

## **Synthesis and application of a phenazine class substrate for high-throughput screening of laccase activity**

Justinas Babinskas<sup>1</sup>, Jerica Sabotič<sup>2</sup>, and Inga Matijošytė<sup>1\*</sup>

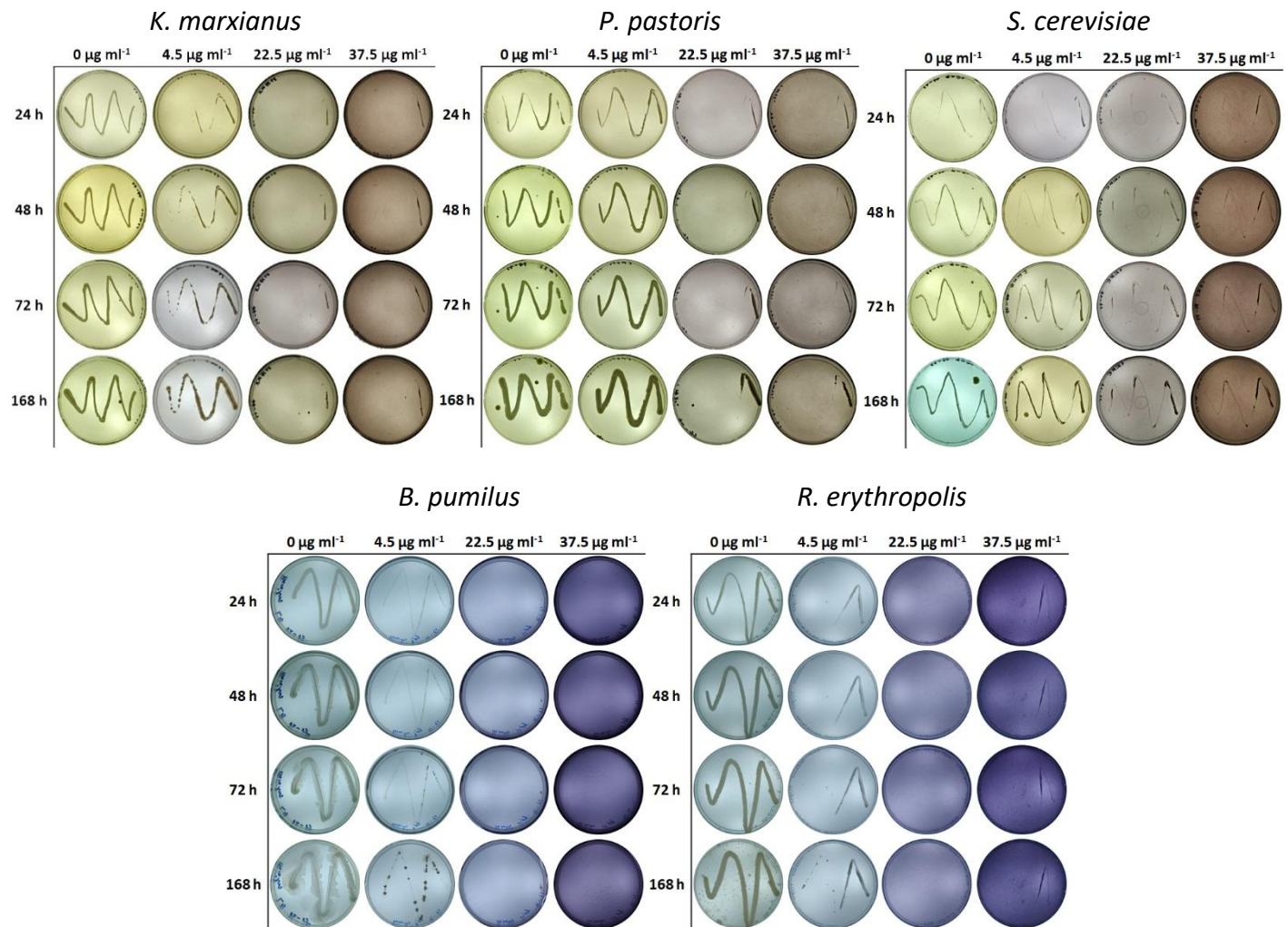
<sup>1</sup> Vilnius University, Life Sciences Center, Institute of Biotechnology, Sector of Applied Biocatalysis, Saulėtekio ave. 7, LT-10257, Vilnius, Lithuania.

<sup>2</sup> Department of Biotechnology, Jožef Stefan Institute, Jamova cesta 39, 1000 Ljubljana, Slovenia.

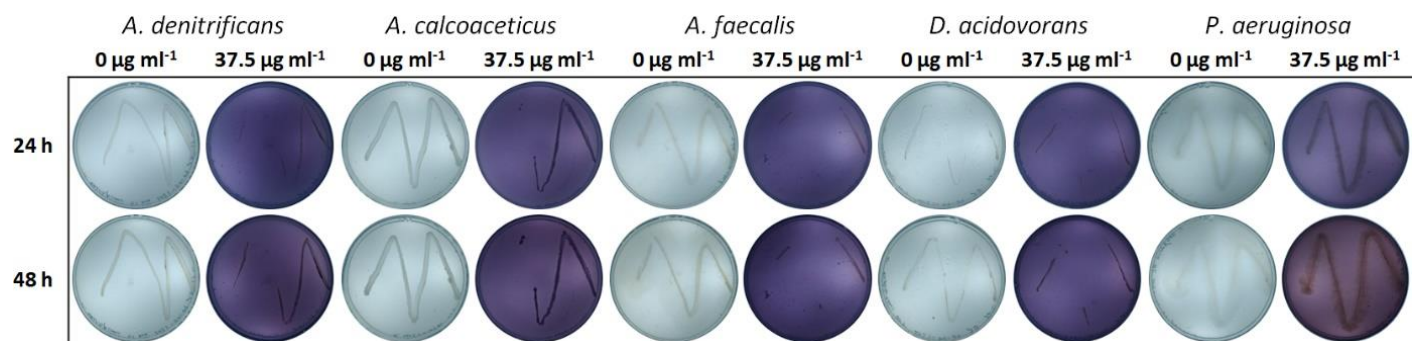
\* Corresponding author: Inga Matijošytė; email [inga.matijosyte@bti.vu.lt](mailto:inga.matijosyte@bti.vu.lt)



**Figure S1.** Results of *E. coli* and *P. putida* growth on  $37.5 \mu\text{g ml}^{-1}$  Ferbamine-LB agar and Ferbamine-TS agar plates after 10 h of incubation, respectively.

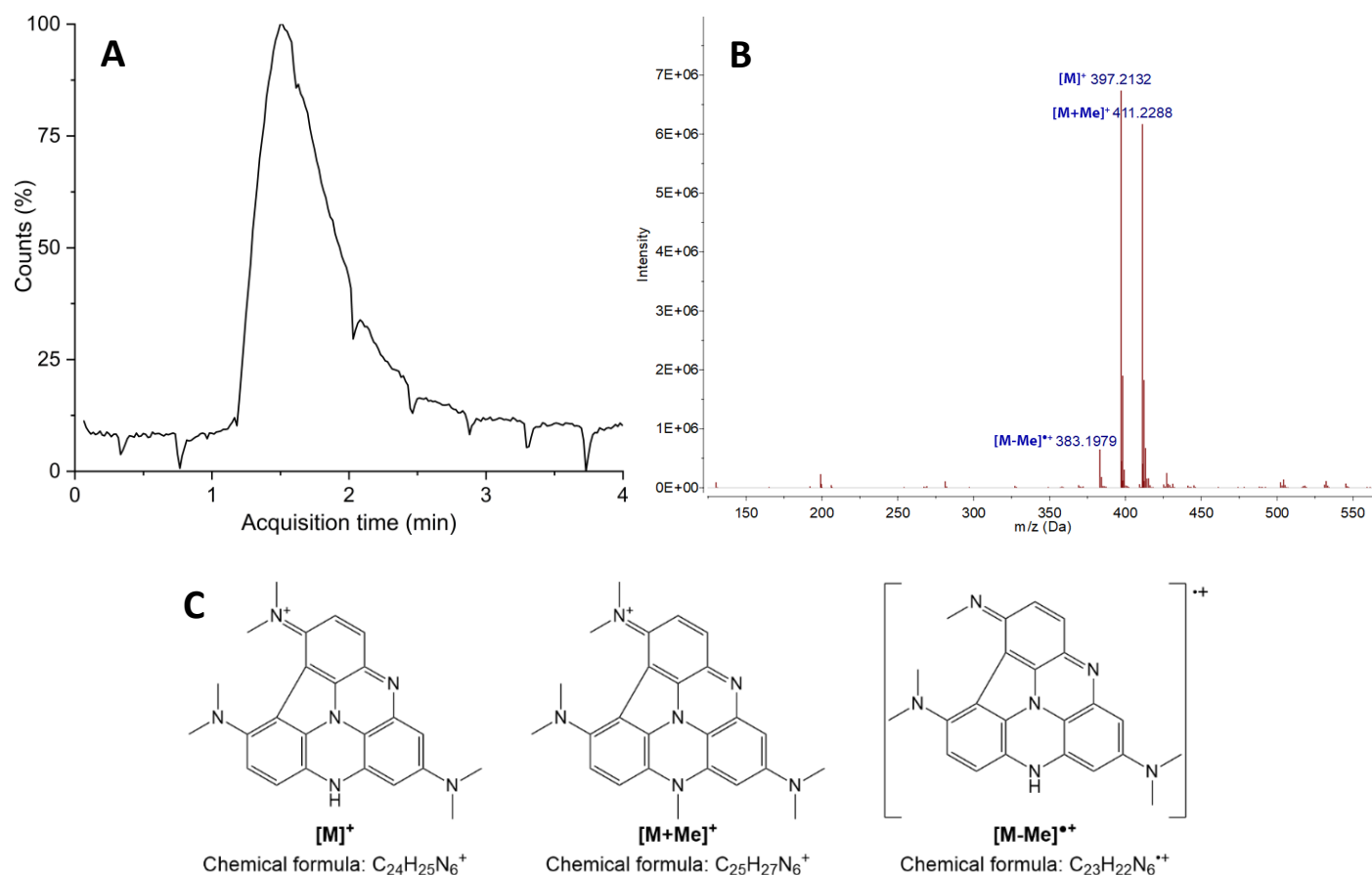


**Figure S2.** Results of Ferbamine concentration ( $0\text{--}37.5 \mu\text{g ml}^{-1}$ ) influence on the growth of *K. lactis*, *P. pastoris*, *S. cerevisiae*, *B. pumilus* and *R. erythropolis* growth on  $0\text{--}37.5 \mu\text{g ml}^{-1}$  Ferbamine and YEPG, LB or nutrient agar plates.



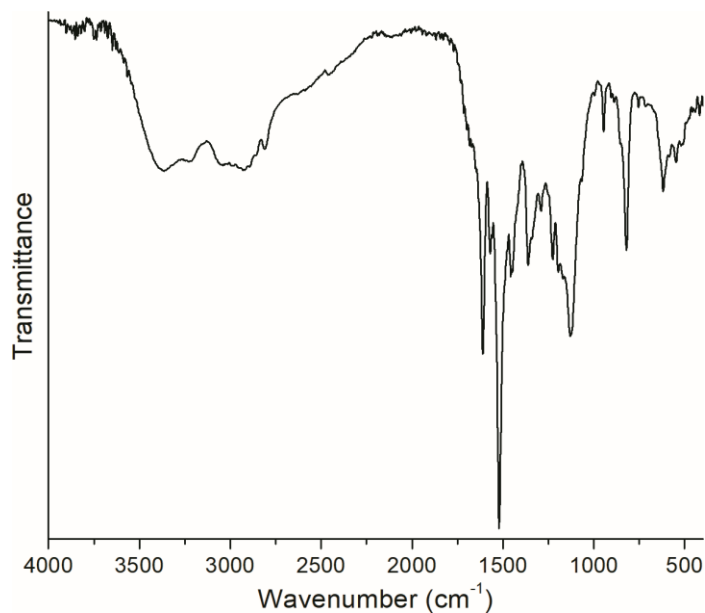
**Figure S3.** Results of Ferbamine concentration (0-37.5  $\mu\text{g ml}^{-1}$ ) influence on the growth of *R. erythropolis*, *A. denitrificans*, *D. acidovorans*, *P. aeruginosa*, *A. faecalis* and *A. calcoaceticus* growth 37.5  $\mu\text{g ml}^{-1}$  Ferbamine-nutrient agar and control plates.

**Liquid chromatography (LC) high-resolution mass spectrometry (HRMS) analysis of Ferbamine synthesis product.** The chromatography and mass spectrometry were carried out at Institute of Chemistry, Vilnius University (Lithuania), using Dual-Esi Q-TOF 6250 spectrometer (Agilent Technologies, Santa Clara, USA). Data analysis was performed by the authors' group.



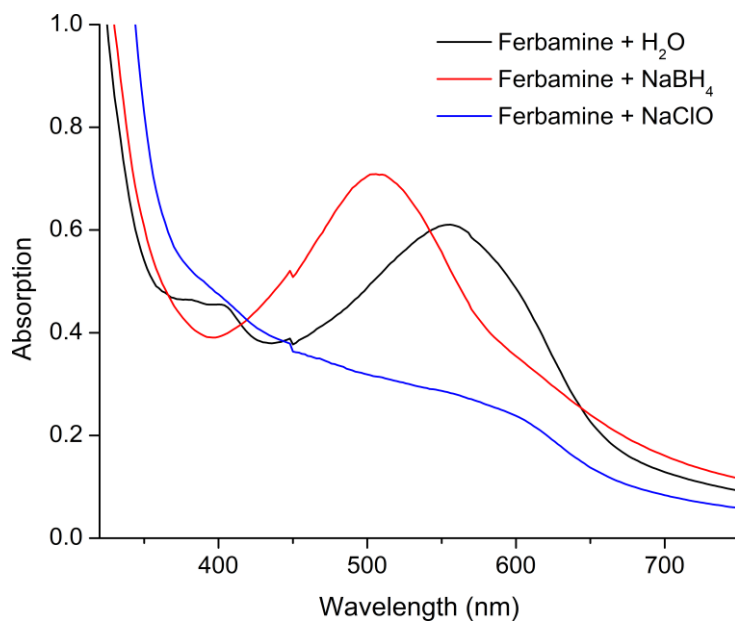
**Figure S4.** Results of Ferbamine LC-HRMS analysis. **A** – LC chromatogram; **B** – HRMS spectra at chromatography acquisition (retention) time 1.515 min; **C** – predicted chemical formula and assigned molecular structures to HRMS signals.

**Attenuated total reflectance Fourier-transform infrared spectroscopy (ATR-FTIR) analysis of Ferbamine synthesis product.**

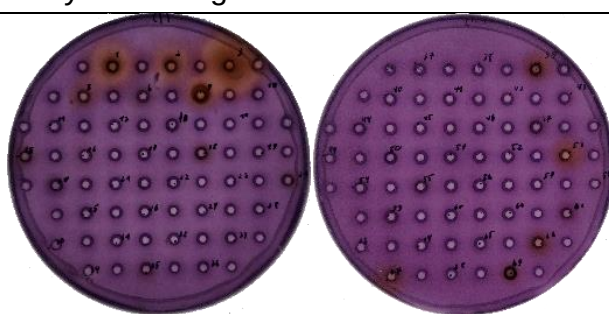


**Figure S5.** ATR-FTIR spectra of Ferbamine.

**Ultraviolet-Visible (UV-VIS) light spectroscopy of native, partially reduced or oxidized Ferbamine solutions.**



**Figure S6.** UV-VIS spectra of partially reduced (Ferbamine + NaBH<sub>4</sub>), oxidized (Ferbamine + NaClO) and control (Ferbamine + H<sub>2</sub>O) Ferbamine solutions.

**Table S1.** Result of laccase activity screening in the collection of extracts from terrestrial fungi.

| Extract No. | Species                                                     | Assays                                   |           |                                                                  |
|-------------|-------------------------------------------------------------|------------------------------------------|-----------|------------------------------------------------------------------|
|             |                                                             | Metol [U ml <sup>-1</sup> ] <sup>a</sup> | Ferbamine | Previously reported laccase <sup>b</sup>                         |
| 3           | <i>Clitocybe nebularis</i>                                  | 9.00                                     | +++       | Yes (Asav 2021)                                                  |
| 39          | <i>Psilocybe cubensis</i>                                   | 5.40                                     | +++       | Yes (Lenz et al. 2020)                                           |
| 6           | <i>Cortinarius violaceus</i>                                | 5.25                                     | +         | No (Chen et al. 2003)                                            |
| 2           | <i>Macrolepiota procera</i>                                 | 4.80                                     | +++       | Yes (Luis et al. 2004)                                           |
| 50          | <i>Gomphidius glutinosus</i>                                | 3.90                                     | -         | Not tested                                                       |
| 53          | <i>Pleurotus eryngii</i>                                    | 2.70                                     | +++       | Yes (Guo et al. 2017)                                            |
| 1           | <i>C. nebularis</i>                                         | 2.55                                     | +++       | Yes (Asav 2021)                                                  |
| 67          | <i>Rhodocybe gemina</i>                                     | 1.65                                     | +         | Not tested                                                       |
| 15          | <i>Tricholoma tigrinum</i>                                  | 1.65                                     | +         | Not tested                                                       |
| 66          | <i>Tricholoma saponaceum</i>                                | 1.50                                     | ++        | Yes (Khaund and Joshi 2014)                                      |
| 33          | <i>Lentinula edodes</i>                                     | 1.13                                     | -         | Yes (Yano et al. 2009)                                           |
| 5           | <i>Ganoderma lucidum</i>                                    | 1.05                                     | ++        | Yes (Jeon et al. 2008)                                           |
| 55          | <i>Gymnopilus penetrans</i>                                 | 0.90                                     | +         | No (Marr et al. 1986)                                            |
| 9           | <i>Cortinarius caperatus</i><br>( <i>Rozites caperata</i> ) | 0.83                                     | +++       | Not tested                                                       |
| 62          | <i>Amanita vaginata</i>                                     | 0.83                                     | +         | Not tested                                                       |
| 47          | <i>Anthurus archeri</i><br>( <i>Clathrus archeri</i> )      | 0.75                                     | +         | Not tested                                                       |
| 18          | <i>Pleurotus ostreatus</i><br>(summer strain)               | 0.75                                     | +         | Yes (Park and Park 2014)                                         |
| 35          | <i>Clitocybe geotropa</i>                                   | 0.68                                     | +         | Not tested                                                       |
| 54          | <i>Amanita spissa</i>                                       | 0.68                                     | -         | No (Chen et al. 2003)                                            |
| 70          | <i>A. vaginata</i>                                          | 0.68                                     | -         | Not tested                                                       |
| 51          | <i>Tricholomopsis rutilans</i>                              | 0.68                                     | -         | Not tested                                                       |
| 59          | <i>Clavariadelphus pistillaris</i>                          | 0.60                                     | +         | Yes (Marr et al. 1986)                                           |
| 40          | <i>Ramaria stricta</i>                                      | 0.53                                     | -         | Yes (ABTS, guaiacol),<br>No (syringaldazine) (Erden et al. 2009) |
| 20          | <i>P. ostreatus</i><br>(winter strain)                      | 0.45                                     | +         | Yes (Park and Park 2014)                                         |
| 36          | <i>Russula alutacea</i>                                     | 0.45                                     | +         | Not tested                                                       |
| 57          | <i>Clitocybe geotropa</i>                                   | 0.38                                     | -         | Not tested                                                       |
| 31          | <i>Paxillius atrotomentosus</i>                             | 0.38                                     | -         | Not tested                                                       |
| 69          | <i>Cortinarius multiformis</i>                              | 0.30                                     | ++        | Not tested                                                       |
| 32          | <i>Clitocybe gibba</i>                                      | 0.30                                     | -         | Not tested                                                       |

|    |                                   |      |   |                                  |
|----|-----------------------------------|------|---|----------------------------------|
| 28 | <i>Lactarius necator</i>          | 0.23 | - | Not tested                       |
| 63 | <i>Amanita rubescens</i>          | 0.15 | - | No (Hutchison 1990)              |
| 58 | <i>Craterellus cornucopioides</i> | 0.15 | - | Not tested                       |
| 43 | <i>P. ostreatus</i>               | 0.15 | - | Yes (Park and Park 2014)         |
| 42 | <i>Xerocomus badius</i>           | 0.15 | - | No (Hutchison 1990)              |
| 65 | <i>Agaricus bisporus</i>          | 0.08 | - | Yes (Mayolo-Deloisa et al. 2011) |
| 41 | <i>A. rubescens</i>               | 0.08 | - | No (Hutchison 1990)              |
| 42 | <i>A. rubescens</i>               | 0.08 | - | No (Hutchison 1990)              |
| 46 | <i>P. atrotomentosus</i>          | 0.08 | - | Not tested                       |
| 24 | <i>Amanita excelsa</i>            | -    | + | Not tested                       |
| 16 | <i>Macrolepiota rachodes</i>      | -    | + | No (Luis et al. 2004)            |
| 8  | <i>Amanita citrina</i>            | -    | - | No (Hutchison 1990)              |
| 61 | <i>A. citrina</i>                 | -    | - | No (Hutchison 1990)              |
| 64 | <i>A. citrina</i>                 | -    | - | No (Hutchison 1990)              |
| 37 | <i>A. rubescens</i>               | -    | - | No (Hutchison 1990)              |
| 60 | <i>A. spissa</i>                  | -    | - | No (Chen et al. 2003)            |
| 68 | <i>A. spissa</i>                  | -    | - | No (Chen et al. 2003)            |
| 21 | <i>Armillaria borealis</i>        | -    | - | Not tested                       |
| 22 | <i>Boletus badius</i>             | -    | - | Not tested                       |
| 11 | <i>Boletus erythropus</i>         | -    | - | Not tested                       |
| 25 | <i>Coprinus comatus</i>           | -    | - | Yes (Bao et al. 2013)            |
| 34 | <i>Fomitopsis pinicola</i>        | -    | - | Yes (Park and Park 2014)         |
| 23 | <i>Kuehneromyces mutabilis</i>    | -    | - | Yes (Gramss 2020)                |
| 14 | <i>L. necator</i>                 | -    | - | Not tested                       |
| 19 | <i>L. edodes</i>                  | -    | - | Yes (Yano et al. 2009)           |
| 56 | <i>Lycoperdon perlatum</i>        | -    | - | Yes (Harkin et al. 1974)         |
| 27 | <i>Sparassis crispa</i>           | -    | - | Not tested                       |
| 10 | <i>Suillus bovinus</i>            | -    | - | No (Timonen and Sen 1998)        |
| 38 | <i>Suillus granulatus</i>         | -    | - | Yes (Cullings et al. 2008)       |
| 17 | <i>Suillus luteus</i>             | -    | - | No (Mucha 2011)                  |
| 30 | <i>Suillus variegatus</i>         | -    | - | Not tested                       |
| 26 | <i>Tricholoma sulphureum</i>      | -    | - | Not tested                       |

<sup>a</sup> 1 U is defined as 1  $\mu$ mol of Metol catalysed per 1 min, at pH 5.0 and 20 °C.

<sup>b</sup> Yes – laccase gene or activity has been previously reported in the species; No – species has been tested, but laccase genes or activity was not detected; Not tested – species has not been tested for laccase genes or activity.

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