

## Supplementary materials

### A systematic approach to determine the outcome of the competition between two microbial species in bioreactor cocultures

Marcin Bizukojć\*, Tomasz Boruta, Anna Ścigaczewska

Lodz University of Technology, Faculty of Process and Environmental Engineering, Department of Bioprocess Engineering, ul. Wólczajska 213, 93-005 Łódź, Poland; marcin.bizukojc@p.lodz.pl

\* corresponding author

**Suppl. Table S1** Compositions of the cultivation media used in the ATSR experiments (Boruta et al. 2021)

| Medium component                                                     | Experiment |       |       |       |       |       |       |       |       |
|----------------------------------------------------------------------|------------|-------|-------|-------|-------|-------|-------|-------|-------|
|                                                                      | ATSR1      | ATSR2 | ATSR3 | ATSR4 | ATSR5 | ATSR6 | ATSR7 | ATSR8 | ATSR9 |
| Glucose (g l <sup>-1</sup> )                                         | 20         | 20    | 20    | 20    | 20    | 0     | 0     | 20    | 0     |
| Lactose (g l <sup>-1</sup> )                                         | 0          | 0     | 20    | 20    | 20    | 20    | 20    | 20    | 20    |
| Yeast extract (g l <sup>-1</sup> )                                   | 5          | 5     | 2     | 4     | 4     | 4     | 4     | 4     | 4     |
| (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> (g l <sup>-1</sup> ) | 0          | 2     | 2     | 0     | 0     | 0     | 0     | 0     | 0     |
| KH <sub>2</sub> PO <sub>4</sub> (g l <sup>-1</sup> )                 | 1.51       | 1.51  | 1.51  | 1.51  | 1.51  | 1.51  | 1.51  | 1.51  | 1.51  |
| NaCl (g l <sup>-1</sup> )                                            | 0.4        | 0.4   | 0.4   | 0.4   | 0.4   | 0.4   | 0.4   | 0.4   | 0.4   |
| MgSO <sub>4</sub> ·7H <sub>2</sub> O (g l <sup>-1</sup> )            | 0.5        | 0.5   | 0.5   | 0.5   | 0.5   | 0.5   | 0.5   | 0.5   | 0.5   |
| Biotin (mg l <sup>-1</sup> )                                         | 0.04       | 0.04  | 0.04  | 0.04  | 0.04  | 0.04  | 0.04  | 0.04  | 0.04  |
| Trace element solution (ml l <sup>-1</sup> )*                        | 1          | 1     | 1     | 1     | 1     | 1     | 1     | 1     | 1     |

\* Trace elements solution of the following composition: MnSO<sub>4</sub> 50 mg l<sup>-1</sup>, ZnSO<sub>4</sub>·7H<sub>2</sub>O 1 g l<sup>-1</sup>, Fe(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O 2 g l<sup>-1</sup>, Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O 100 mg l<sup>-1</sup>, CuSO<sub>4</sub>·5H<sub>2</sub>O 250 mg l<sup>-1</sup> and Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O 50 mg l<sup>-1</sup>.

**Suppl. Table S2** Compositions of the cultivation media used in the ATSN experiments (Boruta et al. 2022)

| Medium component                                                     | Experiment |       |       |       |       |       |  |  |  |
|----------------------------------------------------------------------|------------|-------|-------|-------|-------|-------|--|--|--|
|                                                                      | ATSN3      | ATSN4 | ATSN5 | ATSN6 | ATSN7 | ATSN8 |  |  |  |
| Glucose (g l <sup>-1</sup> )                                         | 0          | 20    | 20    | 0     | 20    | 20    |  |  |  |
| Lactose (g l <sup>-1</sup> )                                         | 20         | 20    | 20    | 20    | 20    | 20    |  |  |  |
| Yeast extract (g l <sup>-1</sup> )                                   | 4          | 4     | 4     | 4     | 4     | 4     |  |  |  |
| (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> (g l <sup>-1</sup> ) | 0          | 2     | 2     | 0     | 0     | 0     |  |  |  |
| KH <sub>2</sub> PO <sub>4</sub> (g l <sup>-1</sup> )                 | 1.51       | 1.51  | 1.51  | 1.51  | 1.51  | 1.51  |  |  |  |
| NaCl (g l <sup>-1</sup> )                                            | 0.4        | 0.4   | 0.4   | 0.4   | 0.4   | 0.4   |  |  |  |
| MgSO <sub>4</sub> ·7H <sub>2</sub> O (g l <sup>-1</sup> )            | 0.5        | 0.5   | 0.5   | 0.5   | 0.5   | 0.5   |  |  |  |
| Biotin (mg l <sup>-1</sup> )                                         | 0.04       | 0.04  | 0.04  | 0.04  | 0.04  | 0.04  |  |  |  |
| Trace element solution (ml l <sup>-1</sup> )*                        | 1          | 1     | 1     | 1     | 1     | 1     |  |  |  |

\* Trace element solution of the following composition: MnSO<sub>4</sub> 50 mg l<sup>-1</sup>, ZnSO<sub>4</sub>·7H<sub>2</sub>O 1 g l<sup>-1</sup>, Fe(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O 2 g l<sup>-1</sup>, Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O 100 mg l<sup>-1</sup>, CuSO<sub>4</sub>·5H<sub>2</sub>O 250 mg l<sup>-1</sup> and Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O 50 mg l<sup>-1</sup>.

**Suppl. Table S3** Compositions of the cultivation media used in the PRSR and PRSN experiments (Boruta et al. 2023a, 2023b)

| Medium component                                                     | Experiment |       |       |       |       |       |  |  |  |
|----------------------------------------------------------------------|------------|-------|-------|-------|-------|-------|--|--|--|
|                                                                      | PRSR1      | PRSR2 | PRSR3 | PRSN1 | PRSN2 | PRSN3 |  |  |  |
| Glucose (g l <sup>-1</sup> )                                         | 10         | 10    | 10    | 10    | 10    | 10    |  |  |  |
| Lactose (g l <sup>-1</sup> )                                         | 40         | 40    | 40    | 40    | 40    | 40    |  |  |  |
| Yeast extract (g l <sup>-1</sup> )                                   | 4          | 4     | 4     | 4     | 4     | 4     |  |  |  |
| (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> (g l <sup>-1</sup> ) | 0          | 2     | 2     | 0     | 0     | 0     |  |  |  |
| KH <sub>2</sub> PO <sub>4</sub> (g l <sup>-1</sup> )                 | 1.51       | 1.51  | 1.51  | 1.51  | 1.51  | 1.51  |  |  |  |
| NaCl (g l <sup>-1</sup> )                                            | 0.4        | 0.4   | 0.4   | 0.4   | 0.4   | 0.4   |  |  |  |
| MgSO <sub>4</sub> ·7H <sub>2</sub> O (g l <sup>-1</sup> )            | 0.5        | 0.5   | 0.5   | 0.5   | 0.5   | 0.5   |  |  |  |
| Biotin (mg l <sup>-1</sup> )                                         | 0.04       | 0.04  | 0.04  | 0.04  | 0.04  | 0.04  |  |  |  |
| Phenylacetic acid (g l <sup>-1</sup> )                               | 0.25       | 0.25  | 0.25  | 0.25  | 0.25  | 0.25  |  |  |  |
| Trace elements solution (ml l <sup>-1</sup> )*                       | 1          | 1     | 1     | 1     | 1     | 1     |  |  |  |

\*Trace element solution of the following composition: MnSO<sub>4</sub> 50 mg l<sup>-1</sup>, ZnSO<sub>4</sub>·7H<sub>2</sub>O 1 g l<sup>-1</sup>, Fe(NO<sub>3</sub>)<sub>3</sub>·9H<sub>2</sub>O 2 g l<sup>-1</sup>, Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O 100 mg l<sup>-1</sup>, CuSO<sub>4</sub>·5H<sub>2</sub>O 250 mg l<sup>-1</sup> and Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O 50 mg l<sup>-1</sup>.

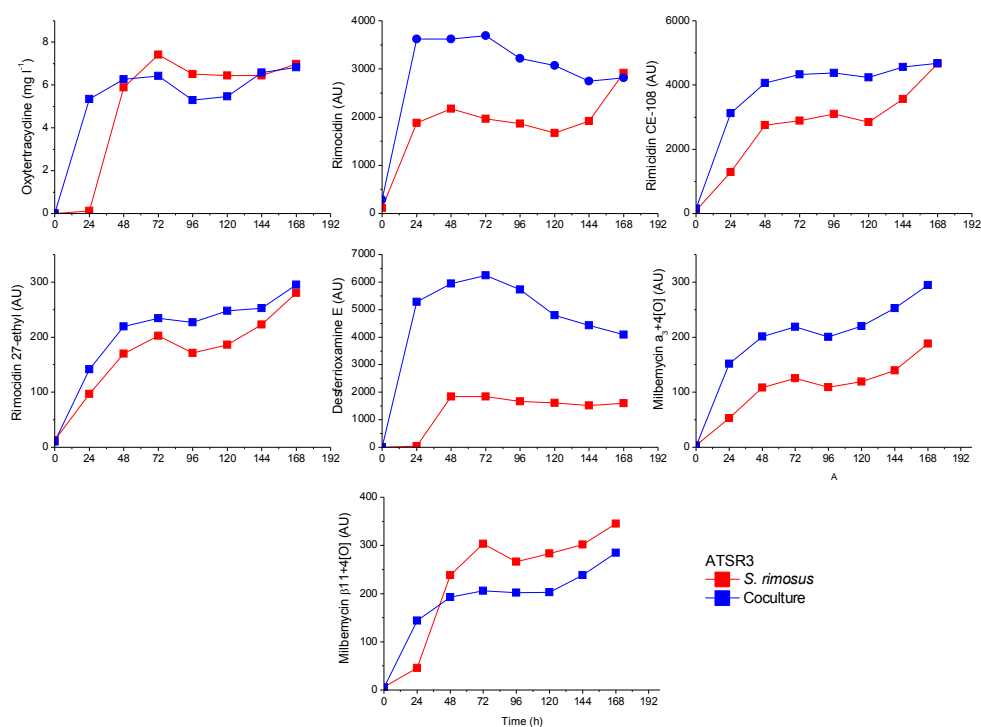
**Suppl. Table S4** Inoculation method in runs ATSR, ASTN, PRSR, PRSN (Boruta et al. 2021, 2022, 2023a, 2023b)

| Exp-<br>eri-<br>mental<br>run | Variant in the run                                                    | Type and volume of inoculum                                                            | Time of<br>inoculation of<br>species                      |
|-------------------------------|-----------------------------------------------------------------------|----------------------------------------------------------------------------------------|-----------------------------------------------------------|
| ATSR1                         | <i>A. terreus</i> monoculture                                         | <i>A. terreus</i> preculture (300 ml)                                                  | 0 h                                                       |
| ATSR2                         | (bioreactor #1)                                                       |                                                                                        |                                                           |
| ATSR3                         | <i>S. rimosus</i>                                                     | <i>S. rimosus</i> preculture (300 ml)                                                  | 0 h                                                       |
| ATSR4                         | monoculture                                                           |                                                                                        |                                                           |
| ATSR5                         | (bioreactor #2)                                                       |                                                                                        |                                                           |
| ATSR6                         | <i>A. terreus</i> + <i>S. rimosus</i><br>coculture<br>(bioreactor #3) | <i>S. rimosus</i> preculture (300 ml) + <i>A. terreus</i> spore<br>suspension (300 ml) | 0 h                                                       |
| ATSR7                         | <i>A. terreus</i> monoculture<br>(bioreactor #1)                      | <i>A. terreus</i> preculture (300 ml)                                                  | 0 h                                                       |
|                               | <i>S. rimosus</i><br>monoculture<br>(bioreactor #2)                   | <i>S. rimosus</i> preculture (300 ml)                                                  | 24 h                                                      |
|                               | <i>A. terreus</i> + <i>S. rimosus</i><br>coculture<br>(bioreactor #3) | <i>S. rimosus</i> preculture (300 ml) + <i>A. terreus</i><br>preculture (300 ml)       | 0 h <i>A. terreus</i><br>24 h <i>S.</i><br><i>rimosus</i> |
| ATSR8                         | <i>A. terreus</i> monoculture<br>(bioreactor #1)                      | <i>A. terreus</i> preculture (300 ml)                                                  | 0 h                                                       |
|                               | <i>S. rimosus</i><br>monoculture<br>(bioreactor #2)                   | <i>S. rimosus</i> preculture (300 ml)                                                  | 24 h                                                      |
|                               | <i>A. terreus</i> + <i>S. rimosus</i><br>coculture<br>(bioreactor #3) | <i>S. rimosus</i> preculture (300 ml) + <i>A. terreus</i><br>preculture (300 ml)       | 0 h <i>A. terreus</i><br>24 h <i>S.</i><br><i>rimosus</i> |
| ATSR9                         | <i>A. terreus</i> monoculture<br>(bioreactor #1)                      | <i>A. terreus</i> spore suspension (300 ml)                                            | 0 h                                                       |
|                               | <i>S. rimosus</i><br>monoculture<br>(bioreactor #2)                   | <i>S. rimosus</i> spore suspension (300 ml)                                            | 0 h                                                       |

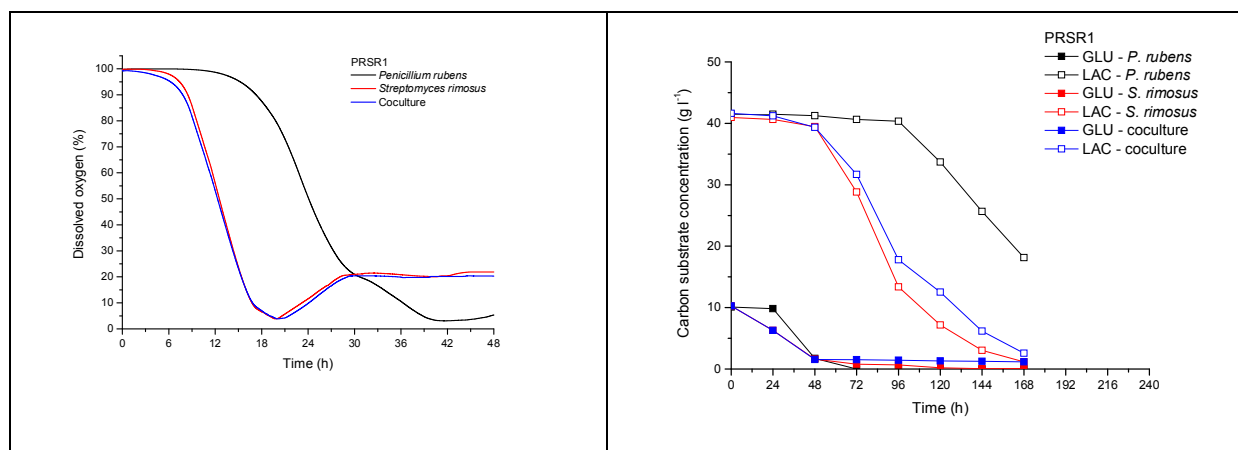
|       |                                                                       |                                                                                              |                                                 |
|-------|-----------------------------------------------------------------------|----------------------------------------------------------------------------------------------|-------------------------------------------------|
|       | <i>A. terreus</i> + <i>S. rimosus</i><br>coculture<br>(bioreactor #3) | <i>S. rimosus</i> spore suspension (300 ml) + <i>A. terreus</i><br>spore suspension (300 ml) | 0 h                                             |
| ATSN3 | <i>A. terreus</i> monoculture<br>(bioreactor #1)                      | <i>A. terreus</i> spore suspension (300 ml)                                                  | 0 h                                             |
|       | <i>S. noursei</i> monoculture<br>(bioreactor #2)                      | <i>S. noursei</i> spore suspension (300 ml)                                                  | 0 h                                             |
|       | <i>A. terreus</i> + <i>S. noursei</i><br>coculture<br>(bioreactor #3) | <i>S. noursei</i> spore suspension (300 ml) + <i>A. terreus</i><br>spore suspension (300 ml) | 0 h                                             |
| ATSN4 | <i>A. terreus</i> monoculture<br>(bioreactor #1)                      | <i>A. terreus</i> spore suspension (300 ml)                                                  | 0 h                                             |
|       | <i>S. noursei</i> monoculture<br>(bioreactor #2)                      | <i>S. noursei</i> spore suspension (300 ml)                                                  | 0 h                                             |
|       | <i>A. terreus</i> + <i>S. noursei</i><br>coculture<br>(bioreactor #3) | <i>S. noursei</i> spore suspension (300 ml) + <i>A. terreus</i><br>spore suspension (300 ml) | 0 h                                             |
| ATSN5 | <i>A. terreus</i> monoculture<br>(bioreactor #1)                      | <i>A. terreus</i> preculture (300 ml)                                                        | 0 h                                             |
|       | <i>S. noursei</i> monoculture<br>(bioreactor #2)                      | <i>S. noursei</i> preculture (300 ml)                                                        | 0 h                                             |
|       | <i>A. terreus</i> + <i>S. noursei</i><br>coculture<br>(bioreactor #3) | <i>S. noursei</i> preculture (300 ml) + <i>A. terreus</i><br>preculture (300 ml)             | 0 h                                             |
| ATSN6 | <i>A. terreus</i> monoculture<br>(bioreactor #1)                      | <i>A. terreus</i> preculture (300 ml)                                                        | 0 h                                             |
|       | <i>S. noursei</i> monoculture<br>(bioreactor #2)                      | <i>S. noursei</i> preculture (300 ml)                                                        | 0 h                                             |
|       | <i>A. terreus</i> + <i>S. noursei</i><br>coculture<br>(bioreactor #3) | <i>S. noursei</i> preculture (300 ml) + <i>A. terreus</i> preculture<br>(300 ml)             | 0 h                                             |
| ATSN7 | <i>A. terreus</i> monoculture<br>(bioreactor #1)                      | <i>A. terreus</i> preculture (300 ml)                                                        | 24 h                                            |
|       | <i>S. noursei</i> monoculture<br>(bioreactor #2)                      | <i>S. noursei</i> preculture (300 ml)                                                        | 0 h                                             |
|       | <i>A. terreus</i> + <i>S. noursei</i><br>coculture<br>(bioreactor #3) | <i>S. noursei</i> preculture (300 ml) + <i>A. terreus</i><br>preculture (300 ml)             | 24 h <i>A. terreus</i><br>0 h <i>S. noursei</i> |
| ATSN8 | <i>A. terreus</i> monoculture<br>(bioreactor #1)                      | <i>A. terreus</i> preculture (300 ml)                                                        | 0 h                                             |

|       |                                                                       |                                                                                             |                                                 |
|-------|-----------------------------------------------------------------------|---------------------------------------------------------------------------------------------|-------------------------------------------------|
|       | <i>S. noursei</i> monoculture<br>(bioreactor #2)                      | <i>S. noursei</i> preculture (300 ml)                                                       | 24 h                                            |
|       | <i>A. terreus</i> + <i>S. noursei</i><br>coculture<br>(bioreactor #3) | <i>S. noursei</i> preculture (300 ml) + <i>A. terreus</i><br>preculture (300 ml)            | 0 h <i>A. terreus</i><br>24 h <i>S. noursei</i> |
| PRSR1 | <i>P. rubens</i> monoculture<br>(bioreactor #1)                       | <i>P. rubens</i> spore suspension (300 ml)                                                  | 0 h                                             |
|       | <i>S. rimosus</i><br>monoculture<br>(bioreactor #2)                   | <i>S. rimosus</i> spore suspension (300 ml)                                                 | 0 h                                             |
|       | <i>P. rubens</i> + <i>S. rimosus</i><br>coculture<br>(bioreactor #3)  | <i>S. rimosus</i> spore suspension (300 ml) + <i>P. rubens</i><br>spore suspension (300 ml) | 0 h                                             |
| PRSR2 | <i>P. rubens</i> monoculture<br>(bioreactor #1)                       | <i>P. rubens</i> spore suspension (300 ml)                                                  | 0 h                                             |
|       | <i>S. rimosus</i><br>monoculture<br>(bioreactor #2)                   | <i>S. rimosus</i> spore suspension (300 ml)                                                 | 24 h                                            |
|       | <i>P. rubens</i> + <i>S. rimosus</i><br>co-culture<br>(bioreactor #3) | <i>S. rimosus</i> spore suspension (300 ml) + <i>P. rubens</i><br>spore suspension (300 ml) | 0 h <i>P. rubens</i><br>24 h <i>S. rimosus</i>  |
| PRSR3 | <i>P. rubens</i> monoculture<br>(bioreactor #1)                       | <i>P. rubens</i> spore suspension (300 ml)                                                  | 0 h                                             |
|       | <i>S. rimosus</i><br>monoculture<br>(bioreactor #2)                   | <i>S. rimosus</i> spore suspension (300 ml)                                                 | 48 h                                            |
|       | <i>P. rubens</i> + <i>S. rimosus</i><br>coculture<br>(bioreactor #3)  | <i>S. rimosus</i> spore suspension (300 ml) + <i>P. rubens</i><br>spore suspension (300 ml) | 0 h <i>P. rubens</i><br>48 h <i>S. rimosus</i>  |
| PRSN1 | <i>P. rubens</i> monoculture<br>(bioreactor #1)                       | <i>P. rubens</i> spore suspension (300 ml)                                                  | 0 h                                             |
|       | <i>S. noursei</i> monoculture<br>(bioreactor #2)                      | <i>S. noursei</i> spore suspension (300 ml)                                                 | 0 h                                             |
|       | <i>P. rubens</i> + <i>S. noursei</i><br>coculture<br>(bioreactor #3)  | <i>S. noursei</i> spore suspension (300 ml) + <i>P. rubens</i><br>spore suspension (300 ml) | 0 h                                             |
| PRSN2 | <i>P. rubens</i> monoculture<br>(bioreactor #1)                       | <i>P. rubens</i> spore suspension (300 ml)                                                  | 0 h                                             |
|       | <i>S. noursei</i> monoculture<br>(bioreactor #2)                      | <i>S. noursei</i> spore suspension (300 ml)                                                 | 24 h                                            |

|       |                                                                      |                                                                                             |                                                |
|-------|----------------------------------------------------------------------|---------------------------------------------------------------------------------------------|------------------------------------------------|
|       | <i>P. rubens</i> + <i>S. noursei</i><br>coculture<br>(bioreactor #3) | <i>S. noursei</i> spore suspension (300 ml) + <i>P. rubens</i><br>spore suspension (300 ml) | 0 h <i>P. rubens</i><br>24 h <i>S. noursei</i> |
| PRSN3 | <i>P. rubens</i> monoculture<br>(bioreactor #1)                      | <i>P. rubens</i> spore suspension (300 ml)                                                  | 0 h                                            |
|       | <i>S. noursei</i> monoculture<br>(bioreactor #2)                     | <i>S. noursei</i> spore suspension (300 ml)                                                 | 48 h                                           |
|       | <i>P. rubens</i> + <i>S. noursei</i><br>coculture<br>(bioreactor #3) | <i>S. noursei</i> spore suspension (300 ml) + <i>P. rubens</i><br>spore suspension (300 ml) | 0 h <i>P. rubens</i><br>48 h <i>S. noursei</i> |



**Suppl. Fig. S1** Metabolic dominance of *S. rimosus* shown on the example of nine secondary metabolites despite lack of its kinetic dominance in ATSR3; upon selected data from Boruta et al. (2021)



**Suppl. Fig. S2** Kinetic dominance of *S. rimosus* over *P. rubens* in PRSR1; upon selected data from Boruta et al. (2023a)