductase (HMBPP reductase)	193	A0A085JZT0	Glutathione S-transferase (Glutathione S-transferase family protein)
194	A0A3G2UV55	M20/M25/M40 family metallo-hydrolase	194	A0A085K951	Glutathione S-transferase (Glutathione S-transferase family protein)
195	A0A084EPE0	30S ribosomal protein S5	195	A0A085K9D0	Acyltransferase
196	A0A085K9R7	tRNA-dihydrouridine synthase	196	A0A085K7C0	UrcA family protein
197	A0A085K5Z4	Universal stress protein (Universal stress protein UspA)	197	A0A3G2UT41	Uncharacterized protein

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198	A0A085K0Z3	DUF2322 family protein	198	A0A085JZ29	DUF4168 domain-containing protein
199	A0A085K986	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase	199	A0A3G2USH6	YIP1 family protein
200	A0A084EQT5	Ornithine carbamoyltransferase (OTCase)	200	A0A3G2UYE6	DUF4142 domain-containing protein
201	A0A084EI43	Elongation factor P (EF-P)	201	A0A085KA97	Uncharacterized protein
202	A0A085JZ22	DNA gyrase inhibitor YacG	202	A0A085KAD0	Uncharacterized protein
203	A0A085KAK4	Peptidase M2 family protein (Peptidyl-dipeptidase)	203	A0A3G2ULH4	DUF1330 domain-containing protein
204	A0A085JZY7	ETC complex I subunit (ETC complex subunit I)	204	A0A085K7Z5	Membrane protein
205	A0A3G2UKZ6	Uncharacterized protein	205	A0A3G2UMV2	LPD7 domain-containing protein
206	A0A3G2UKW0	Type II toxin-antitoxin system Phd/YefM family antitoxin	206	A0A3G2ULT0	Uncharacterized protein
207	A0A085KAV1	Carboxylating nicotinate-nucleotide diphosphorylase	207	A0A085K318	Uncharacterized protein
208	A0A291N0P8	GLOBIN domain-containing protein	208	A0A085K0K5	Heavy metal resistance protein (Periplasmic heavy metal sensor)
209	A0A085K7J1	Signal recognition particle protein (Fifty-four homolog)	209	A0A3G2UKY4	DUF779 domain-containing protein
210	A0A084ER82	Peptide methionine sulfoxide reductase MsrA (Protein-methionine-S-oxide reductase)	210	A0A084ERZ8	Acyl carrier protein (Phosphopantetheine-binding protein)
211	A0A085K319	Asparaginase (L-asparaginase/ GlutRNAGln amidotransferase subunit D)	211	A0A085K344	YciI family protein
212	A0A085K908	DUF3297 family protein (Glutathione peroxidase)	212	A0A3G2UPY7	ABC transporter permease
213	A0A085K3G5	Porphobilinogen deaminase (PBG)	213	A0A085K2L9	Membrane protein
214	A0A3G2UPI8	Glycerol kinase	214	A0A3G2UPM5	Aldehyde dehydrogenase
215	A0A084EPR1	Ribosomal silencing factor RsfS	215	A0A3G2UV94	MFS transporter
216	A0A085K5J5	Acetyl-CoA C-acyltransferase	216	A0A3G2UZG5	TetR family transcriptional regulator
217	A0A3G2UYN8	Probable malate:quinone oxidoreductase	217	A0A3G2ULN3	RraA family protein
218	A0A085K583	tRNA uridine 5-carboxymethylaminomethyl modification enzyme MnmG (Glucose-inhibited division protein A)	218	A0A3G2ULJ3	2-hydroxychromene-2-carboxylate isomerase
219	A0A085K6N4	Fumarylacetoacetase	219	A0A084EL78	17 kD surface antigen (Glycine zipper 2TM domain-containing protein)
220	A0A085KAL4	Mannose-1-phosphate guanylyltransferase (Nucleotidyltransferase family protein)	220	A0A084EHX2	Uncharacterized protein
221	A0A084ER14	CTP synthase (EC 6.3.4.2) (Cytidine 5'-triphosphate synthase) (Cytidine triphosphate synthetase) (CTP synthetase) (CTPS) (UTP-ammonia ligase)	221	A0A3G2UX17	Polysaccharide biosynthesis tyrosine autokinase
222	A0A085KAN0	LuxR family transcriptional regulator (TatD family deoxyribonuclease)	222	A0A3G2UQ13	Uncharacterized protein
223	A0A3G2UKB9	Alpha/beta fold hydrolase	223	A0A3G2UYK7	Sulfate adenylyltransferase subunit 2
224	A0A085K5J6	Nitronate monooxygenase	224	A0A084ELP5	Envelope stress response membrane protein PspB (Phage-shock protein)
225	A0A3G2UNZ2	NAD(P)/FAD-dependent oxidoreductase	225	A0A085K082	Universal stress protein (Universal stress protein UspA)
226	A0A3G2UUR1	3-methyl-2-oxobutanoate dehydrogenase (2-methylpropanoyl-transferring) subunit alpha	226	A0A085JZ27	Membrane protein (OmpA family protein)
227	A0A085K6N7	Cobalamin biosynthesis protein CobW	227	A0A085K2D3	Threonine synthase
228	A0A085KAG9	Alanine racemase	228	A0A3G2UNS7	Conjugal transfer protein
229	A0A3G2USH2	Type I restriction endonuclease subunit S	229	A0A085K2Q2	Ribosomal RNA small subunit methyltransferase H

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230	A0A085JYX9	Glyoxalase (Lactoylglutathione lyase)	230	A0A085K5D9	Malic enzyme
231	A0A3G2UQP5	S-(hydroxymethyl)glutathione dehydrogenase	231	A0A085K678	Periplasmic serine endoprotease DegP-like
232	A0A085K5J3	3-hydroxy-2-methylbutyryl-CoA dehydrogenase (SDR family NAD(P)-dependent oxidoreductase)	232	A0A3G2UWP4	Uncharacterized protein
233	A0A084EKT4	50S ribosomal protein L25 (General stress protein CTC)	233	A0A085K7F1	Lipoprotein-releasing system ATP-binding protein LolD
234	A0A085K9A4	Enoyl-[acyl-carrier-protein] reductase [NADH]	234	A0A3G2UYI6	Biotin/lipoyl-binding protein
235	A0A085K8Q7	Lysine--tRNA ligase	235	A0A085K5Z3	GCN5 family acetyltransferase (GCN5-related N-acetyltransferase) (GNAT family N-acetyltransferase) (N-acetyltransferase)
236	A0A3G2UL15	Molybdopterin dinucleotide-binding protein	236	A0A085K6F9	LysR family transcriptional regulator (Transcriptional regulator)
237	A0A085K092	M20/M25/M40 family metallo-hydrolase (Peptidase M20)	237	A0A085K376	3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ
238	A0A085K9Q7	Nucleoside triphosphate hydrolase	238	A0A084EQU7	Ubiquinol-cytochrome c reductase iron-sulfur subunit
239	A0A085K1E3	Glycosyl transferase family 1 (Glycosyltransferase family 1 protein)	239	A0A085K6Y6	Acetyl-CoA C-acetyltransferase
240	A0A084EJK6	DUF2093 domain-containing protein	240	A0A085JZ59	Arylesterase (GDSL family lipase)
241	A0A084ETG6	MucR family transcriptional regulator (Transcriptional regulator)	241	A0A085K2Q1	Transcriptional regulator MraZ
242	A0A0J9D2S7	Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit B (Asp/Glu-ADT subunit B)	242	A0A084EQH0	Glycine zipper 2TM domain-containing protein
243	A0A085K3F8	Ribose-phosphate pyrophosphokinase (RPPK)	243	A0A3G2URY7	FtsX-like permease family protein
244	A0A085K3D2	Histidinol-phosphate aminotransferase	244	A0A085K292	Autotransporter domain-containing protein (Serine protease)
245	A0A291MVR2	NADH-quinone oxidoreductase subunit F	245	A0A3G2UNN3	Sigma-70 family RNA polymerase sigma factor
246	A0A3G2UWP0	Acyl-CoA dehydrogenase	246	A0A085K184	DNA-binding response regulator (Transcriptional regulator) (Two-component system response regulator)
247	A0A084EQE2	Elongation factor Ts (EF-Ts)	247	A0A085K6B7	Uncharacterized protein
248	A0A0J9CVL9	Cytoplasmic protein (DUF1013 domain-containing protein)	248	A0A085K714	Efflux transporter outer membrane subunit (RND transporter)
249	A0A085K5Z2	Membrane protein (OmpW family protein) (Outer membrane protein W)	249	A0A3G2UNU8	YnbE family lipoprotein
250	A0A3G2UYD7	Ribosomal protein S12 methylthiotransferase RimO (S12 MTTase) (S12 methylthiotransferase)	250	A0A3G2ULB5	Cbb3-type cytochrome c oxidase subunit
251	A0A085KAF1	GTPase Era	251	A0A085JYY0	SIMPL domain-containing protein
252	A0A085K3C1	Phosphoribosylformylglycinamidine cyclo-ligase	252	A0A3G2UNN5	General stress protein
253	A0A084ENT3	Ribonucleoside-diphosphate reductase subunit beta	253	A0A3G2UT87	Tautomerase
254	A0A085JZY1	Glycine dehydrogenase (aminomethyl-transferring)	–	–	–
255	A0A3G2UP12	Methylcrotonoyl-CoA carboxylase	–	–	–
256	A0A085K230	2-methylisocitrate lyase (Isocitrate lyase/ phosphoenolpyruvate mutase family protein)	–	–	–
257	A0A085KAS4	5-methyltetrahydrofolate-homocysteine methyltransferase	–	–	–
258	A0A085KAV2	Peptidase S9 (S9 family peptidase)	–	–	–

(待续)

(续表 5)

259	A0A3G2UME0	S9 family peptidase	–	–	–
260	A0A085JZ23	Ribonuclease	–	–	–
261	A0A084EPY4	Peptide deformylase (PDF)	–	–	–
262	A0A085K2B6	50S ribosomal protein L5	–	–	–
263	A0A085K1D3	ATP synthase subunit delta (ATP synthase F(1) sector subunit delta) (F-type ATPase subunit delta) (F-ATPase subunit delta)	–	–	–
264	A0A085K6E7	Cysteine desulfurase	–	–	–
265	A0A085K9G6	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase (BPG-dependent PGAM) (PGAM) (Phosphoglyceromutase) (dPGM)	–	–	–
266	A0A085K5J2	Acetyl-CoA C-acyltransferase	–	–	–
267	A0A085K3B7	Helicase	–	–	–
268	A0A3G2UP86	Amidohydrolase	–	–	–
269	A0A3G2UM60	CoA ester lyase	–	–	–
270	A0A3G2UNI9	DUF952 domain-containing protein	–	–	–
271	A0A084ER03	Acyl dehydratase (Dehydratase) (MaoC family dehydratase)	–	–	–
272	A0A085KA38	Transketolase	–	–	–
273	A0A085K264	Aspartate-tRNA(Asp/Asn) ligase (EC 6.1.1.23) (Aspartyl-tRNA synthetase) (AspRS) (Non-discriminating aspartyl-tRNA synthetase) (ND-AspRS)	–	–	–
274	A0A085K8P6	MBL fold metallo-hydrolase	–	–	–
275	A0A084ECG8	50S ribosomal protein L20	–	–	–
276	A0A3G2V108	Argininosuccinate lyase (ASAL)	–	–	–
277	A0A085K0X5	LexA repressor	–	–	–
278	A0A084EH78	ATP-binding protein (Putative ATPase)	–	–	–
279	A0A084ES93	2-nitropropane dioxygenase (2-nitropropane dioxygenase-like enzyme)	–	–	–
280	A0A085K044	Electron transfer flavoprotein subunit alpha (Electron transfer flavoprotein subunit beta)	–	–	–
281	A0A085K538	Peptidase C69 (TldD/PmbA family protein)	–	–	–
282	A0A084EPE7	50S ribosomal protein L14	–	–	–
283	A0A085K7E7	Ribose 5-phosphate isomerase (Ribose 5-phosphate isomerase B)	–	–	–
284	A0A085K717	3-oxoacyl-ACP synthase (3-oxoacyl-[acyl-carrier-protein] reductase)	–	–	–
285	A0A085K6F3	Branched-chain-amino-acid aminotransferase	–	–	–
286	A0A085K959	Metalloprotease TldD (Protease TldD)	–	–	–
287	A0A085KAI9	Alginate_exp domain-containing protein	–	–	–
288	A0A0J9CY82	Electron transfer flavoprotein subunit beta (Electron transfer flavoprotein subunit beta/FixA family protein)	–	–	–
289	A0A085K152	Uncharacterized protein	–	–	–
290	A0A085K2D8	Ribonuclease PH (RNase PH)	–	–	–
291	A0A084EPP4	50S ribosomal protein L17	–	–	–
292	A0A085KAG3	Methylmalonyl-CoA mutase	–	–	–
293	A0A3G2UQG4	Acyl-CoA dehydrogenase	–	–	–

(待续)

(续表 5)

294	A0A3G2UTU5	Sigma-54-dependent Fis family transcriptional regulator	–	–	–
295	A0A084EHW5	Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit C (Asp/Glu-ADT subunit C)	–	–	–
296	A0A3G2UQS8	N-acetyl-gamma-glutamyl-phosphate reductase (AGPR)	–	–	–
297	A0A085K7R0	Uracil-DNA glycosylase (UDG)	–	–	–
298	A0A085K3G0	Glutamate-tRNA ligase	–	–	–
299	A0A084EQ83	Ferredoxin	–	–	–
300	A0A3G2UMX9	GatB/YqeY domain-containing protein	–	–	–
301	A0A085K7A7	CocE/NonD family hydrolase (Glutaryl-7-ACA acylase)	–	–	–
302	A0A084ETQ3	GTP-binding protein TypA (Translational GTPase TypA)	–	–	–
303	A0A085KA41	Alpha-L-rhamnosidase	–	–	–
304	A0A3G2UR06	MBL fold hydrolase	–	–	–
305	A0A085K554	DUF4167 domain-containing protein	–	–	–
306	A0A3G2UUK6	UPF0246 protein EBF16_04420	–	–	–
307	A0A3G2USF9	Transcriptional regulator	–	–	–
308	A0A085K1G3	Valine-tRNA ligase	–	–	–
309	A0A085K5B9	Shikimate dehydrogenase (NADP(+)) (SDH)	–	–	–
310	A0A085K9B6	Cytokinin riboside 5'-monophosphate phosphoribohydrolase	–	–	–
311	A0A085K3I0	Bifunctional purine biosynthesis protein PurH [Includes: Phosphoribosylaminoimidazolecarboxamide formyltransferase	–	–	–
312	A0A177JX07	Carbamoyl-phosphate synthase large chain	–	–	–
313	A0A085K3J0	Single-stranded DNA-binding protein (SSB)	–	–	–
314	A0A085K9E6	UPF0178 protein EBF16_12115	–	–	–
315	A0A085K8S9	Uncharacterized protein	–	–	–
316	A0A3G2V0V0	TPR_REGION domain-containing protein	–	–	–
317	A0A3G2UNL7	Bifunctional protein PutA [Includes: Proline dehydrogenase	–	–	–
318	A0A084EPP3	DNA-directed RNA polymerase subunit alpha (RNAP subunit alpha)	–	–	–
319	A0A085K7E6	Serine hydroxymethyltransferase (SHMT) (Serine methylase)	–	–	–
320	A0A085KAG1	Acyl-CoA carboxylase subunit beta (Methylmalonyl-CoA carboxyltransferase)	–	–	–
321	A0A3G2V1J1	Shikimate 5-dehydrogenase	–	–	–
322	A0A0J9CZF5	Acyl-CoA thioesterase	–	–	–
323	A0A085KA49	Phosphoribosylformylglycinamidine synthase subunit PurQ (FGAM synthase)	–	–	–
324	A0A085K5F7	Phosphoheptose isomerase	–	–	–
325	A0A3G2V241	S9 family peptidase	–	–	–
326	A0A085K5D7	Bifunctional uridylyltransferase/uridylyl-removing enzyme (UTase/UR) (Bifunctional [protein-PII] modification enzyme) (Bifunctional nitrogen sensor protein) [Includes: [Protein-PII] uridylyltransferase (PII uridylyltransferase) (UTase)	–	–	–
327	A0A085K7D5	Agmatine deiminase family protein	–	–	–

Note: –: No data.

2.4.2 差异蛋白的生物信息学分析

分别对表达差异蛋白在分子功能、生物学过程以及KEGG信号通路层面进行了分析,如图6所示。在分子功能方面，除了催化和结合功能外，有近10%的表达差异蛋白具有转运的功能，这说明在菲的环境下，细菌需要表达更多的转运蛋白将菲、无机盐等物质转运至胞内。表达差异蛋白参与的生物学过程主要集中在代谢过程、细胞生长和生物调控等方面。KEGG 信号通路分析结果显示大量的蛋白参与了各类物质的代谢和合成。根据以上结果推测鞘脂菌 SJTF-8 需要表达更多可在多样化环境中发挥代谢功能的蛋白、转运蛋白以及生物调控蛋白等来适应菲的胁迫。

2.4.3 差异调控蛋白的比较

分别对 DIA 和 DDA 测试结果中 A 组发现的表达差异调控蛋白做了统计分析,发现利用 DIA 技术定量到 39 个调控蛋白(CV 值≤20%)，数量是 DDA 中调控蛋白(CV 值≤20%)的 3 倍以上，证明了 DIA 技术方法对低丰度差异蛋白检出的优势。如表 6 所示，我们还发现在这 39 个调控蛋白中，有 21 个调控蛋白在 DDA 所有定量结果中虽然被检测到，但 CV 值均大于 20%,进一步说明 DDA 对低丰度蛋白定量检测的重现性差,这也更加凸显出 DIA 的方法对低丰度调控蛋白检测的优势。本研究的菲胁迫下 SJTF-8 蛋白质组的定量结果为鞘脂菌对菲的降解途径调控机制的研究提供了新线索和方向。

3 讨论与结论

DIA 技术的出现为高通量、全面地定量蛋白质组学开辟了一个新的领域，已被广泛应用于高等生物样品如人类体液、组织、小鼠/大鼠和斑马鱼等动物模型的研究中[15,23-24]。Lin 等对血清蛋白的研究发现，与 DDA 技术相比，DIA 法检测到的多肽和蛋白质的数量增加一倍且具有较好的重现性[25]；Muntel 等对 87 份尿液样品的检测中也发现，相较于 DDA 法，DIA 明显提高了检测通量以及定量能力,使得高效地发现和验证生物标记物成为可能[14]。但是在低等生物中 DIA 技术的应用研究较少，目前仅在幽门螺旋杆菌、结核分枝杆菌和酵母的研究中得到应用[26-28]。Selevsek 等将 DIA 运用于酵母的研究中，发现 DIA 技术不仅与多重反应监测技术有相当的精度、重复型和准确性，还允许在多个样品中定

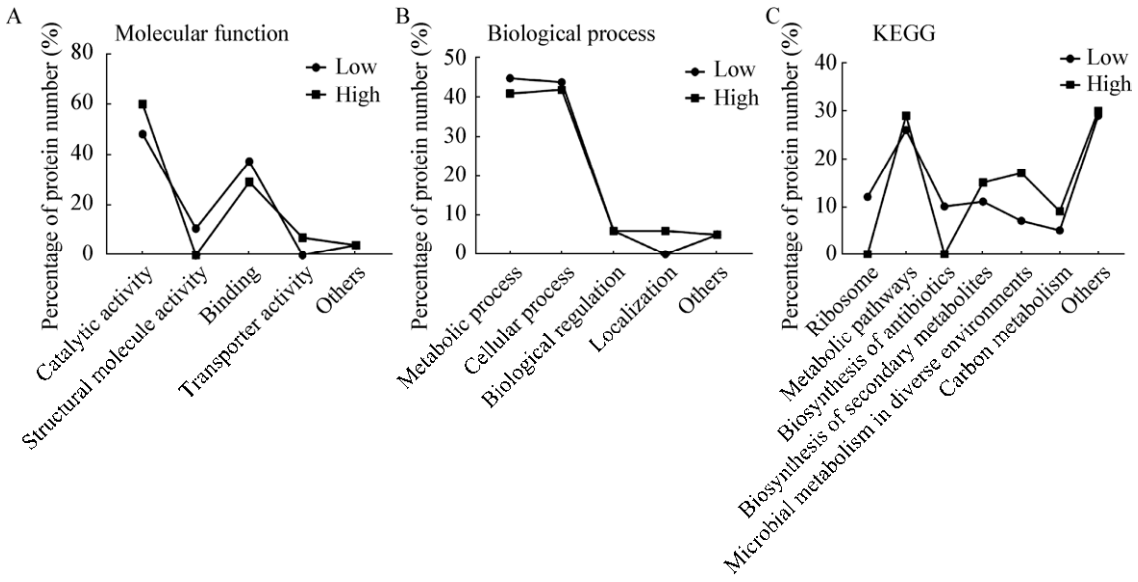

图 6 DDA 和 DIA 的共同得到的差异蛋白生物信息学分析

Figure 6 Bioinformatics analysis of differentially expressed proteins both from DDA and DIA

表 6 DDA 中 CV 值≥20%的 21 个调控蛋白

Table 6 21 regulatory proteins with CV≥20% in DDA

No.	Accessions	Protein names
1	A0A3G2UX28	Transcriptional regulator
2	A0A3G2UTF0	Type II toxin-antitoxin system ParD family antitoxin
3	A0A3G2V104	LysR family transcriptional regulator
4	A0A3G2UPX2	Transcriptional regulator
5	A0A3G2UTK0	MerR family transcriptional regulator
6	A0A3G2UTM0	PAS domain-containing sensor histidine kinase
7	A0A085K5Z7	Crp/Fnr family transcriptional regulator (cAMP-binding protein-catabolite transcription activator)
8	A0A085K957	Nitrogen regulatory protein P-II 1 (P-II family nitrogen regulator)
9	A0A085K7C3	Histidine kinase (Putative regulator of cell autolysis) (Sensor histidine kinase)
10	A0A085K417	FadR family transcriptional regulator (GntR family transcriptional regulator)
11	A0A084EUT7	Histidine phosphotransferase (Hpt domain-containing protein)
12	A0A085K446	LacI family DNA-binding transcriptional regulator (LacI family transcriptional regulator)
13	A0A085K6A6	FadR family transcriptional regulator (GntR family transcriptional regulator)
14	A0A085K0Y0	Histidine kinase (Sensor histidine kinase)
15	A0A085K5M0	MarR family transcriptional regulator
16	A0A085K4Q1	ArsR family transcriptional regulator
17	A0A085K9N9	Uncharacterized protein
18	A0A085K560	DeoR faimly transcriptional regulator (PLP-dependent aminotransferase family protein)
19	A0A085JZV3	Chemotaxis protein CheY (DNA-binding response regulator) (Two-component system response regulator)
20	A0A3G2ULP2	Glutaredoxin family protein
21	A0A3G2UQ46	Transcriptional regulator

量大量蛋白质，提高了传统方法的效率[27]。除此之外，Schubert 等对结核分枝杆菌的研究也让我们看到了将 DIA 运用到绝对定量蛋白质组学中的可能性[28]。

本研究选取低等生物鞘脂菌 SJTF-8 为分析对象，从缺失值、重现性和定性定量蛋白数目等方面比较了 DDA 和 DIA 技术的定量特点，发现 DIA 技术的重现性和对低丰度蛋白的定量能力明显优于 DDA 技术。因此 DIA 在实际可用的表达差异蛋白检出方面具备明显优势，尤其有利于发现菲胁迫下细胞诱导表达的低丰度调控蛋白。然而，DIA 相较于 DDA 技术在蛋白质组定性定量总体数量方面不具备显著优势，这可能是由于本实验以微生物蛋白质组为研究对象，鞘氨醇类菌的蛋白家族数量有限，远少于高等生物，现有的仪器在 DDA 模式下就可以采集到较为全面的蛋白信息。因此，对于蛋白成分简单的样品，使用 DDA 采集模式不仅可以获得相当体量的蛋白质组定性定量结果，而且具有样品前处理及质谱采集方法设置简单等优势。科研人员可以根据研究对象的特点和研究目的合理选择 DDA 和 DIA 技术进行蛋白质组相对定量分析。

鞘氨醇类菌(包含鞘氨醇单胞菌、鞘脂菌、鞘氨醇盒菌以及新鞘脂菌属)是最常见的菲降解菌之一，在微生物修复多环芳烃的污染方面具有重要意义[29]。人们对于鞘氨醇类菌降解菲的代谢途径已做了较为广泛的研究[3]。但是，关于多环芳烃降解的调控机制、代谢产物聚集等方面的机理尚不清楚[1]。鞘氨醇类菌的蛋白质组研究，可以为其在微生物修复方面的基因调控机制提供清晰的思路。然而，关于鞘氨醇类菌在多环芳烃环境下的蛋白质组学研究则少之又少，而且鉴定到的蛋白数量也有一定限制[1,30-31]。

本实验以鞘氨醇类菌中鞘脂菌为研究对象，通过 DDA 和 DIA 两种技术方法进行菲胁迫下鞘脂菌蛋白质组非标记相对定量分析，共得到 580 个表达差异蛋白，更完整地优化出鞘脂菌 *Sphingobium yanoikuyae* SJTF-8 在菲胁迫下表达差异的蛋白。另外，GO 和 KEGG 分析表明表达差异蛋白在细胞代谢、转运和调控等方面都发挥一定功能，为深入研究鞘脂菌的多环芳烃降解过程提供了理论依据，也为更好地治理有机物污染提供了新的研究思路。

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