Supplementary Material 3 : Detailed proteomic analysis. On pages 2 and 3 are given the parameters used in Xtandem for protein identification.

After reduction, alkylation and trypsin digestion in gel, protein samples were reconstituted in 2% ACN, 0.1% formic acid buffer and analyzed on a TimsTof Pro mass spectrometer (Bruker, Billerika USA) coupled to a nanoElute chromatography (Bruker). Samples were loaded on a nanoEase trap column (inner diameter 0.18mm, length 20mm, particle size 5µm, from Waters) and eluted on an aurora column (internal diameter 0.075mm, length 25cm, particule size 1.6µm, ionOptics) with a two step linear gradient at 250nl.min^{-1}. The first step was performed from 4 % to 20 % of buffer B (0.1 % formic acid in acetonitrile) in buffer A (0,1 % formic acid in water) in 65 min. The second step was performed from 20 % to 32 % of buffer B in buffer A, in 5min. Columns were washed with 84 % of buffer B for 12 min.

Electrospray ionization was performed with a Captive Spray interface at 2kV. Peptide ions were analyzed using otof Control 6.0.3 (Bruker) on PASEF Mode with tims activated, mz range set from 100 m/z to 1700 m/z, and mobility ramp set from 0.60 Vs/cm² to 1.6 Vs/cm². Acquisition cycle was performed as 1 MS of 100 ms, followed by 10 PASEF frames of 100ms. For each PASEF frame, 10 MS/MS spectra were fragmented using a linear ramp energy from 20 eV to 59 eV. Isolation was set to 2 m/z for precursor mass smaller than 700 m/z, and 3m/z for the others. The dynamic exclusion was set to 20 seconds for precursors with 20k count global spectrum intensity. Lower abundant precursors were re-analyzed if the intensity was 4 times more intense than in their previous scan.

Identification parameter

Protein identification

Xtandem Run information

param	value
version	X! Tandem Piledriver (2015.04.01.1)
sequence source #1	Ctyphae_annotation_2023_proteins.fasta
sequence source #2	contaminants_standarts.fasta
sequence source #3	ncbi_C_typhae_220509.fasta

Spectra filtering

param	value
parent monoisotopic mass error minus	50
parent monoisotopic mass error plus	50
parent monoisotopic mass error units	ppm
parent monoisotopic mass isotope error	yes
maximum parent charge	5
fragment mass type	monoisotopic
fragment monoisotopic mass error	50
fragment monoisotopic mass error units	ppm
total peaks	100
neutral loss mass	18.01057
neutral loss window	0.05
dynamic range	100.0
minimum fragment mz	150.0
minimum parent m+h	500.0
minimum peaks	15

Enzymatic cleavage

param	value
cleavage site	[RK]|{P}
cleavage semi	no
quick pyrolidone	yes
stP bias	yes
quick acetyl	yes

Amino acid modification

param	value
modification mass	57.02146@C
potential modification mass	15.99491@M

Refinement module

param	value
use refine	yes
maximum valid expectation value	0.01
spectrum synthesis	yes
unanticipated cleavage	no
modification mass	57.02146@C
point mutations	no
potential modification mass	15.99491@M
use potential modifications for full refinement	yes
cleavage semi	yes
spectrum synthesis	yes

Scoring

param	value
minimum ion count	4
maximum missed cleavage sites	1
cyclic permutation	yes
include reverse	yes
b ions	yes
y ions	yes