Online Resource 1

For in-gel digestion analysis:

Protein identification by mass spectrometry analysis. The excised spots containing the proteins were processed according with Bonilla et al. (2016). The proteins in solution were reduced and alkylated with DTT and Iodoacetamide respectively, followed by digestion using trypsin sequencing grade (Promega, Madison Wi) The tryptic digested peptides were in-line desalted as described previously (Bonilla et al., 2016). Peptide analysis was performed using nanoUPLC-ESI-MS/MS. The peptides were in-line desalted and concentrated with an RP-Trap column Symmetry C18, 5 µm, 180 µm (id) X 20 mm nanoAcquity UPLC column (Waters Corp., Milford, MA), and separated using a BEH130 C18 RP, 1.7 µm , 100 µm (id) X 100 mm nanoAcquity UPLC column (Waters Corp., Milford, MA). The standard gradient used was: 0-2 min, 3% B isocratic; 2-40 min, 3-80 % B linear. Mobile phase A was water/formic acid (99.9:0.1, v/v), and phase B was acetonitrile/formic acid (99.9:0.1 v/v). The solvent flow rate was 400 nl/min. The separation was performed using nanoAcquity Ultra Performance LC nanoAcquity UPLC (Waters Corp., Milford, MA). The eluted ions were analyzed by one full precursor MS scan (400-2000 m/z) followed by four MS/MS scans of the most abundant ions detected in the precursor MS scan while operating under dynamic exclusion or direct data acquisition system Spectra obtained in the positive ion mode with nano ESI Q-Tof Synapt G1 HDMS mass spectrometer (Waters, Milford, MA) were deconvoluted, and analyzed using the MassLynx software 4.1 (Micromass, UK). A peak list (PKL format) was generated to identify +1 or multiple charged precursor ions from the mass spectrometry data file (**Online Resource 2**). The instrument was calibrated in MS/MS mode using 100 fmole of (Glu1)-Fibrinopeptide B human with a root mean square residual of 7.484 e-4 amu or 7.76 e-1 ppm. Parent mass (MS) and fragment mass (MS/MS) peak ranges were 400-2000 Da and 65-2000 Da, respectively.

- Bonilla JO, Callegari EA, Delfini CD, Estevez MC, Villegas LB (2016) Simultaneous chromate and sulfate removal by *Streptomyces* sp. MC1. Changes in intracellular protein profile induced by Cr(VI). J Basic Microbiol 56(11):1212-1221. https://doi.org/10.1002/jobm.201600170

Intracellular proteomic analysis of *Streptomyces* sp. MC1 when exposed to Cr(VI) by gel-based and gel-free methods. Current Microbiology. José O. Bonilla, Eduardo A. Callegari, María C. Estevez, Liliana B. Villegas.

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