

***Lactobacillus plantarum* attenuates anxiety-related behavior and protects against stress-induced dysbiosis in adult zebrafish**

*Daniel J. Davis<sup>1#</sup>, Holly M. Doerr<sup>1#</sup>, Agata K. Grzelak<sup>1</sup>, Susheel B. Busi<sup>1</sup>, Eldin Jasarevic<sup>2</sup>, Aaron C. Ericsson<sup>1,3</sup>, and Elizabeth C. Bryda<sup>1,\*</sup>*

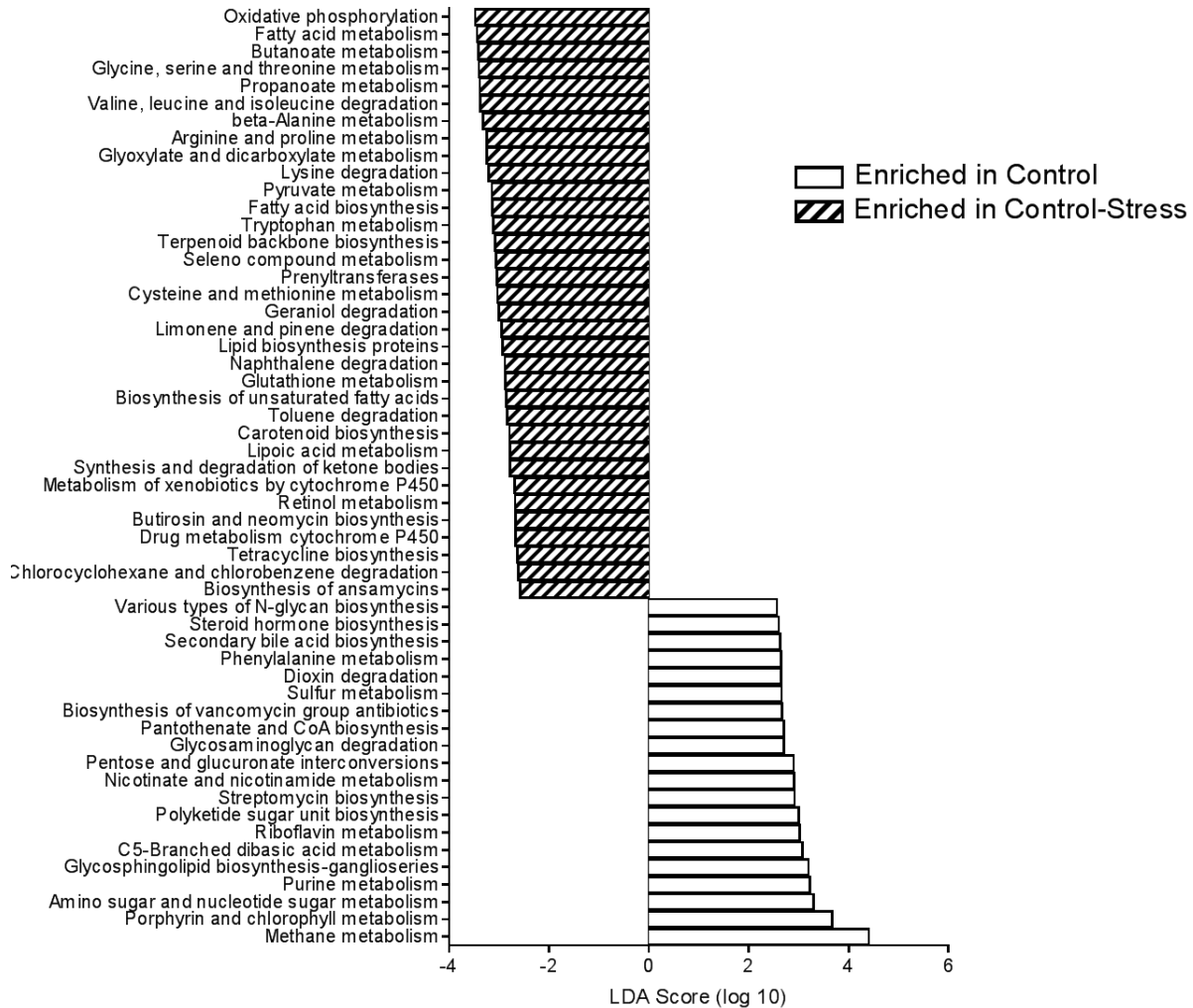
<sup>1</sup> Department of Veterinary Pathobiology, University of Missouri, Columbia, MO 65201, USA

<sup>2</sup> Center for Host-Microbial Interactions, University of Pennsylvania, Philadelphia, PA 19104, USA

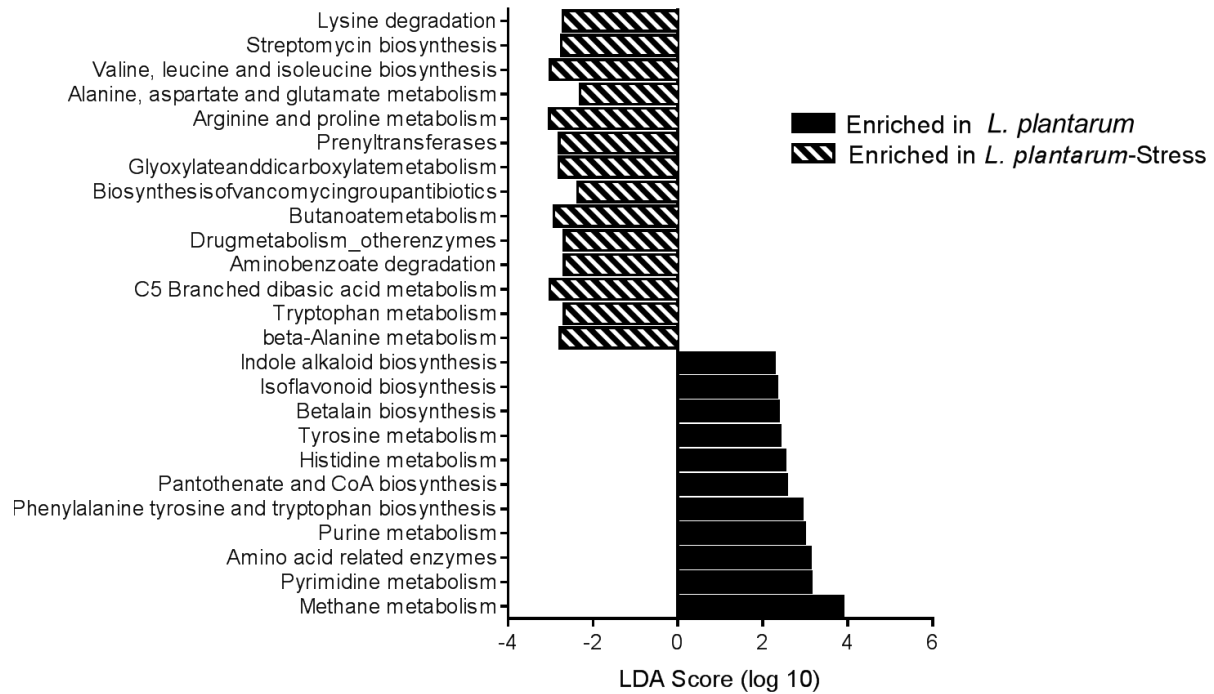
<sup>3</sup> University of Missouri Metagenomics Center (MUMC), University of Missouri, Columbia, MO 65201, USA

\*Correspondence: [brydae@missouri.edu](mailto:brydae@missouri.edu)

# Co-first authors



**Supplementary Figure 1** PICRUSt analysis reveals stress-induced functional alterations of the GM. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) predicted functional profile alteration of microbial communities in control zebrafish subjected to chronic unpredictable stress (CUS). ( $n = 12$  fish/group). (Linear discriminant analysis; LDA).



**Supplementary Figure 2** PICRUSt analysis reveals stress-induced functional alterations of the GM in *L. plantarum* treated zebrafish. Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) predicted functional profile alteration of microbial communities in *L. plantarum* treated zebrafish subjected to chronic unpredictable stress (CUS). ( $n = 12$  fish/group). (Linear discriminant analysis; LDA).



	<b>Morning</b>	<b>Evening</b>
<b>Day 1</b>	Chasing (8 minutes)	Tank changes (x6)
<b>Day 2</b>	Over-crowding (60 minutes)	Dorsal body exposure (2 minutes)
<b>Day 3</b>	Social isolation (30 minutes)	Tank changes (x6)
<b>Day 4</b>	Dorsal body exposure (2 minutes)	Chasing (8 minutes)
<b>Day 5</b>	Tank changes (x6)	Dorsal body exposure (2 minutes)

***Supplementary Table 3* Chronic unpredictable stress (CUS) paradigm.**

<b>Gene Symbol</b>	<b>Forward Primer</b>	<b>Reverse Primer</b>	<b>Reference</b>
<i>gad1</i>	5'-AACTCAGGCGATTGTTGCAT-3'	5'-TGAGGACATTTCCAGCCTTC-3'	<i>Hortopan et al (2010)</i>
<i>gabra1</i>	5'-TCAGGCAGAGCTGGAAGGAT-3'	5'-TGCCGTTGTGGAAGAACGT-3'	<i>Hortopan et al (2010)</i>
<i>slc6a4a</i>	5'-TAACCACTACAGTTTGGCTTGATG-3'	5'-AACAGTTAACCGAGCTTGTGAT-3'	<i>Wong et al (2013)</i>
<i>slc6a4b</i>	5'-GCCGAGGAGTTTTACACGAGGA-3'	5'-ACATATGGCAGGGTGGCAGT-3'	<i>Wong et al (2013)</i>
<i>oxtl</i>	5'-ATTCGACAGTGTATGCCGTG-3'	5'-TCACACGGAGAAGGGAGAAA-3'	<i>Wong et al (2013)</i>
<i>npy</i>	5'-ACTACATCAACCTCATAACAAGGC-3'	5'-GATGAGATCACCATGCCAAATGAT-3'	<i>Wong et al (2013)</i>
<i>ef1a</i>	5'-CCTCTGGTCGCTTTGC-3'	5'-GGTGTGATTGAGGGAAATTCA-3'	<i>Wong et al (2013)</i>

**Supplementary Table 4 Complete list of primer sequences used for qPCR.**