

Article

Effects of the Coculture Initiation Method on the Production of Secondary Metabolites in Bioreactor Cocultures of *Penicillium rubens* and *Streptomyces rimosus*

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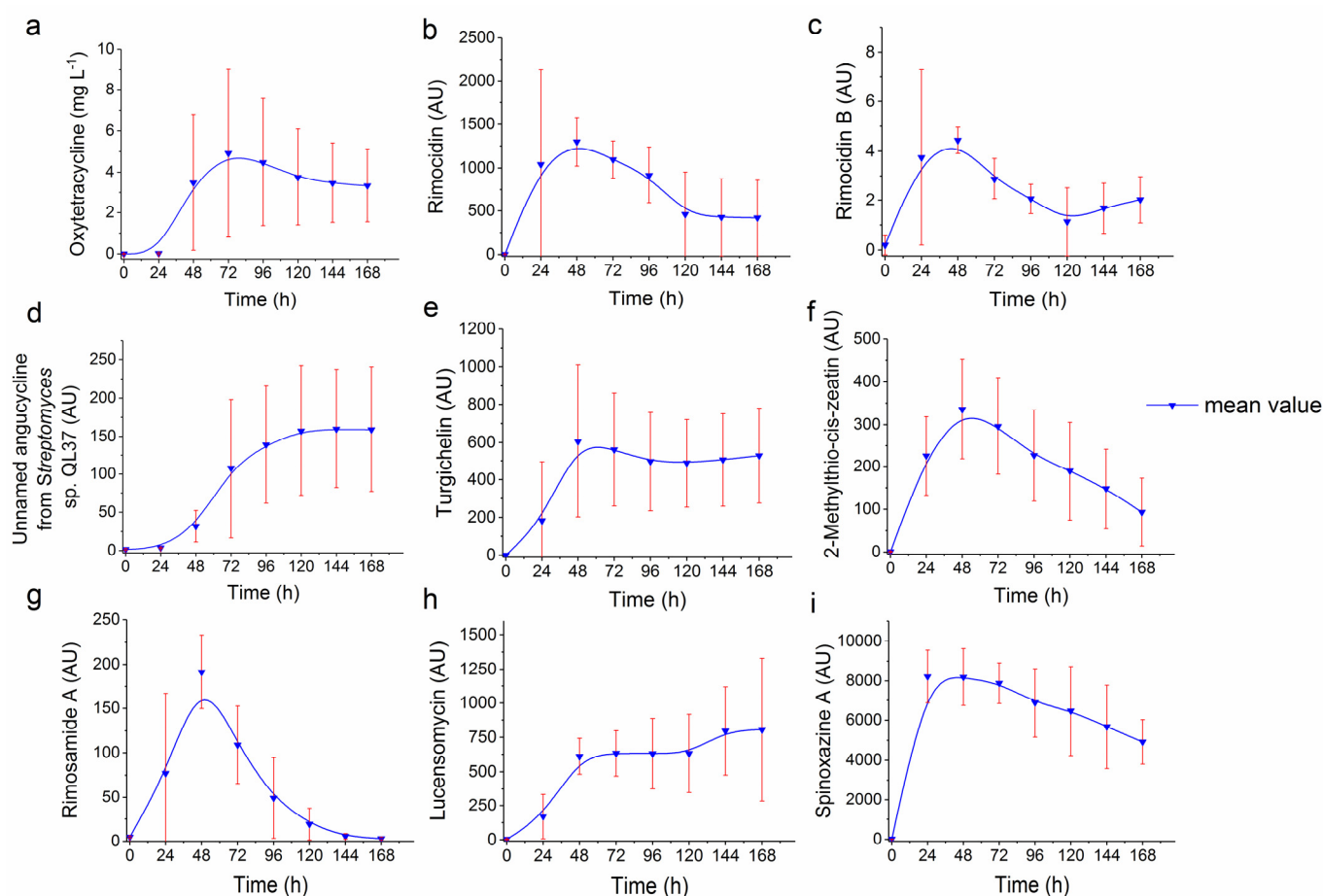


Figure S1. Replicability analysis of the levels of secondary metabolites produced by *S. rimosus*, namely, oxytetracycline (a) and the metabolites tentatively identified as rimocidin (b), rimocidin B (c), unnamed angucycline from *Streptomyces* sp. QL37 (d), turgichelin (e), 2-methylthio-cis-zeatin (f), rimosamide A (g), lu-

censomycin (h), and spinoxazine A (i). The confidence intervals for the mean values are depicted. A significance level of $\alpha=0.05$ was used for the calculations. The replicability analysis was based on the data collected during the PR®R1, PR®R2 and PR®R3 monocultures of *S. rimosus*. AU-auxiliary units.

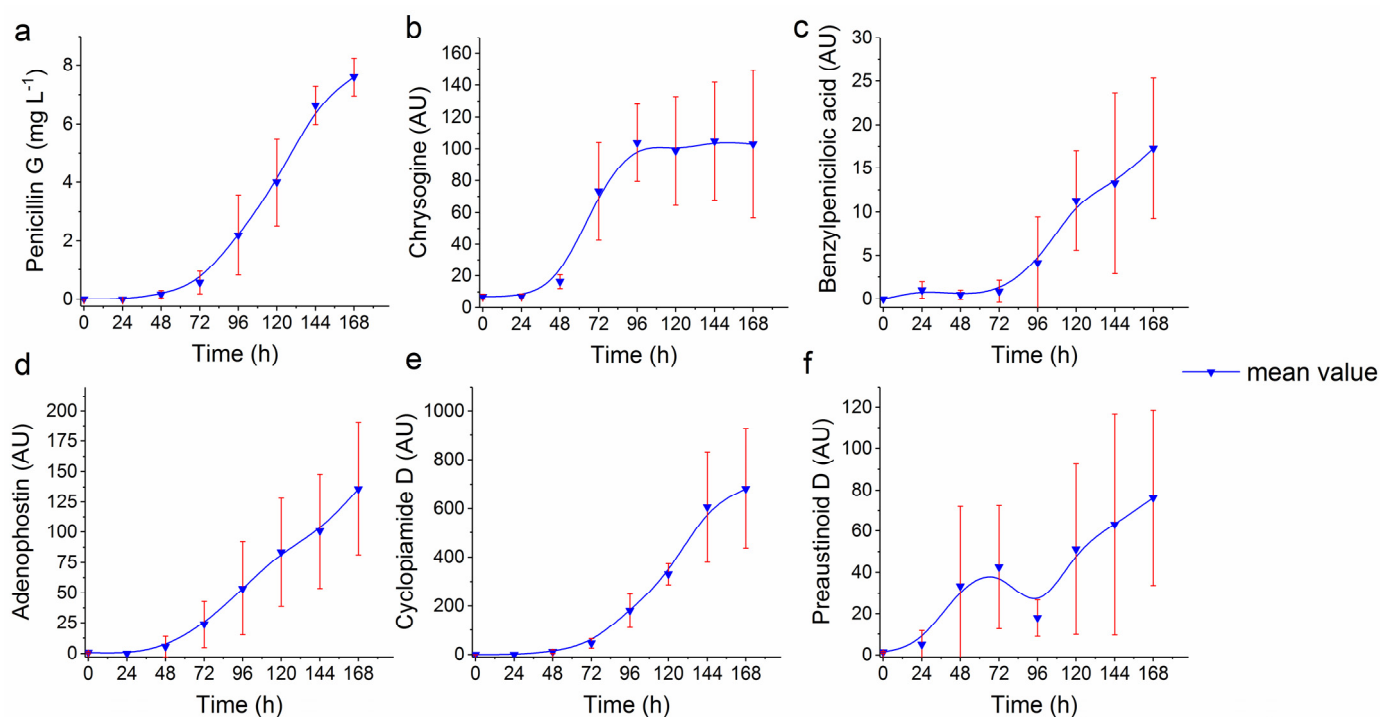


Figure S2. Replicability analysis of the levels of secondary metabolites produced by *P. rubens*, namely, penicillin G (a) and the metabolites tentatively identified as chrysogine (b), benzylpenicilloic acid (c), adenophostin B (d), cyclopiamide D (e), and preaustinoid D (f). The confidence intervals for the mean values are depicted. A significance level of $\alpha=0.05$ was used for the calculations. The replicability analysis was based on the data collected during the PR®R1, PR®R2 and PR®R3 monocultures of *P. rubens*. AU-auxiliary units.

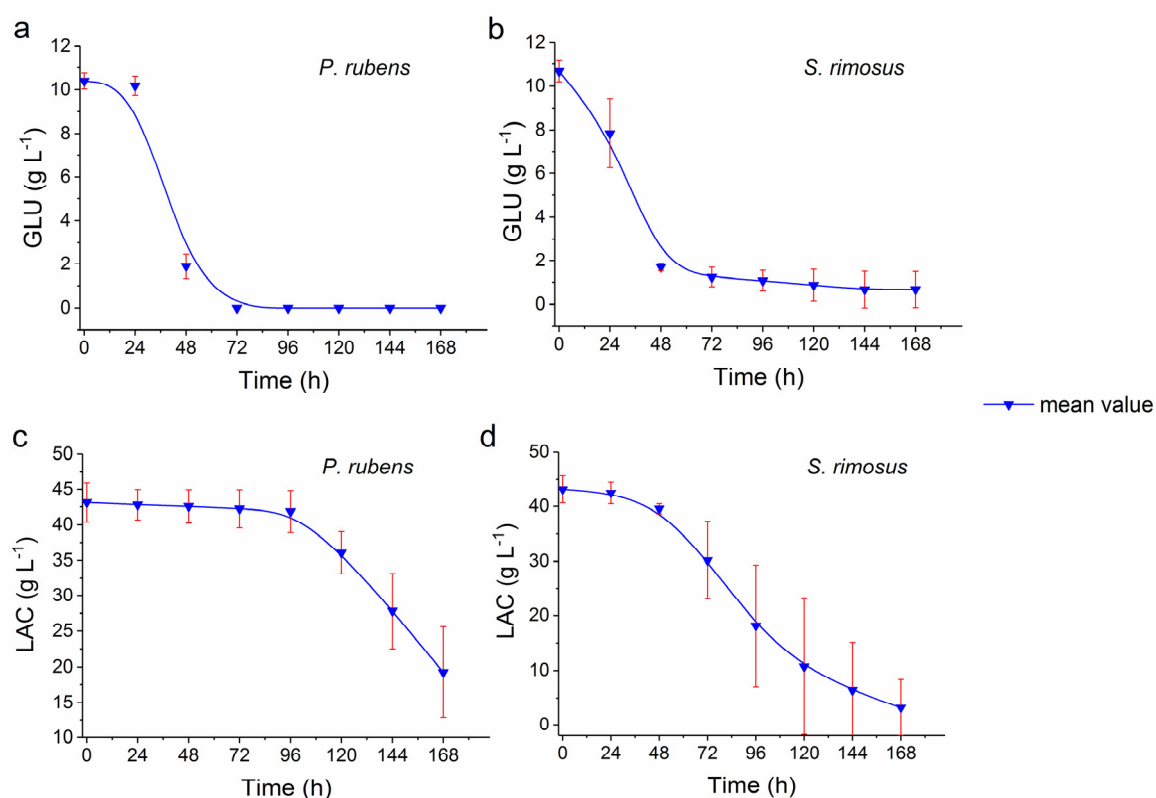


Figure S3. Replicability analysis of the levels of glucose (a, b) and lactose (c, d) in the monocultures of *P. rubens* (a, c) and *S. rimosus* (b, d). The confidence intervals for the mean values are depicted. A significance level of $\alpha=0.05$ was used for the calculations. The replicability analysis was based on the data collected during the PR®R1, PR®R2 and PR®R3 monocultures of *P. rubens* and *S. rimosus*. AU-auxiliary units.

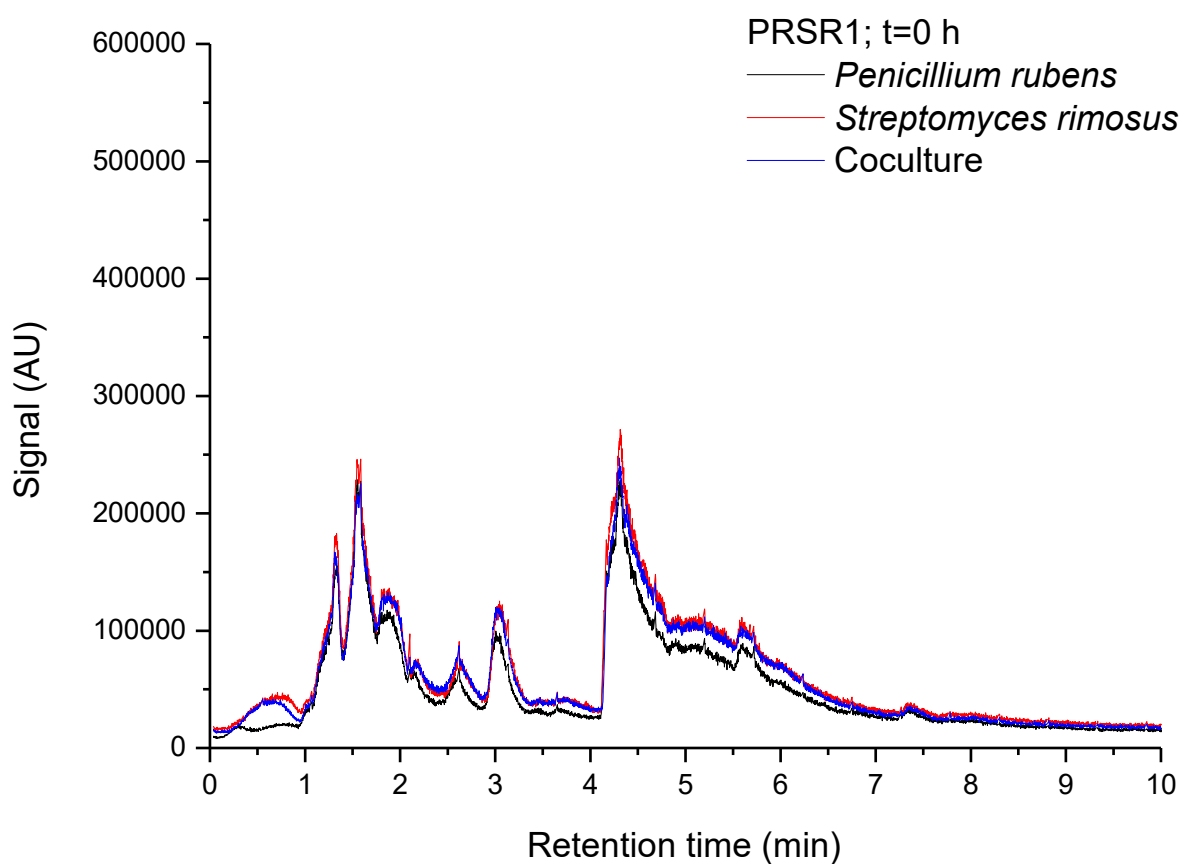


Figure S4 Alignments of total ion chromatograms (TICs) recorded in the PRSR1 experiment at t=0 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units.

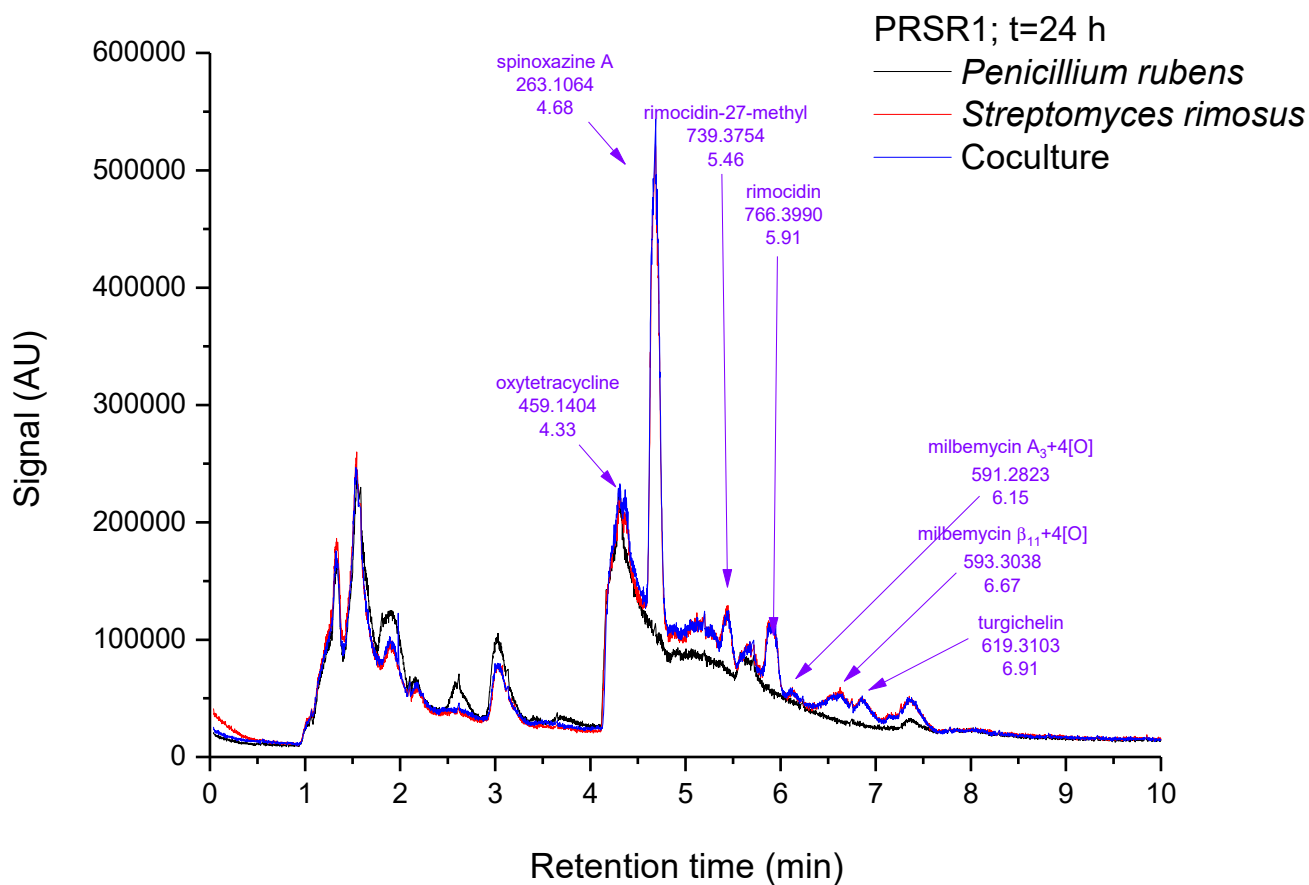


Figure S5 Alignments of total ion chromatograms (TICs) recorded in the PR[®]R1 experiment at t=24 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units. The names of metabolites that were found both in the monoculture of *S. rimosus* and the coculture are shown in violet. The metabolites were tentatively identified on the basis of M⁺ data, however, their identities were not confirmed due to a lack of analytical standards. The exception was the confirmed identity of oxytetracycline.

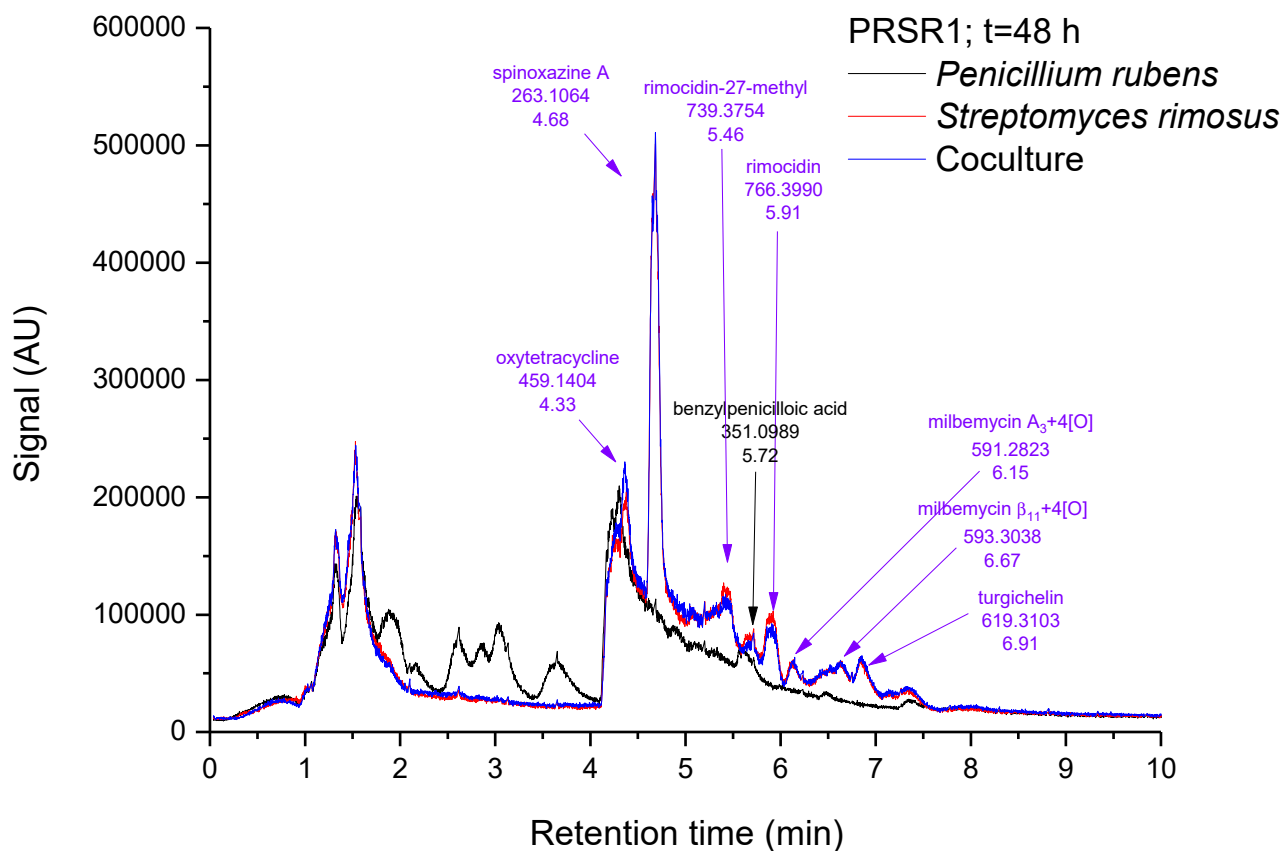


Figure S6 Alignments of total ion chromatograms (TICs) recorded in the PR[®]R1 experiment at t=48 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units. The names of metabolites that were found both in the monoculture of *S. rimosus* and the coculture are shown in violet. The name of the metabolite that was found solely in the monoculture of *P. rubens* is shown in black. The metabolites were tentatively identified on the basis of M[®] data, however, their identities were not confirmed due to a lack of analytical standards. The exception was the confirmed identity of oxytetracycline.

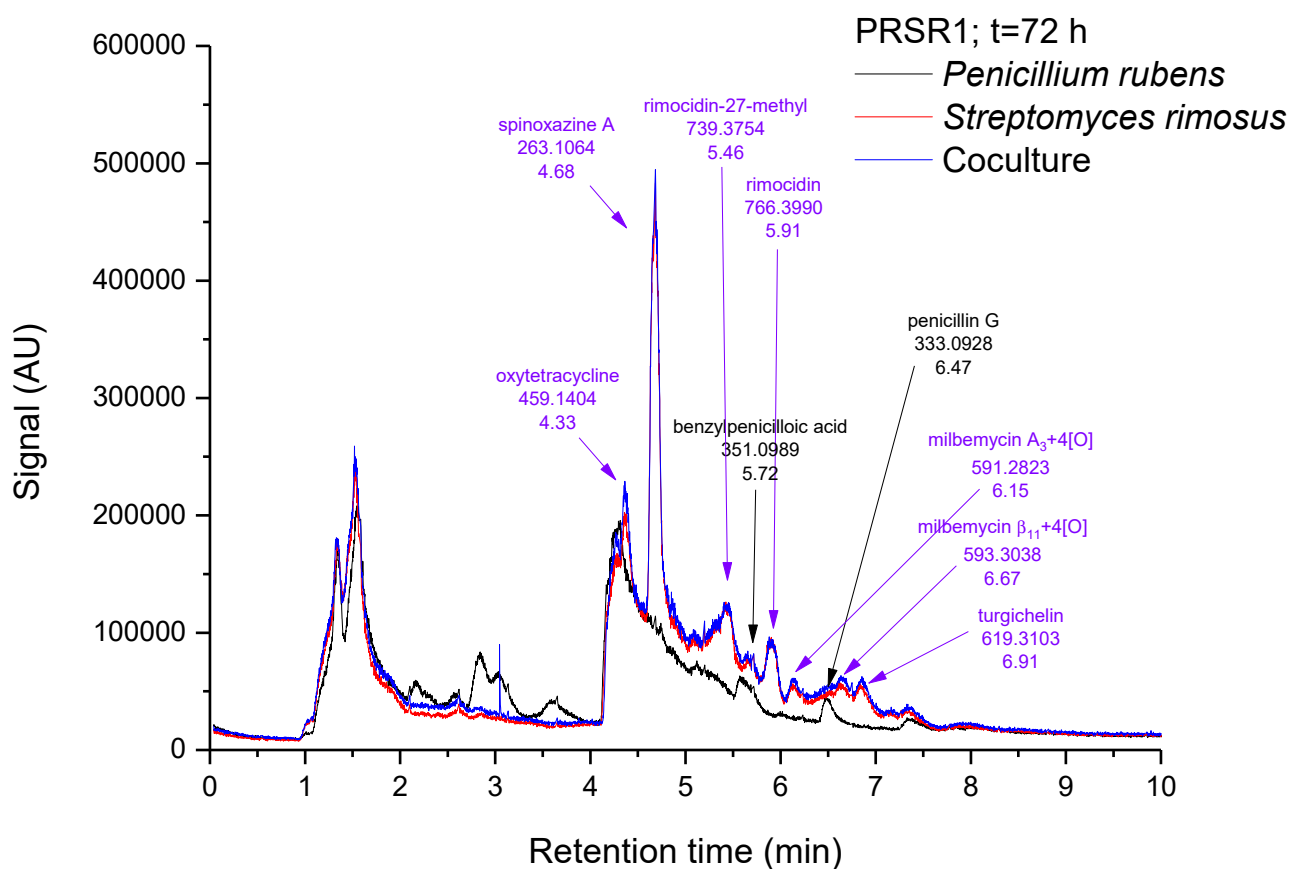


Figure S7 Alignments of total ion chromatograms (TICs) recorded in the PR[®]R1 experiment at t=72 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units. The names of metabolites that were found both in the monoculture of *S. rimosus* and the coculture are shown in violet. The names of the metabolites that were found solely in the monoculture of *P. rubens* are shown in black. The metabolites were tentatively identified on the basis of M⁺ data, however, their identities were not confirmed due to a lack of analytical standards. The exceptions were the confirmed identities of oxytetracycline and penicillin G.

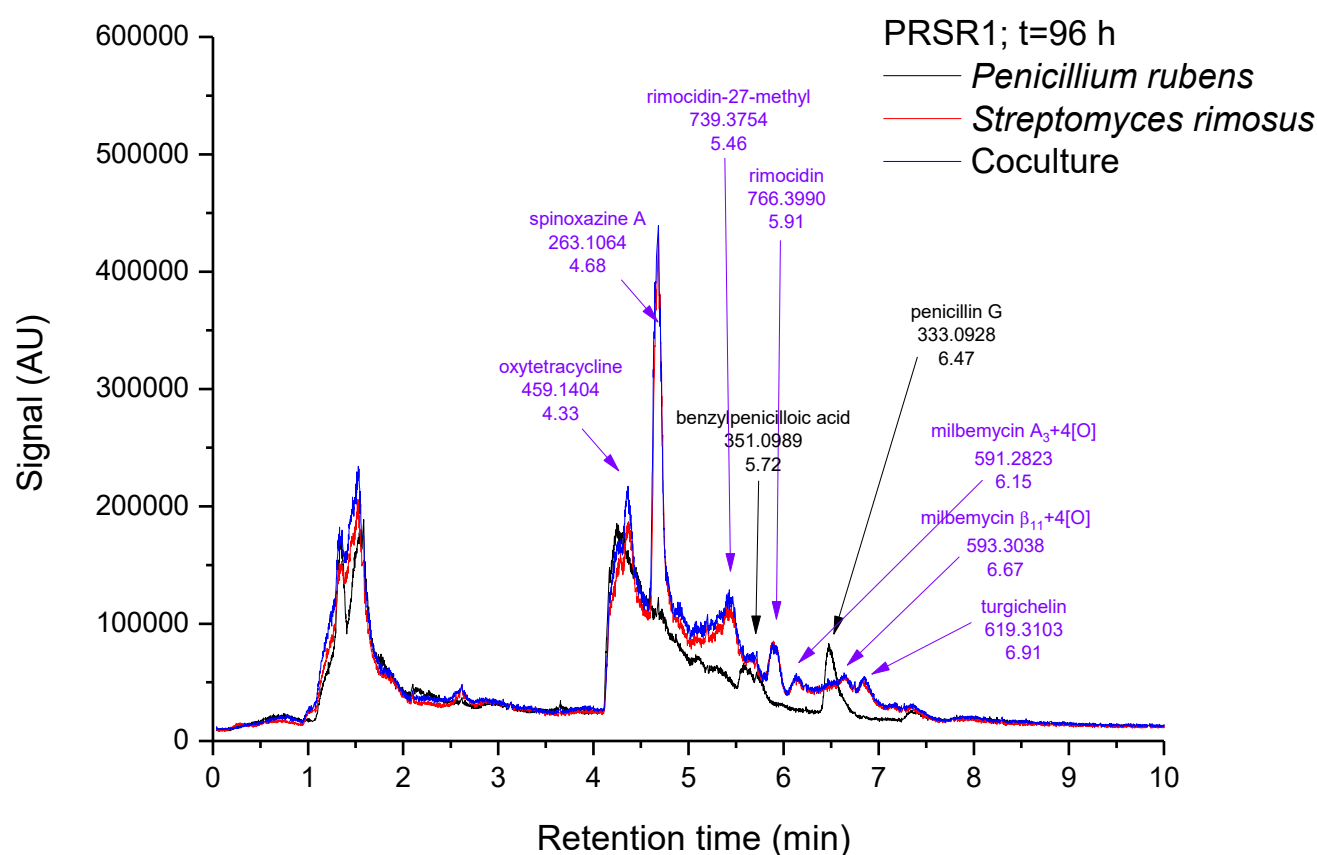


Figure S8 Alignments of total ion chromatograms (TICs) recorded in the PR®R1 experiment at t=96 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units. The names of metabolites that were found both in the monoculture of *S. rimosus* and the coculture are shown in violet. The names of the metabolites that were found solely in the monoculture of *P. rubens* are shown in black. The metabolites were tentatively identified on the basis of M⁺ data, however, their identities were not confirmed due to a lack of analytical standards. The exceptions were the confirmed identities of oxytetracycline and penicillin G.

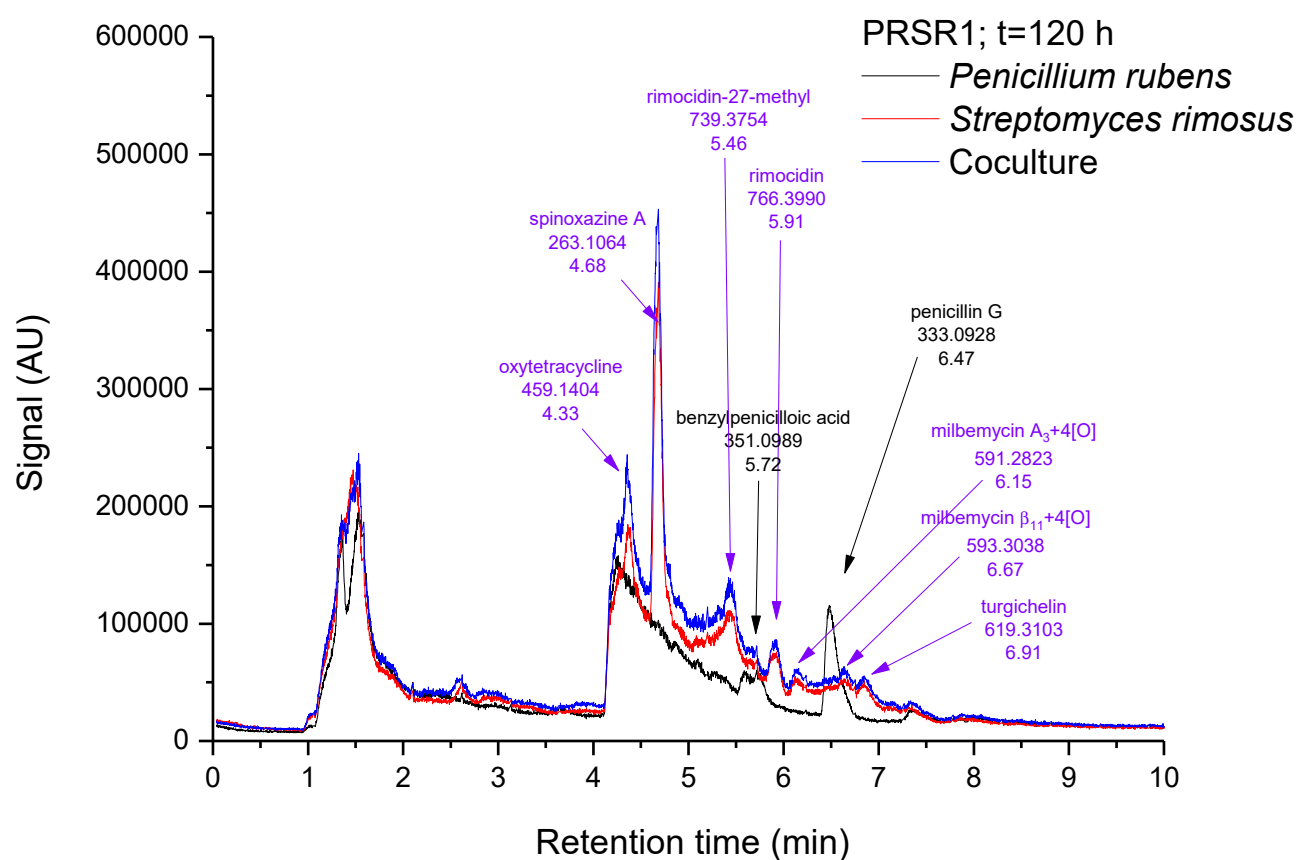


Figure S9 Alignments of total ion chromatograms (TICs) recorded in the PRSR1 experiment at t=120 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units. The names of metabolites that were found both in the monoculture of *S. rimosus* and the coculture are shown in violet. The names of the metabolites that were found solely in the monoculture of *P. rubens* are shown in black. The metabolites were tentatively identified on the basis of M^+ data, however, their identities were not confirmed due to a lack of analytical standards. The exceptions were the confirmed identities of oxytetracycline and penicillin G.

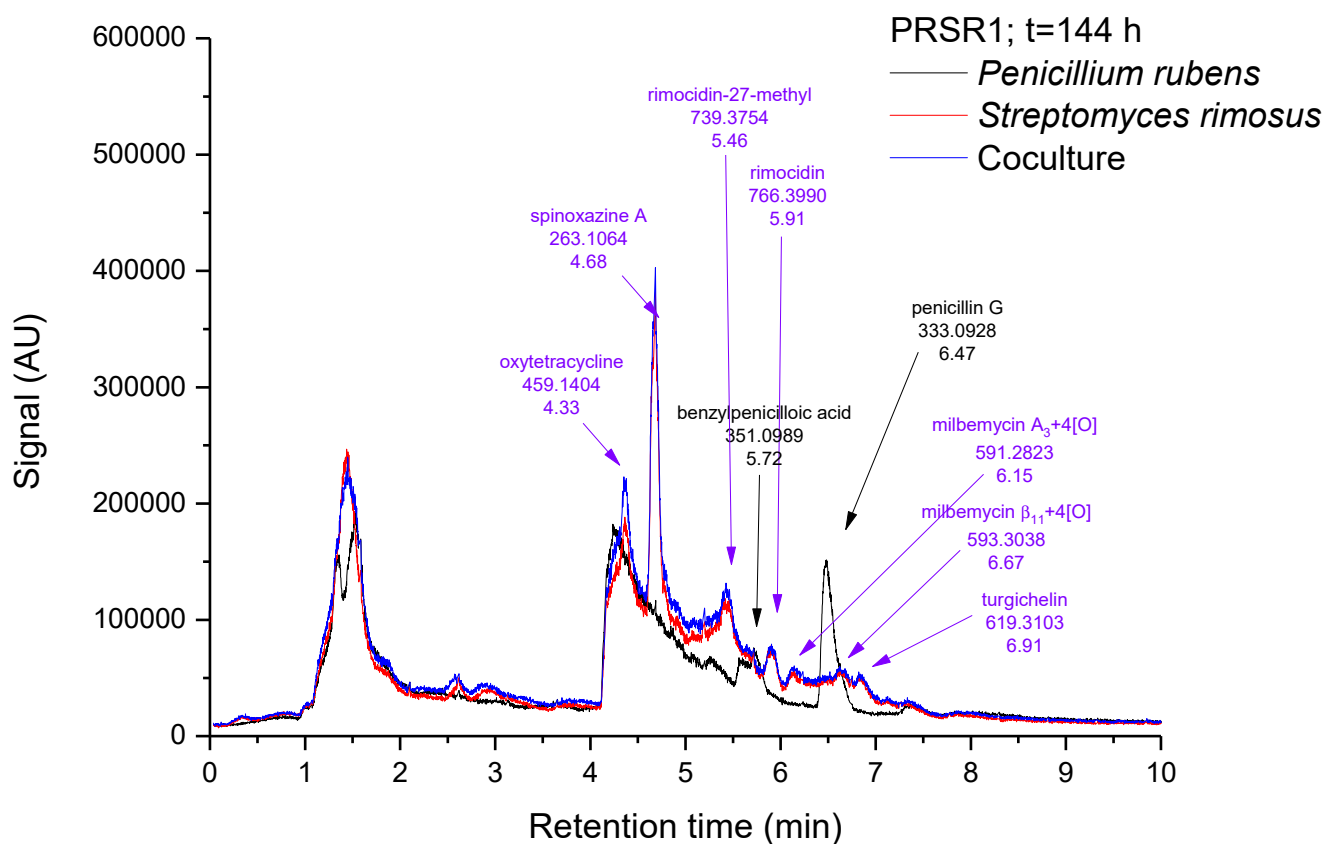


Figure S10 Alignments of total ion chromatograms (TICs) recorded in the PR[®]R1 experiment at t=144 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units. The names of metabolites that were found both in the monoculture of *S. rimosus* and the coculture are shown in violet. The names of the metabolites that were found solely in the monoculture of *P. rubens* are shown in black. The metabolites were tentatively identified on the basis of M⁺ data, however, their identities were not confirmed due to a lack of analytical standards. The exceptions were the confirmed identities of oxytetracycline and penicillin G.

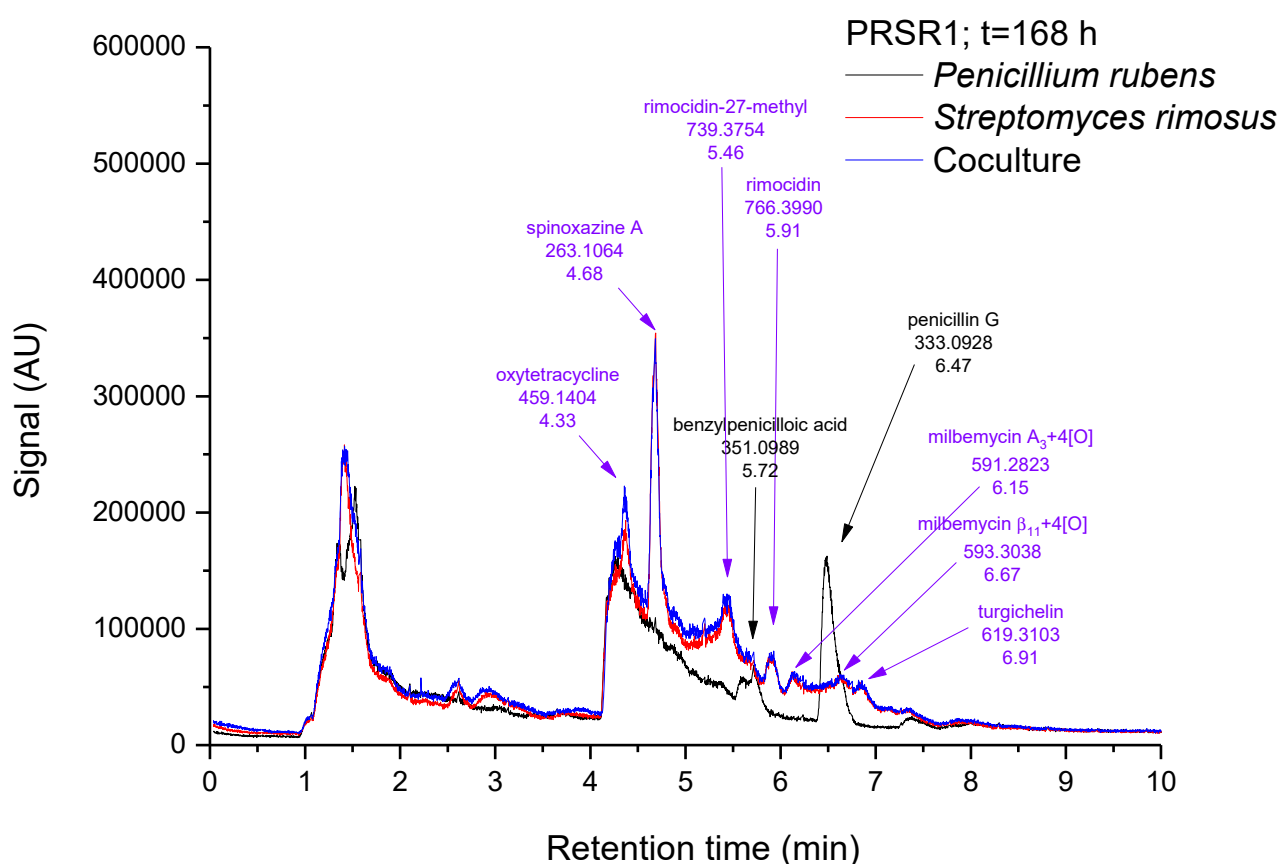


Figure S11 Alignments of total ion chromatograms (TICs) recorded in the PRSR1 experiment at t=168 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units. The names of metabolites that were found both in the monoculture of *S. rimosus* and the coculture are shown in violet. The names of the metabolites that were found solely in the monoculture of *P. rubens* are shown in black. The metabolites were tentatively identified on the basis of M^+ data, however, their identities were not confirmed due to a lack of analytical standards. The exceptions were the confirmed identities of oxytetracycline and penicillin G.

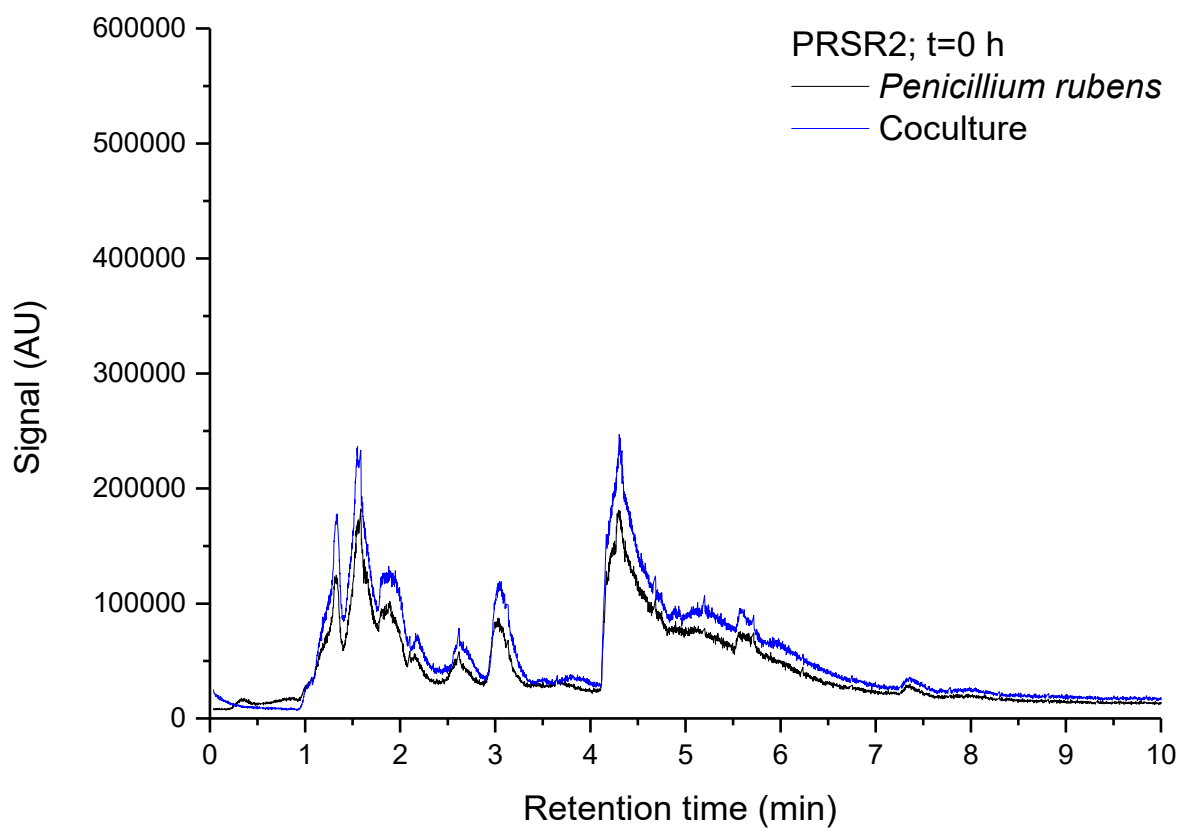


Figure S12 Alignments of total ion chromatograms (TICs) recorded in the PR®R2 experiment at $t=0$ h for *P. rubens* monoculture (black line) and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU-auxiliary units.

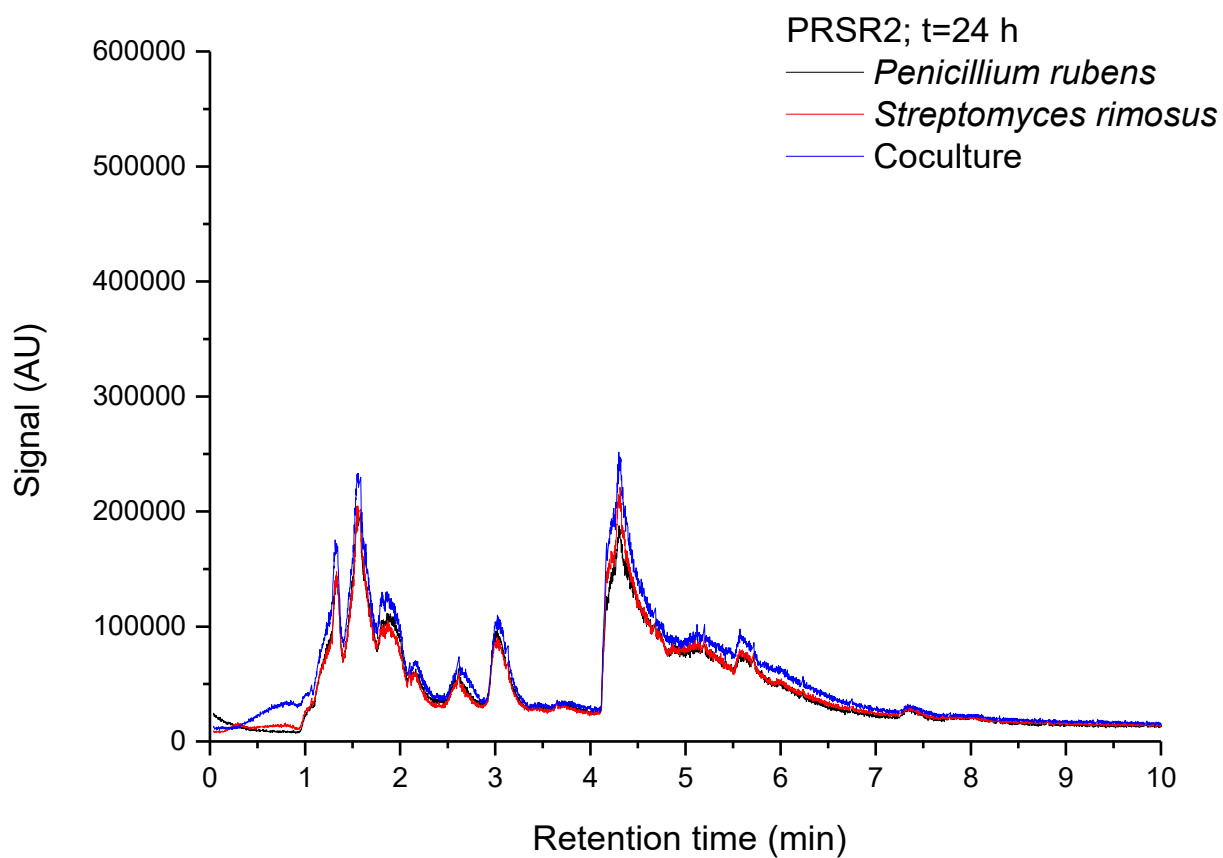


Figure S13 Alignments of total ion chromatograms (TICs) recorded in the PRSR2 experiment at t=24 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units.

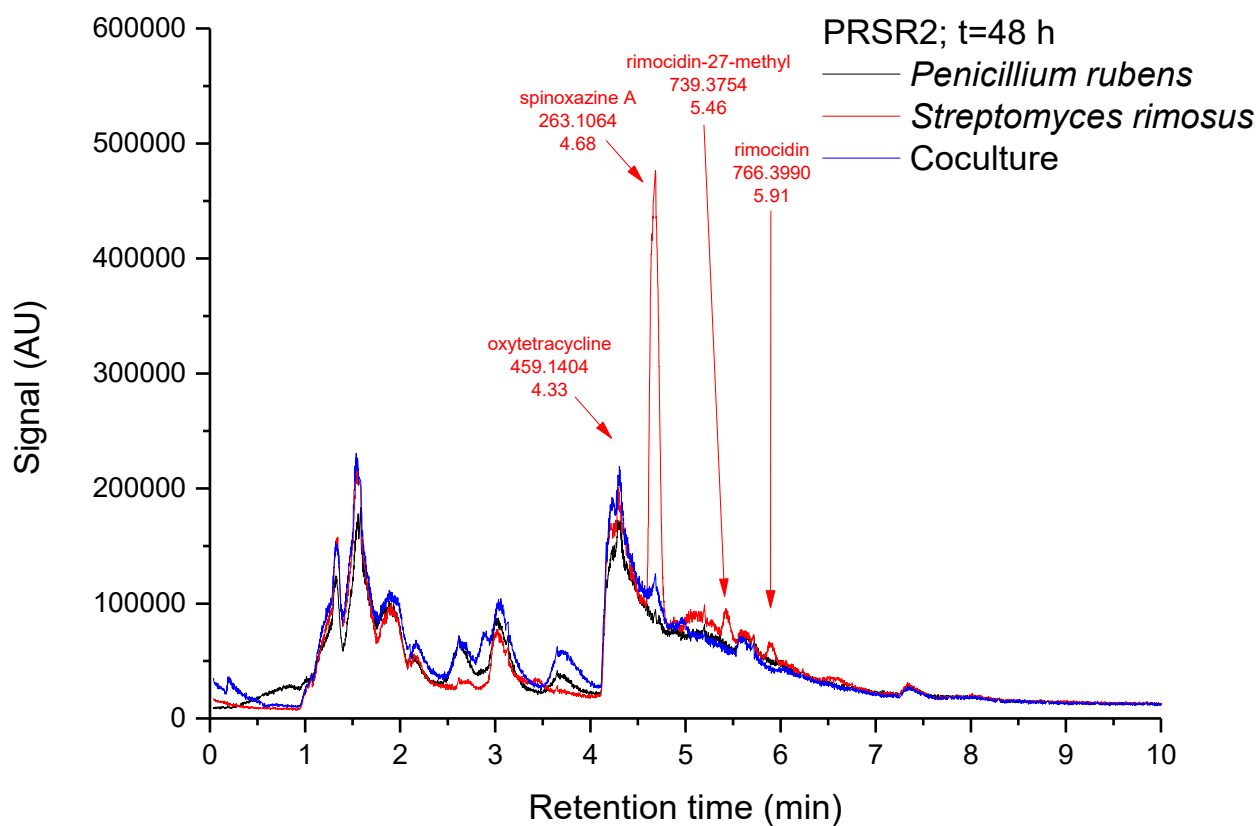


Figure S14 Alignments of total ion chromatograms (TICs) recorded in the PRSR2 experiment at t=48 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units. The names of the metabolites that were found solely in the monoculture of *S. rimosus* are shown in red. The metabolites were tentatively identified on the basis of m/z data, however, their identities were not confirmed due to a lack of analytical standards. The exception was the confirmed identity of oxytetracycline.

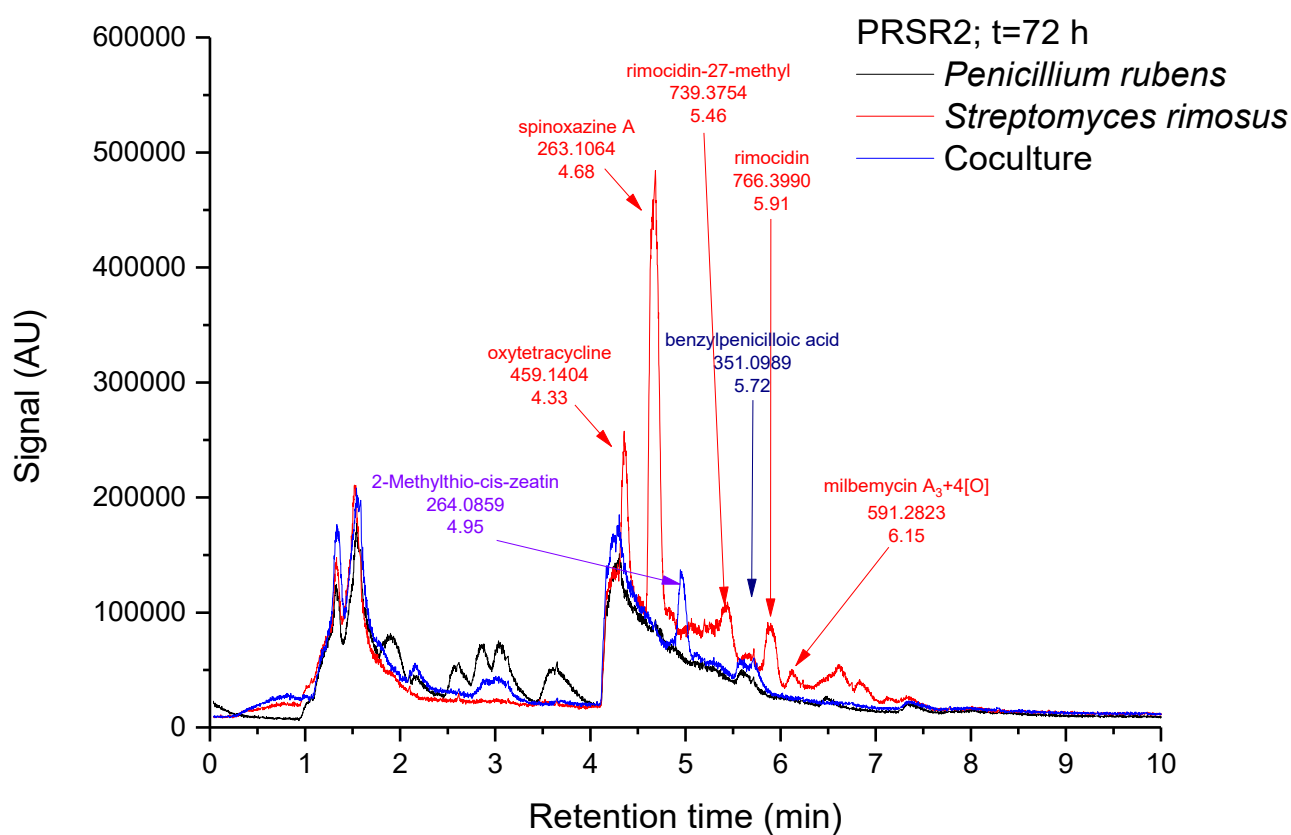


Figure S15 Alignments of total ion chromatograms (TICs) recorded in the PRSR2 experiment at t=72 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units. The names of the metabolites that were found solely in the monoculture of *S. rimosus* are shown in red. The name of the metabolite that was found both in the monoculture of *S. rimosus* and the coculture is shown in violet. The name of the metabolite that was found both in the monoculture of *P. rubens* and the coculture is shown in navy blue. The metabolites were tentatively identified on the basis of MS data, however, their identities were not confirmed due to a lack of analytical standards. The exception was the confirmed identity of oxytetracycline.

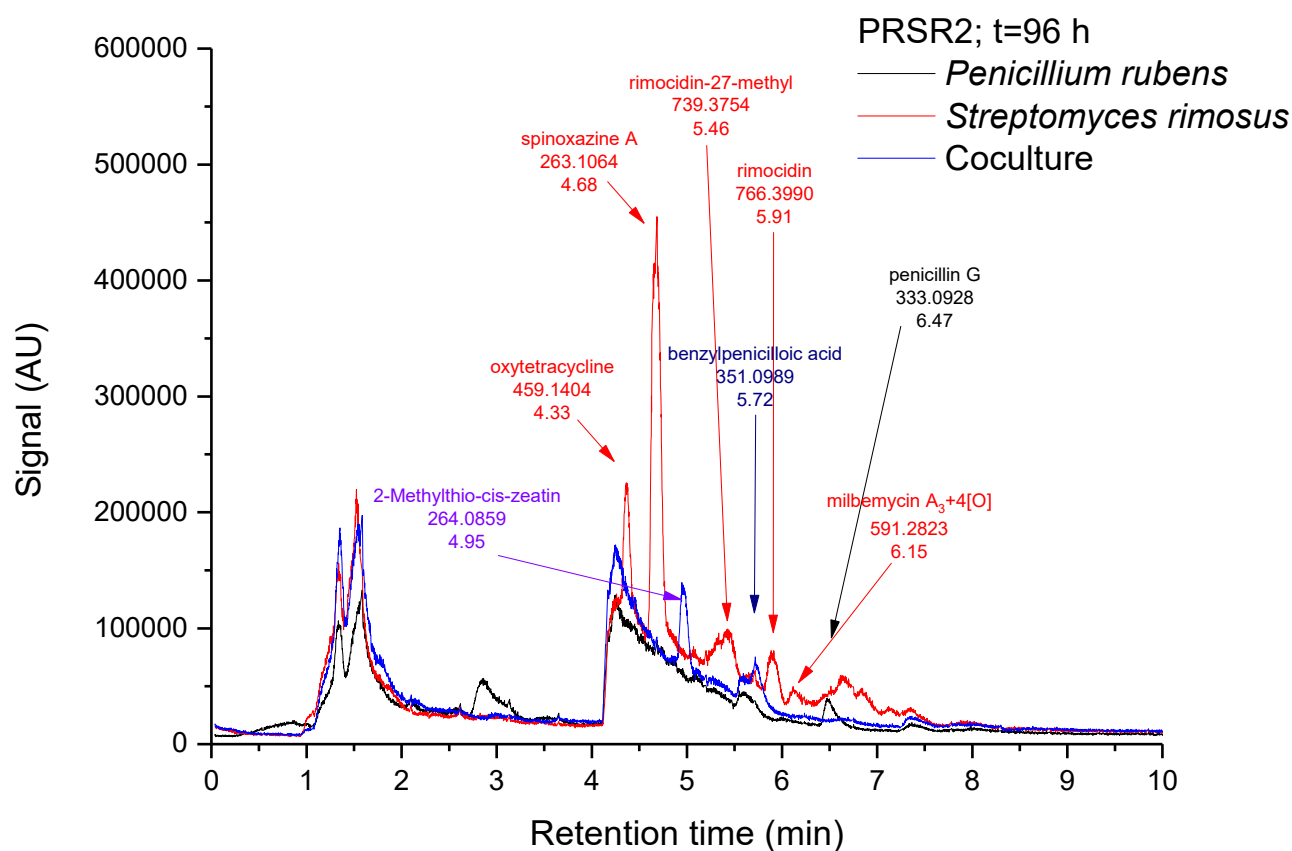


Figure S16 Alignments of total ion chromatograms (TICs) recorded in the PRSR2 experiment at t=96 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units. The names of the metabolites that were found solely in the monoculture of *S. rimosus* are shown in red. The name of the metabolite that was found both in the monoculture of *S. rimosus* and the coculture is shown in violet. The name of the metabolite that was found both in the monoculture of *P. rubens* and the coculture is shown in navy blue. The name of the metabolite that was found solely in the monoculture of *P. rubens* is shown in black. The metabolites were tentatively identified on the basis of MS data, however, their identities were not confirmed due to a lack of analytical standards. The exceptions were the confirmed identities of oxytetracycline and penicillin G.

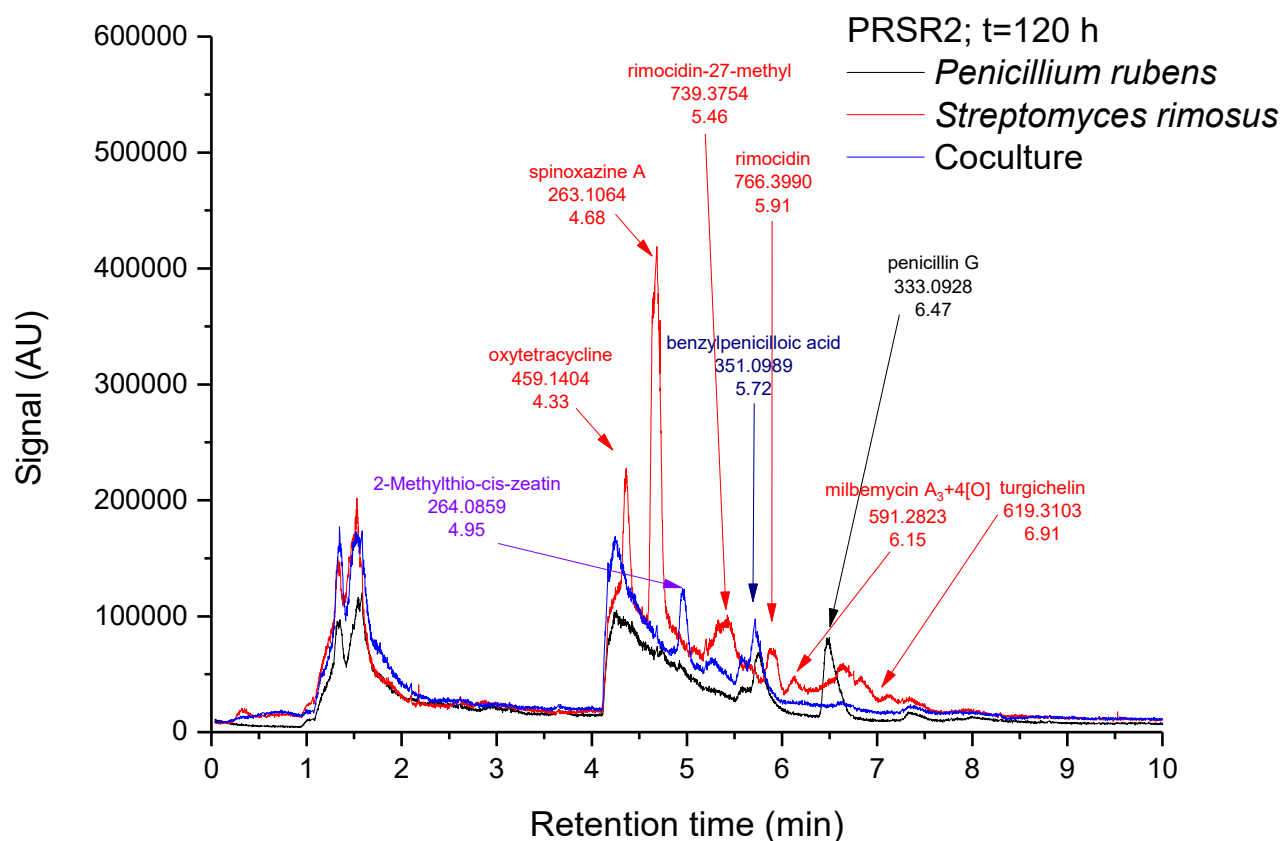


Figure S17 Alignments of total ion chromatograms (TICs) recorded in the PR[®]R2 experiment at t=120 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units. The names of the metabolites that were found solely in the monoculture of *S. rimosus* are shown in red. The name of the metabolite that was found both in the monoculture of *S. rimosus* and the coculture is shown in violet. The name of the metabolite that was found both in the monoculture of *P. rubens* and the coculture is shown in navy blue. The name of the metabolite that was found solely in the monoculture of *P. rubens* is shown in black. The metabolites were tentatively identified on the basis of M[®] data, however, their identities were not confirmed due to a lack of analytical standards. The exceptions were the confirmed identities of oxytetracycline and penicillin G.

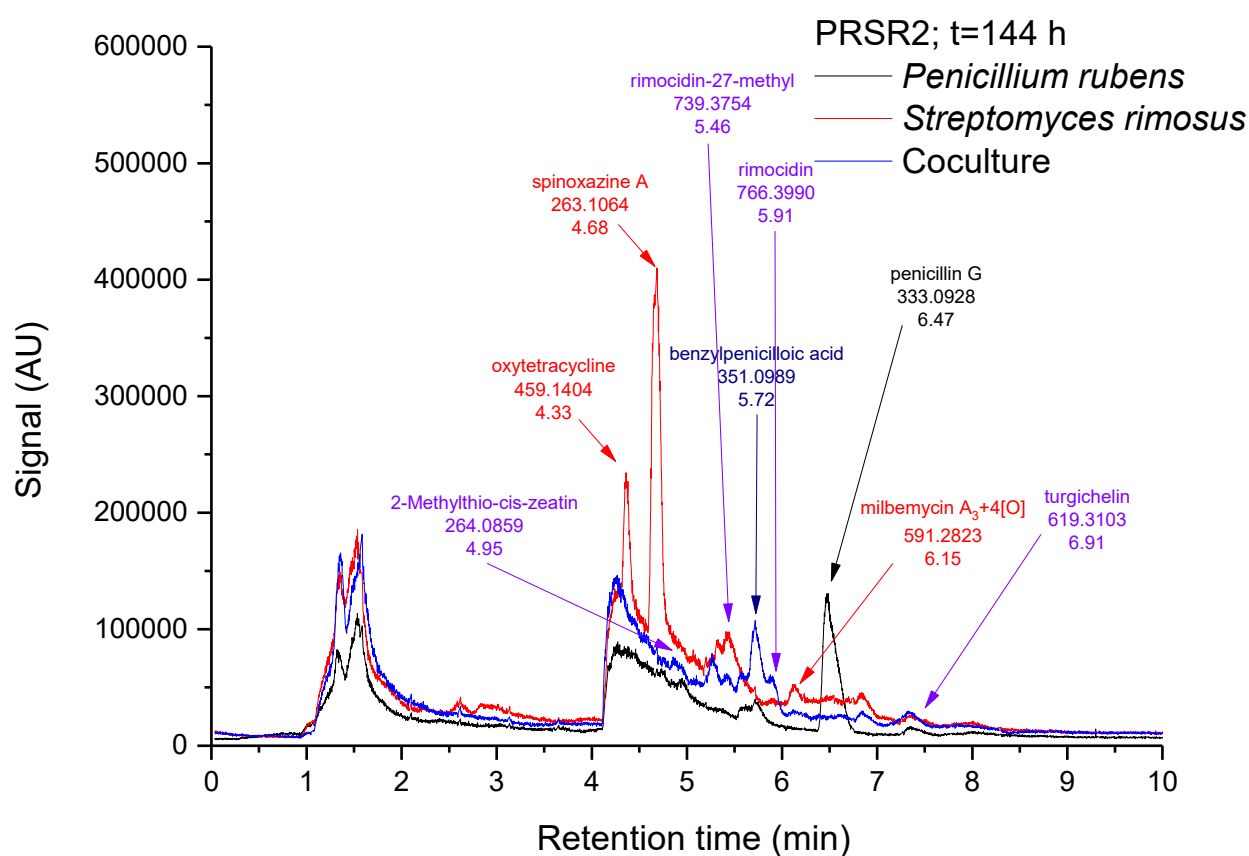


Figure S18 Alignments of total ion chromatograms (TICs) recorded in the PR[®]R2 experiment at t=144 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units. The names of the metabolites that were found solely in the monoculture of *S. rimosus* are shown in red. The names of the metabolites that were found both in the monoculture of *S. rimosus* and the coculture are shown in violet. The name of the metabolite that was found both in the monoculture of *P. rubens* and the coculture is shown in navy blue. The name of the metabolite that was found solely in the monoculture of *P. rubens* is shown in black. The metabolites were tentatively identified on the basis of M[®] data, however, their identities were not confirmed due to a lack of analytical standards. The exceptions were the confirmed identities of oxytetracycline and penicillin G.

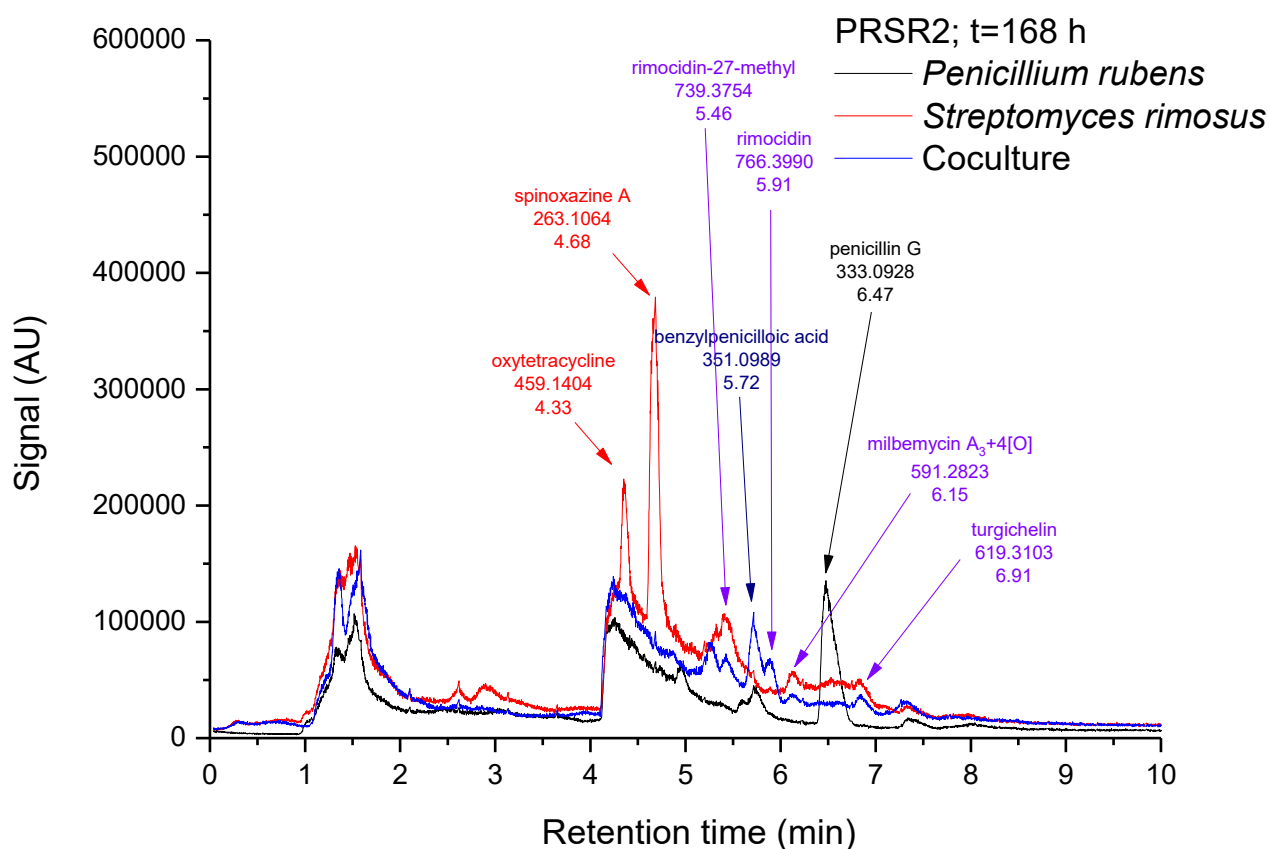


Figure S19 Alignments of total ion chromatograms (TICs) recorded in the PR[®]R2 experiment at t=168 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units. The names of the metabolites that were found solely in the monoculture of *S. rimosus* are shown in red. The names of the metabolites that were found both in the monoculture of *S. rimosus* and the coculture are shown in violet. The name of the metabolite that was found both in the monoculture of *P. rubens* and the coculture is shown in navy blue. The name of the metabolite that was found solely in the monoculture of *P. rubens* is shown in black. The metabolites were tentatively identified on the basis of M[®] data, however, their identities were not confirmed due to a lack of analytical standards. The exceptions were the confirmed identities of oxytetracycline and penicillin G.

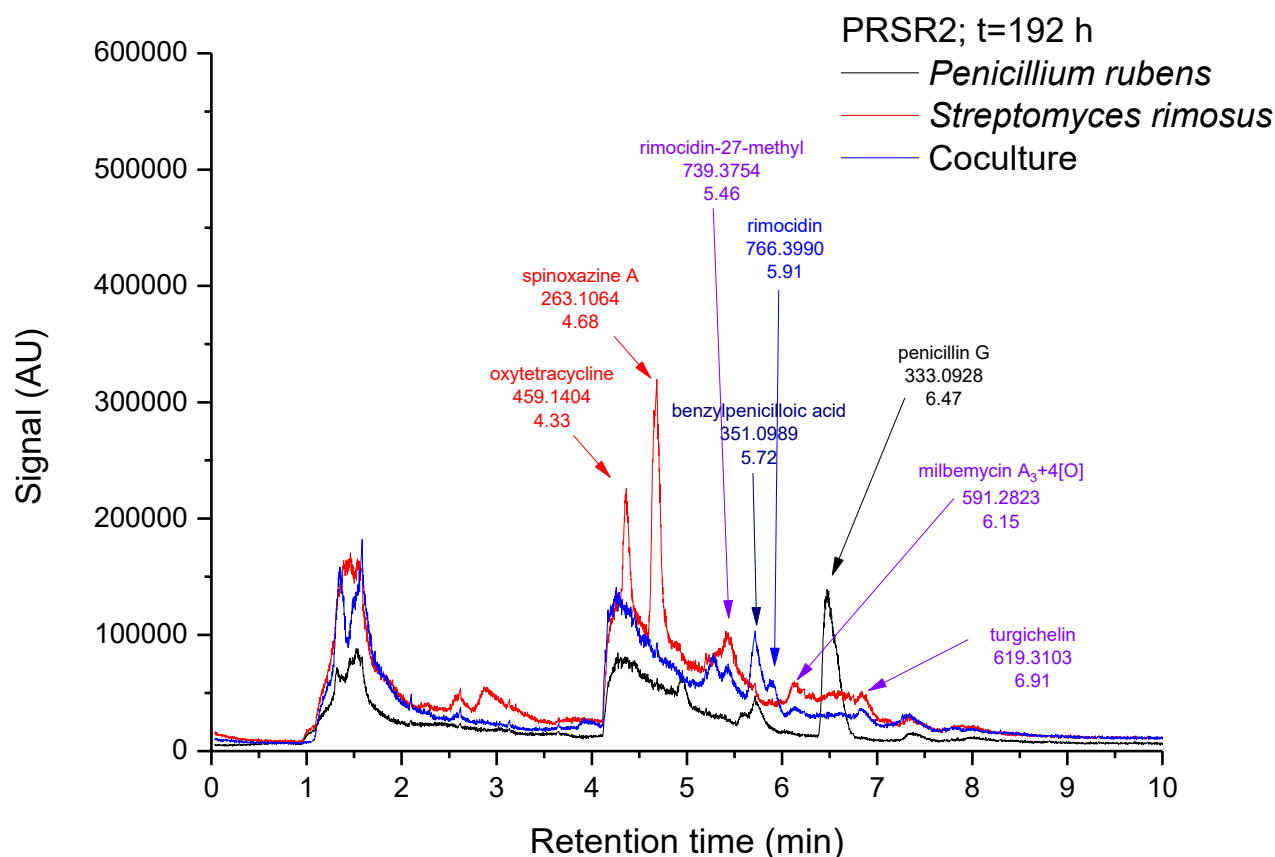


Figure S20 Alignments of total ion chromatograms (TICs) recorded in the PRSR2 experiment at t=192 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units. The names of the metabolites that were found solely in the monoculture of *S. rimosus* are shown in red. The names of the metabolites that were found both in the monoculture of *S. rimosus* and the coculture are shown in violet. The name of the metabolite that was found both in the monoculture of *P. rubens* and the coculture is shown in navy blue. The name of the metabolite that was found solely in the monoculture of *P. rubens* is shown in black. The name of the metabolite that was found solely in the coculture is shown in blue. The metabolites were tentatively identified on the basis of MS data, however, their identities were not confirmed due to a lack of analytical standards. The exceptions were the confirmed identities of oxytetracycline and penicillin G.

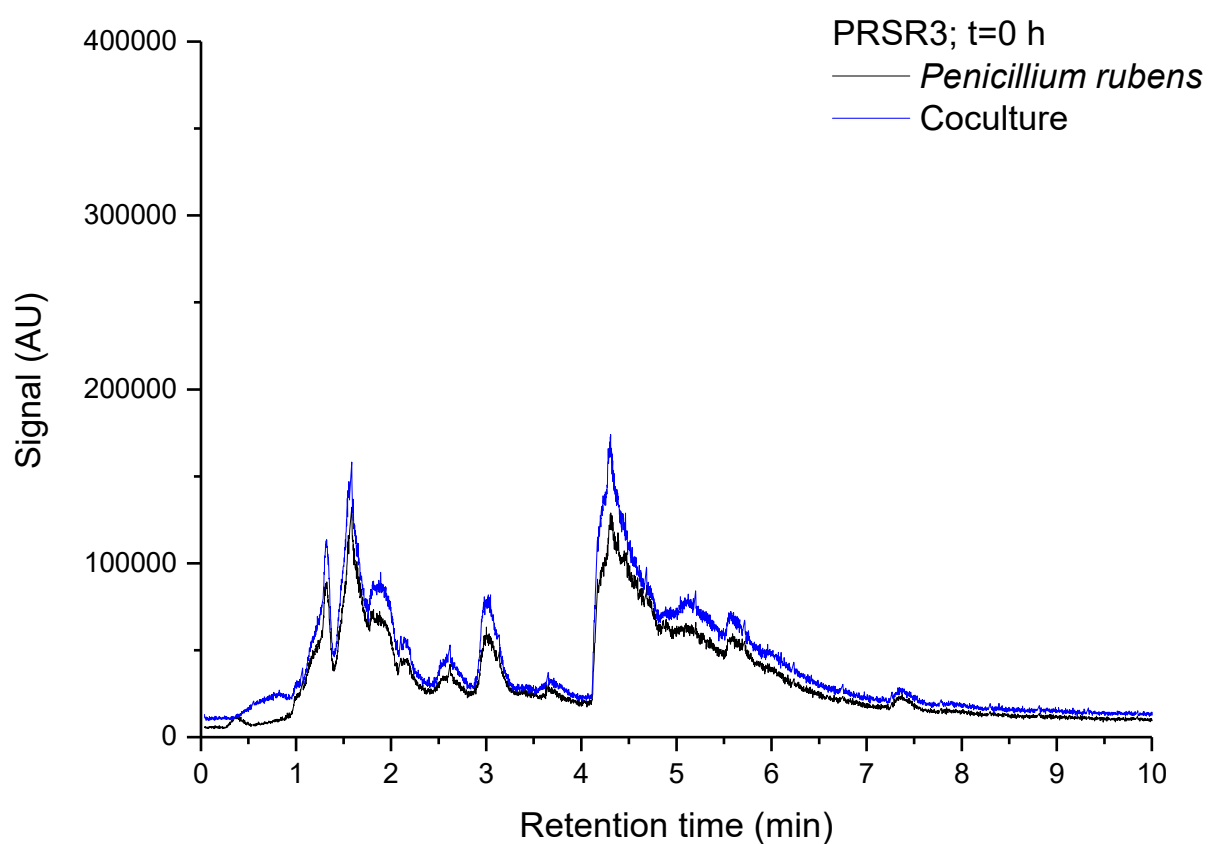


Figure S21 Alignments of total ion chromatograms (TICs) recorded in the PR®R3 experiment at t=0 h for *P. rubens* monoculture (black line) and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU-auxiliary units.

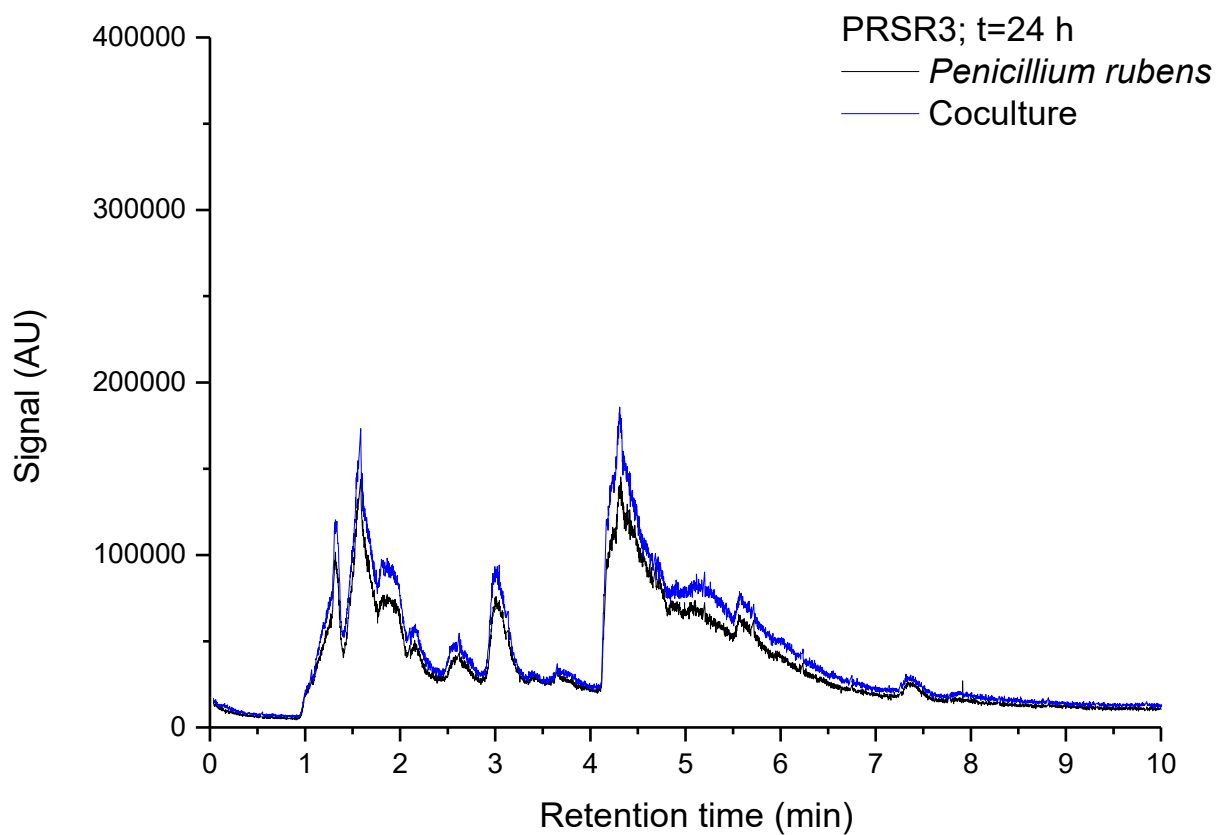


Figure S22 Alignments of total ion chromatograms (TICs) recorded in the PRSR3 experiment at t=24 h for *P. rubens* monoculture (black line) and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU-auxiliary units.

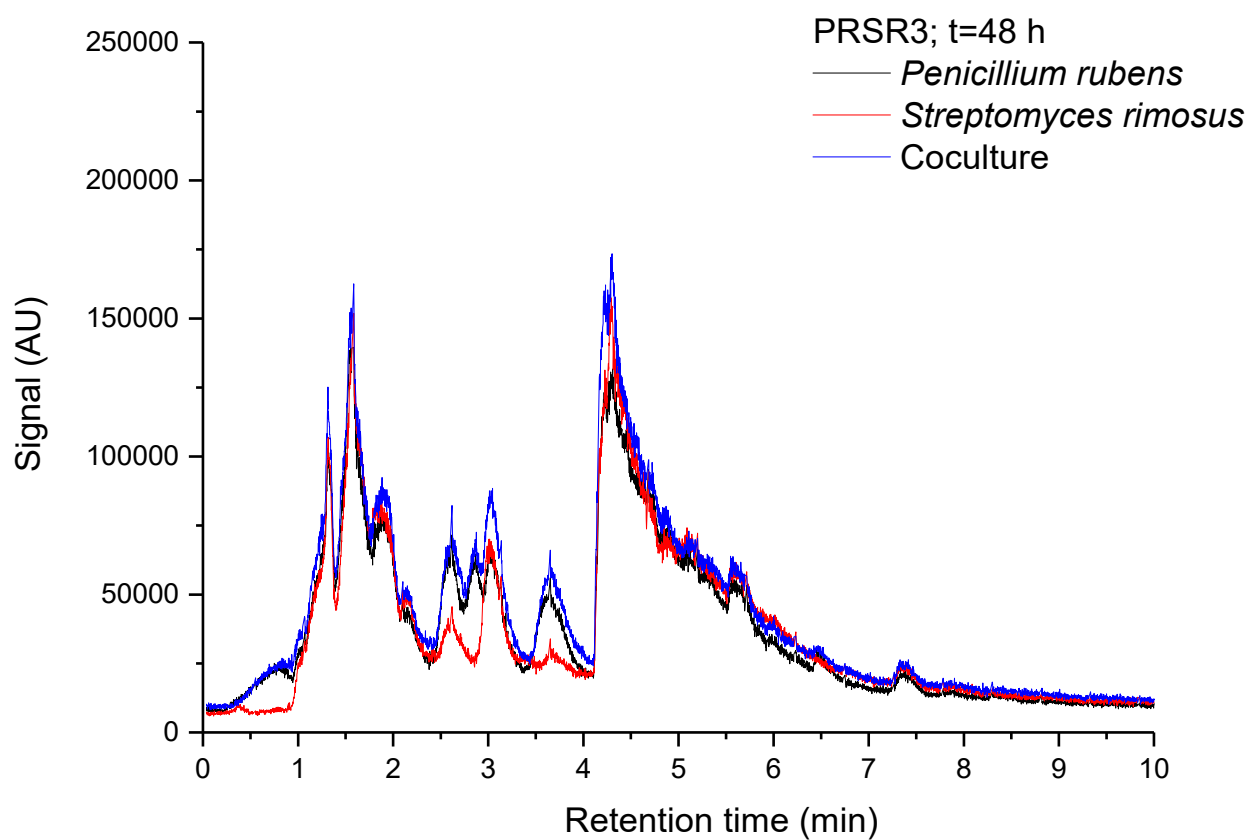


Figure S23 Alignments of total ion chromatograms (TICs) recorded in the PRSR3 experiment at t=48 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units.

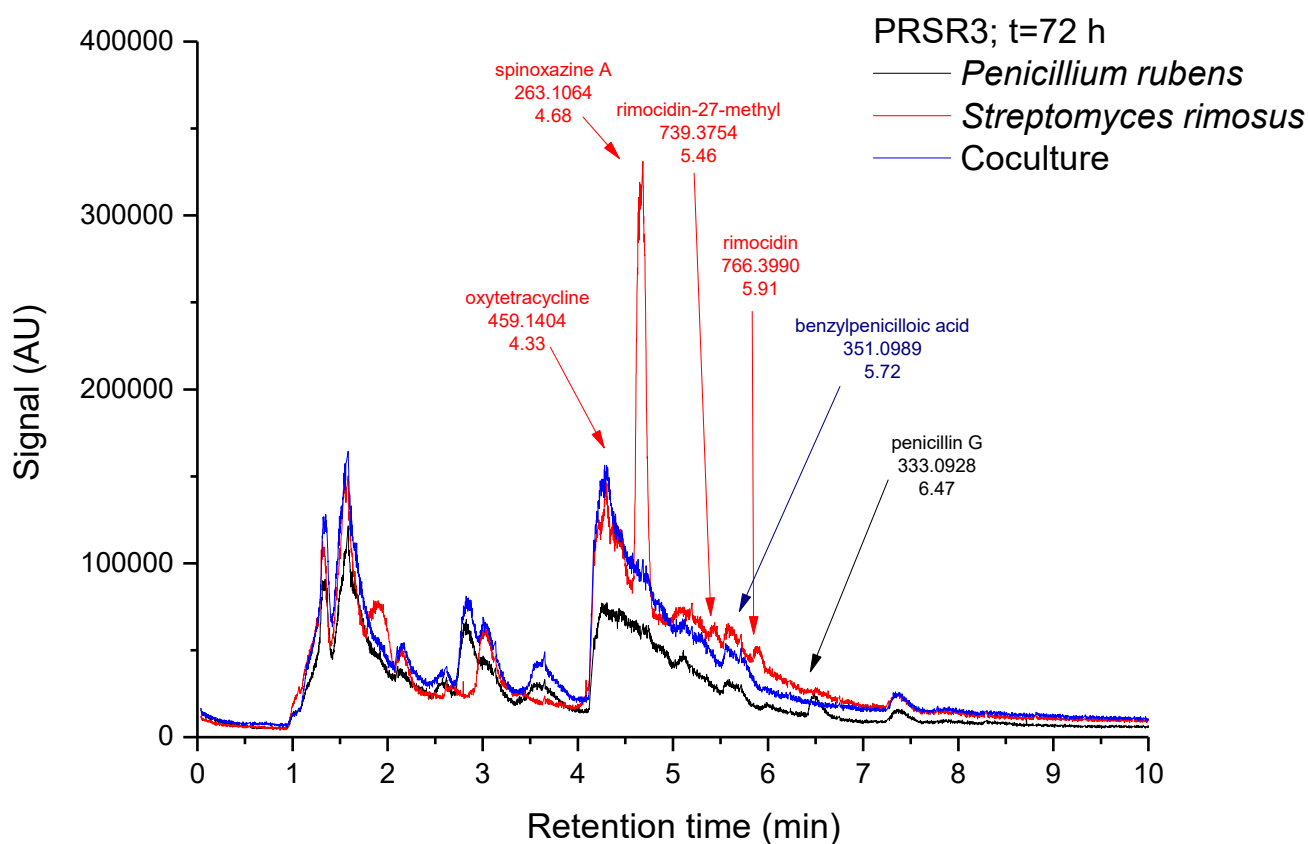


Figure S24 Alignments of total ion chromatograms (TICs) recorded in the PR[®]R3 experiment at t=72 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units. The names of the metabolites that were found solely in the monoculture of *S. rimosus* are shown in red. The name of the metabolite that was found both in the monoculture of *P. rubens* and the coculture is shown in navy blue. The name of the metabolite that was found solely in the monoculture of *P. rubens* is shown in black. The metabolites were tentatively identified on the basis of M⁺ data, however, their identities were not confirmed due to a lack of analytical standards. The exceptions were the confirmed identities of oxytetracycline and penicillin G.

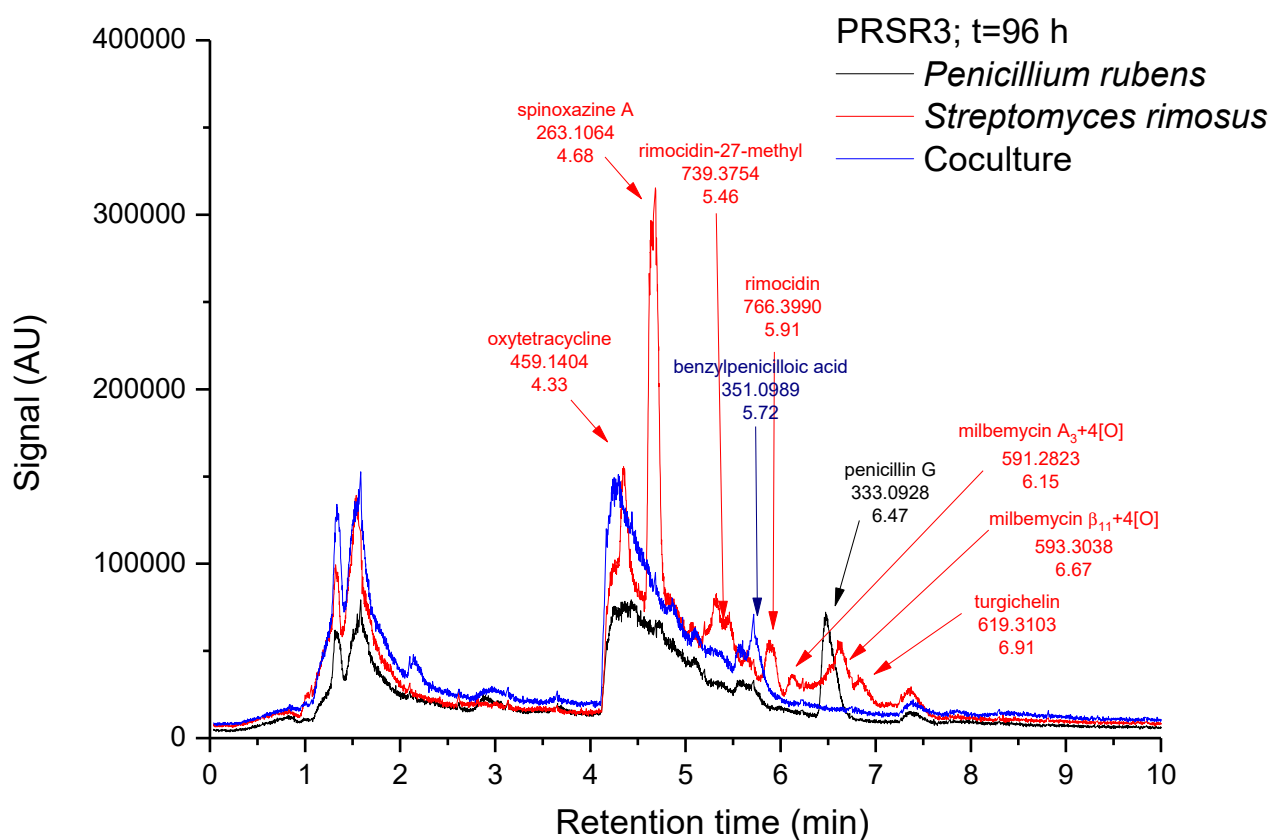


Figure S25 Alignments of total ion chromatograms (TICs) recorded in the PR[®]R3 experiment at t=96 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units. The names of the metabolites that were found solely in the monoculture of *S. rimosus* are shown in red. The name of the metabolite that was found both in the monoculture of *P. rubens* and the coculture is shown in navy blue. The name of the metabolite that was found solely in the monoculture of *P. rubens* is shown in black. The metabolites were tentatively identified on the basis of M⁺ data, however, their identities were not confirmed due to a lack of analytical standards. The exceptions were the confirmed identities of oxytetracycline and penicillin G.

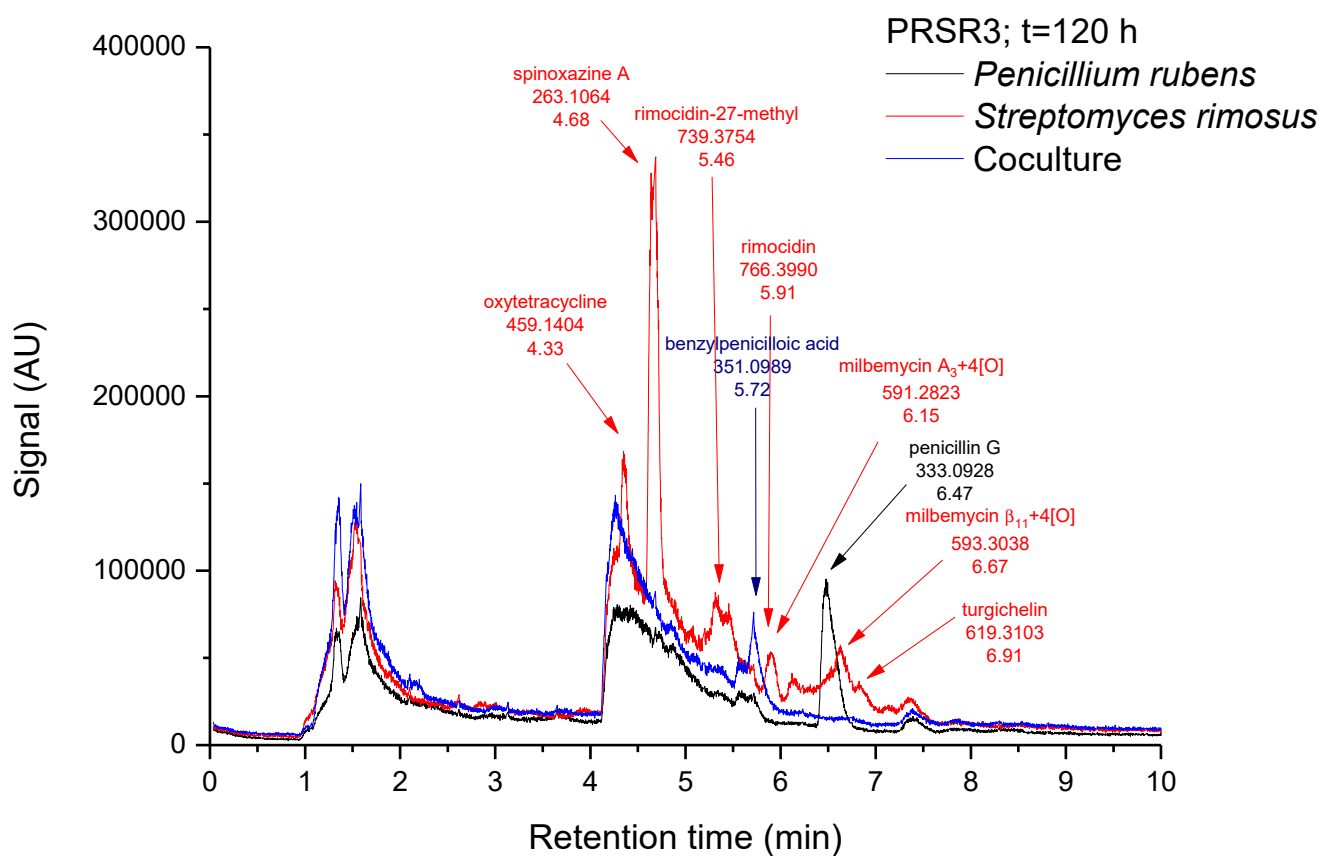


Figure S26 Alignments of total ion chromatograms (TICs) recorded in the PR[®]R3 experiment at t=120 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units. The names of the metabolites that were found solely in the monoculture of *S. rimosus* are shown in red. The name of the metabolite that was found both in the monoculture of *P. rubens* and the coculture is shown in navy blue. The name of the metabolite that was found solely in the monoculture of *P. rubens* is shown in black. The metabolites were tentatively identified on the basis of M[®] data, however, their identities were not confirmed due to a lack of analytical standards. The exceptions were the confirmed identities of oxytetracycline and penicillin G.

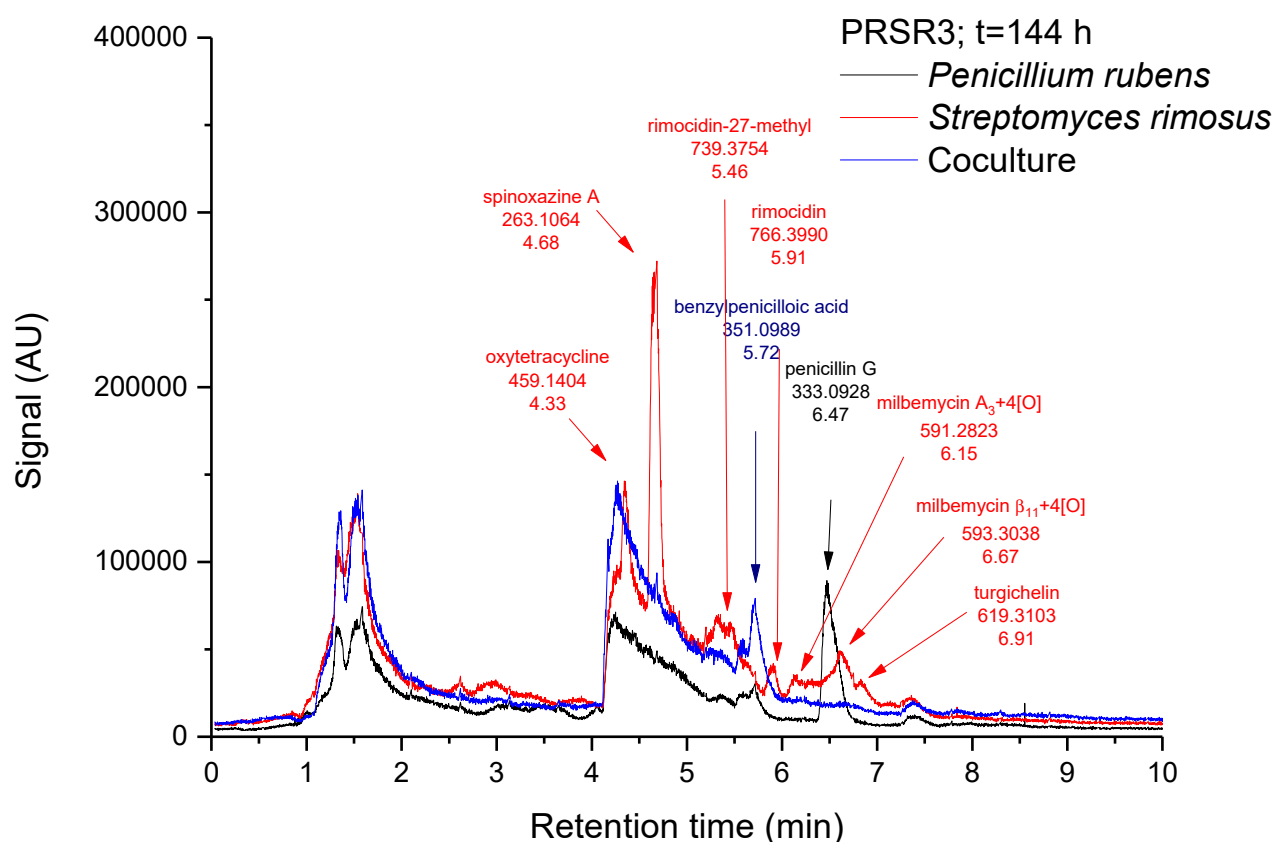


Figure S27 Alignments of total ion chromatograms (TICs) recorded in the PR[®]R3 experiment at t=144 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units. The names of the metabolites that were found solely in the monoculture of *S. rimosus* are shown in red. The name of the metabolite that was found both in the monoculture of *P. rubens* and the coculture is shown in navy blue. The name of the metabolite that was found solely in the monoculture of *P. rubens* is shown in black. The metabolites were tentatively identified on the basis of M[®] data, however, their identities were not confirmed due to a lack of analytical standards. The exceptions were the confirmed identities of oxytetracycline and penicillin G.

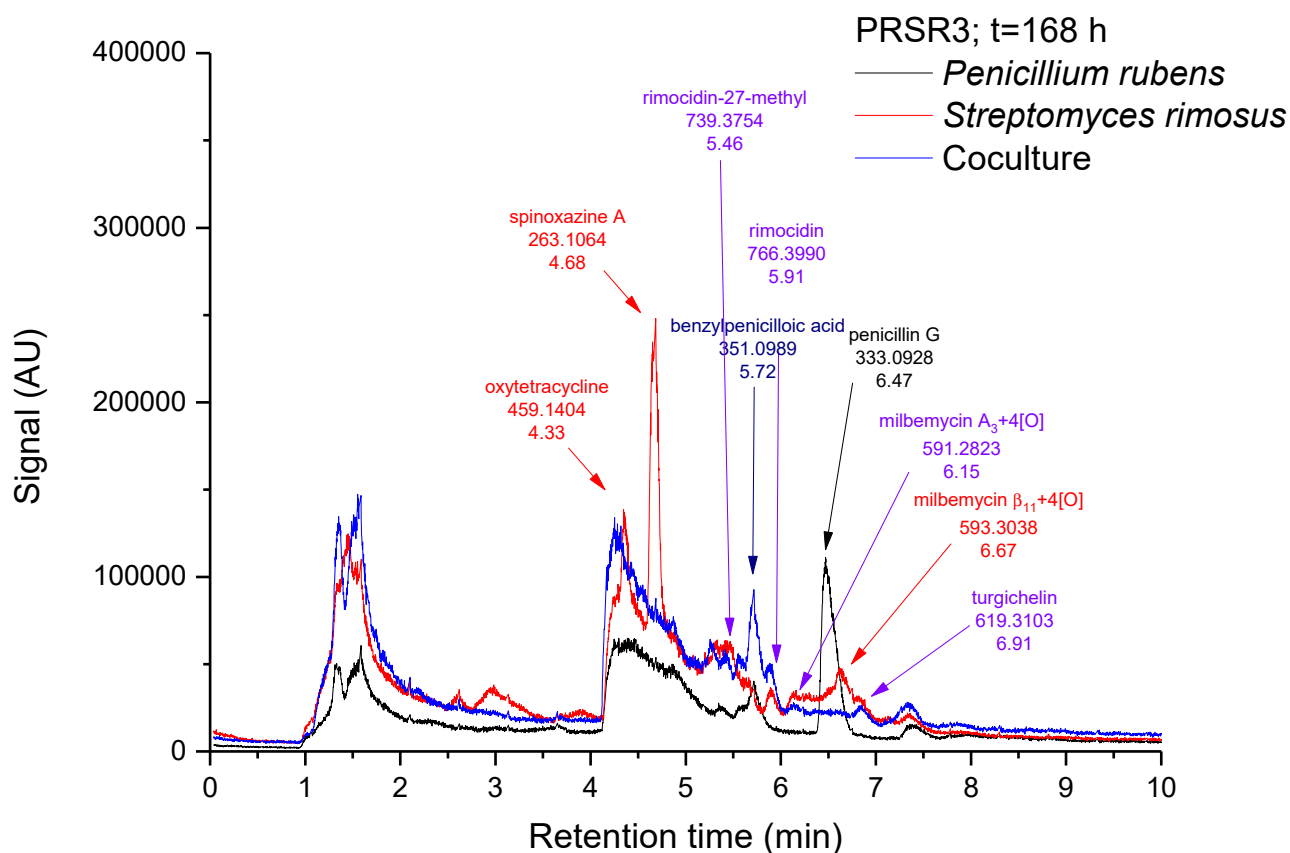


Figure S28 Alignments of total ion chromatograms (TICs) recorded in the PR®R3 experiment at t=168 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units. The names of the metabolites that were found solely in the monoculture of *S. rimosus* are shown in red. The names of the metabolites that were found both in the monoculture of *S. rimosus* and the coculture are shown in violet. The name of the metabolite that was found both in the monoculture of *P. rubens* and the coculture is shown in navy blue. The name of the metabolite that was found solely in the monoculture of *P. rubens* is shown in black. The metabolites were tentatively identified on the basis of M® data, however, their identities were not confirmed due to a lack of analytical standards. The exceptions were the confirmed identities of oxytetracycline and penicillin G.

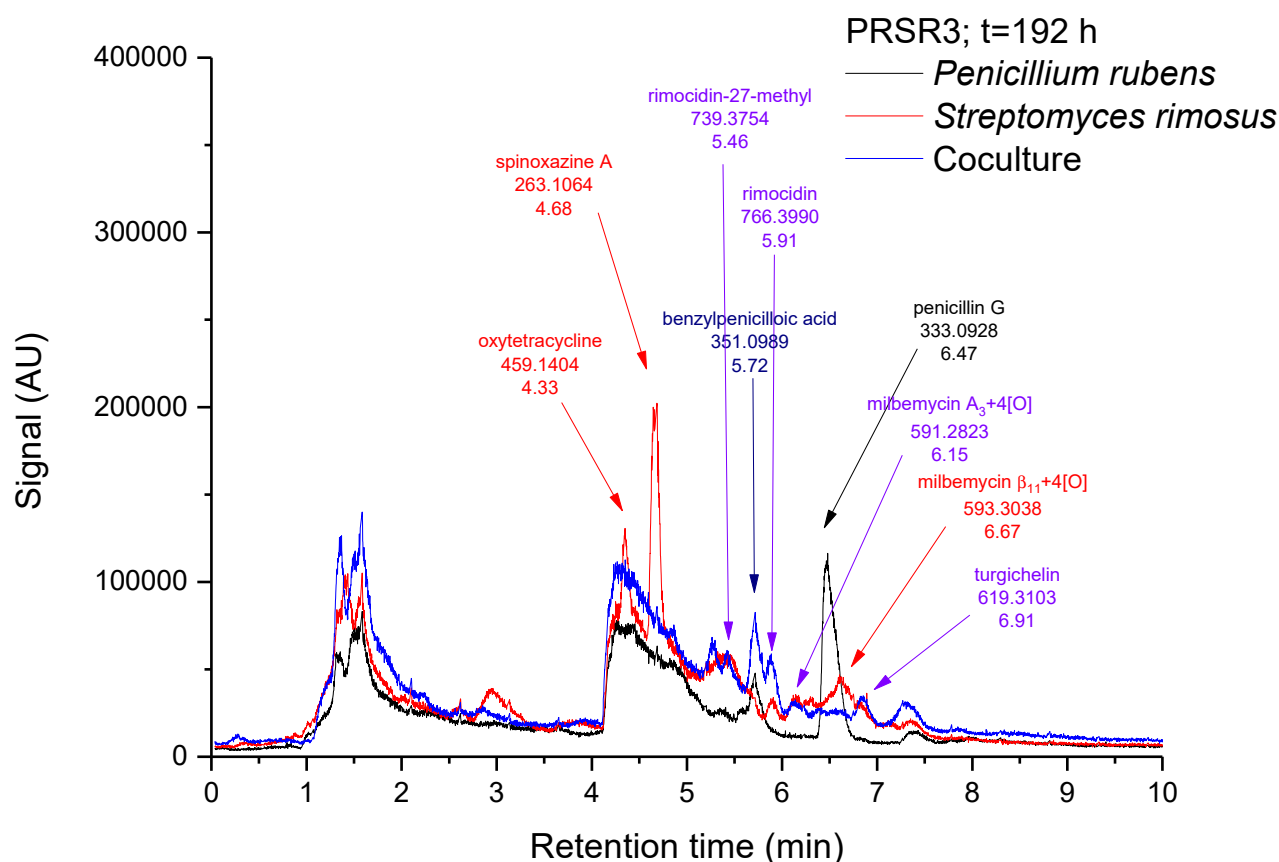


Figure S29 Alignments of total ion chromatograms (TICs) recorded in the PR[®]R3 experiment at t=192 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units. The names of the metabolites that were found solely in the monoculture of *S. rimosus* are shown in red. The names of the metabolites that were found both in the monoculture of *S. rimosus* and the coculture are shown in violet. The name of the metabolite that was found both in the monoculture of *P. rubens* and the coculture is shown in navy blue. The name of the metabolite that was found solely in the monoculture of *P. rubens* is shown in black. The metabolites were tentatively identified on the basis of M[®] data, however, their identities were not confirmed due to a lack of analytical standards. The exceptions were the confirmed identities of oxytetracycline and penicillin G.

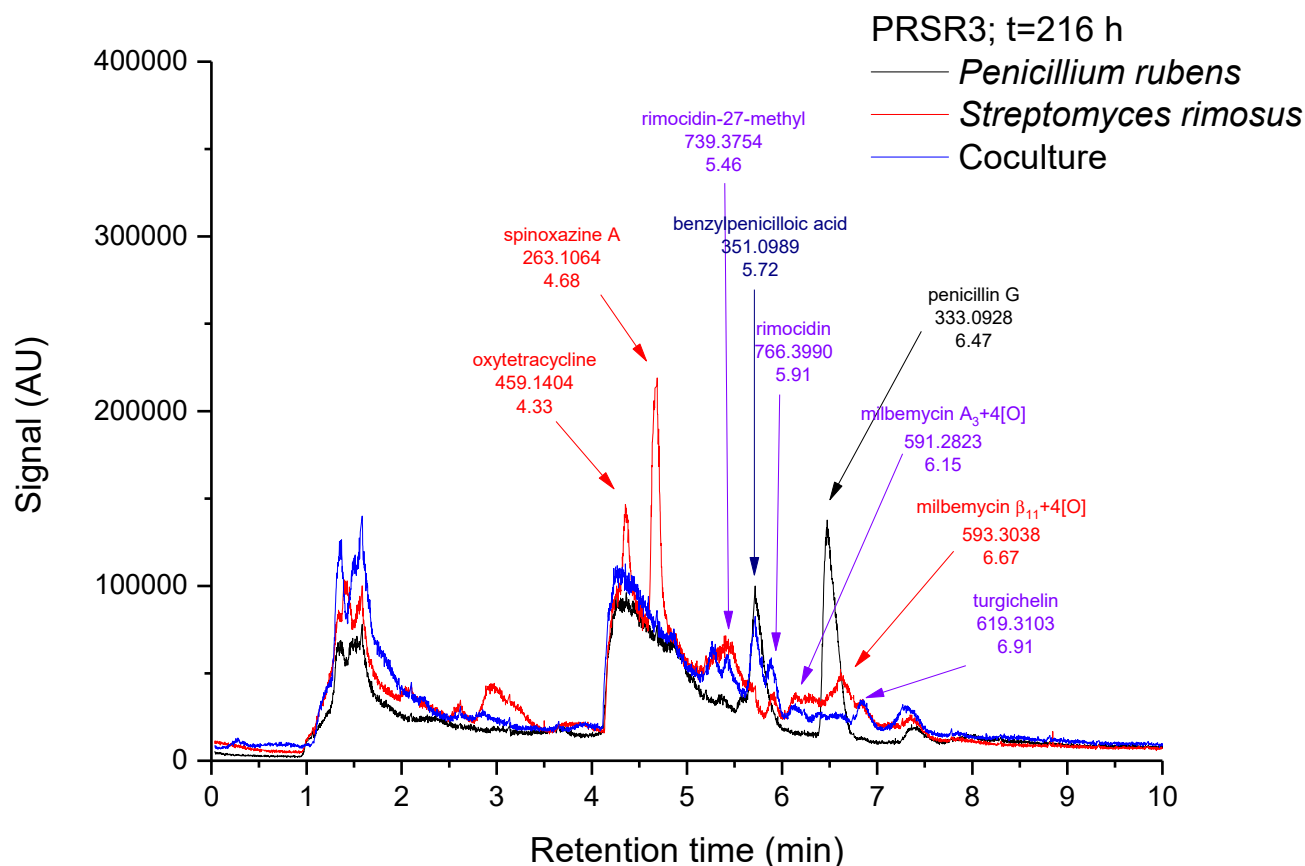


Figure S30 Alignments of total ion chromatograms (TICs) recorded in the PR[®]R3 experiment at t=216 h for *P. rubens* monoculture (black line), *S. rimosus* monoculture (red line), and the “*P. rubens* vs. *S. rimosus*” coculture (blue line). AU—auxiliary units. The names of the metabolites that were found solely in the monoculture of *S. rimosus* are shown in red. The names of the metabolites that were found both in the monoculture of *S. rimosus* and the coculture are shown in violet. The name of the metabolite that was found both in the monoculture of *P. rubens* and the coculture is shown in navy blue. The name of the metabolite that was found solely in the monoculture of *P. rubens* is shown in black. The metabolites were tentatively identified on the basis of M[®] data, however, their identities were not confirmed due to a lack of analytical standards. The exceptions were the confirmed identities of oxytetracycline and penicillin G.

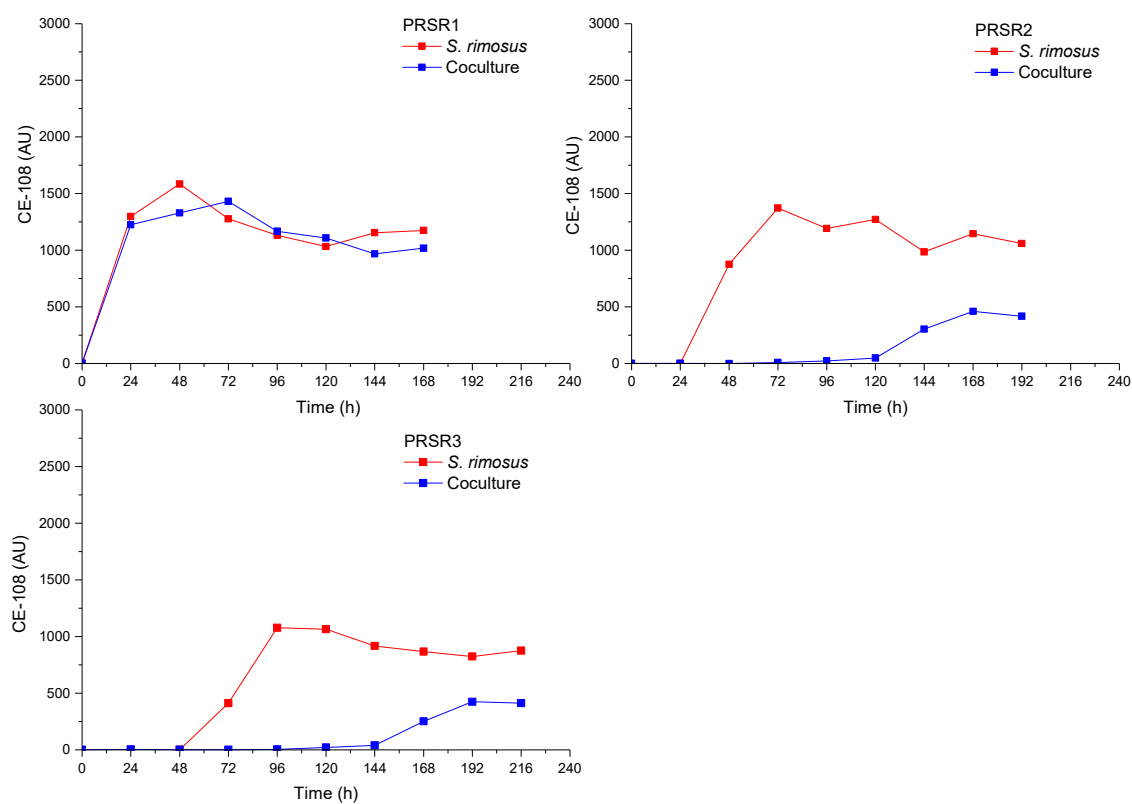


Figure S31 PR[®]R1, PR[®]R2 and PR[®]R3 profiles of CE-108 levels in the “*S. rimosus* vs. *P. rubens*” cocultures and the corresponding monocultures of *S. rimosus*. AU-auxiliary units. The metabolite was tentatively identified on the basis of MS data, however, its identity was not confirmed due to a lack of analytical standard.

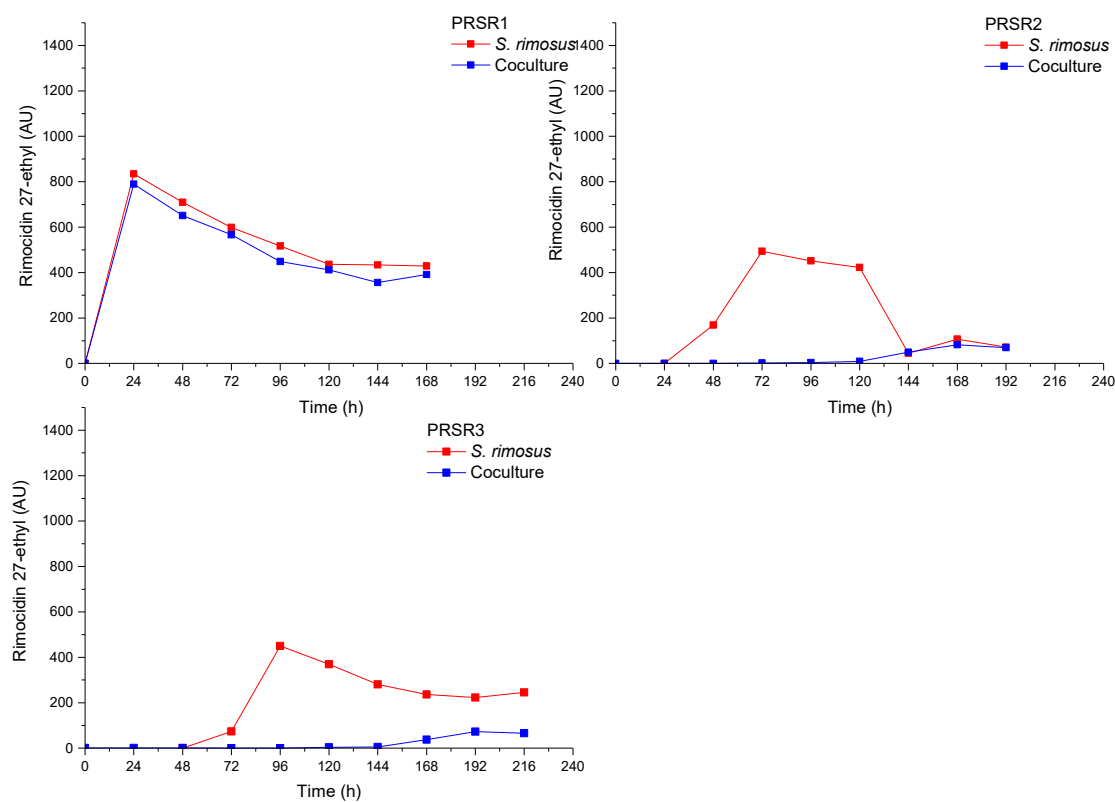


Figure S32 PR[®]R1, PR[®]R2 and PR[®]R3 profiles of rimocidin (27-ethyl) levels in the “*S. rimosus* vs. *P. rubens*” cocultures and the corresponding monocultures of *S. rimosus*. AU-auxiliary units. The metabolite was tentatively identified on the basis of M⁺ data, however, its identity was not confirmed due to a lack of analytical standard.

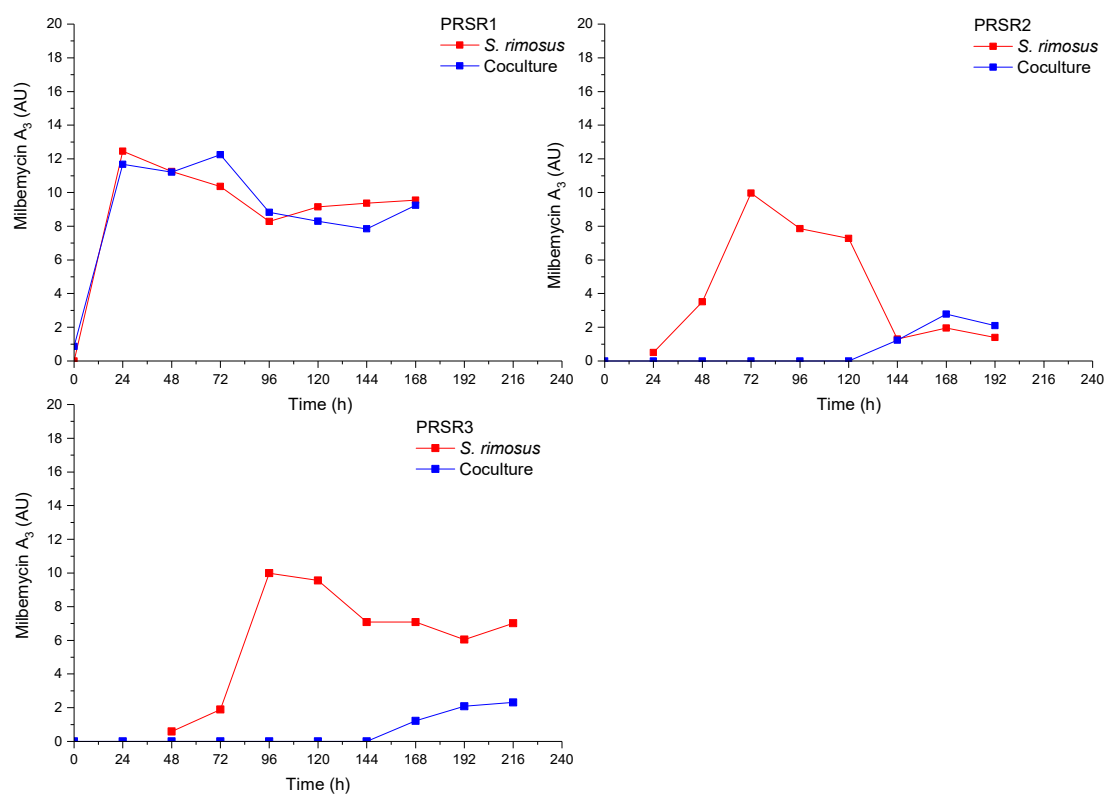


Figure S33 PR[®]R1, PR[®]R2 and PR[®]R3 profiles of milbemycin A₃ levels in the “*S. rimosus* vs. *P. rubens*” cocultures and the corresponding monocultures of *S. rimosus*. AU-auxiliary units. The metabolite was tentatively identified on the basis of M⁺ data, however, its identity was not confirmed due to a lack of analytical standard.

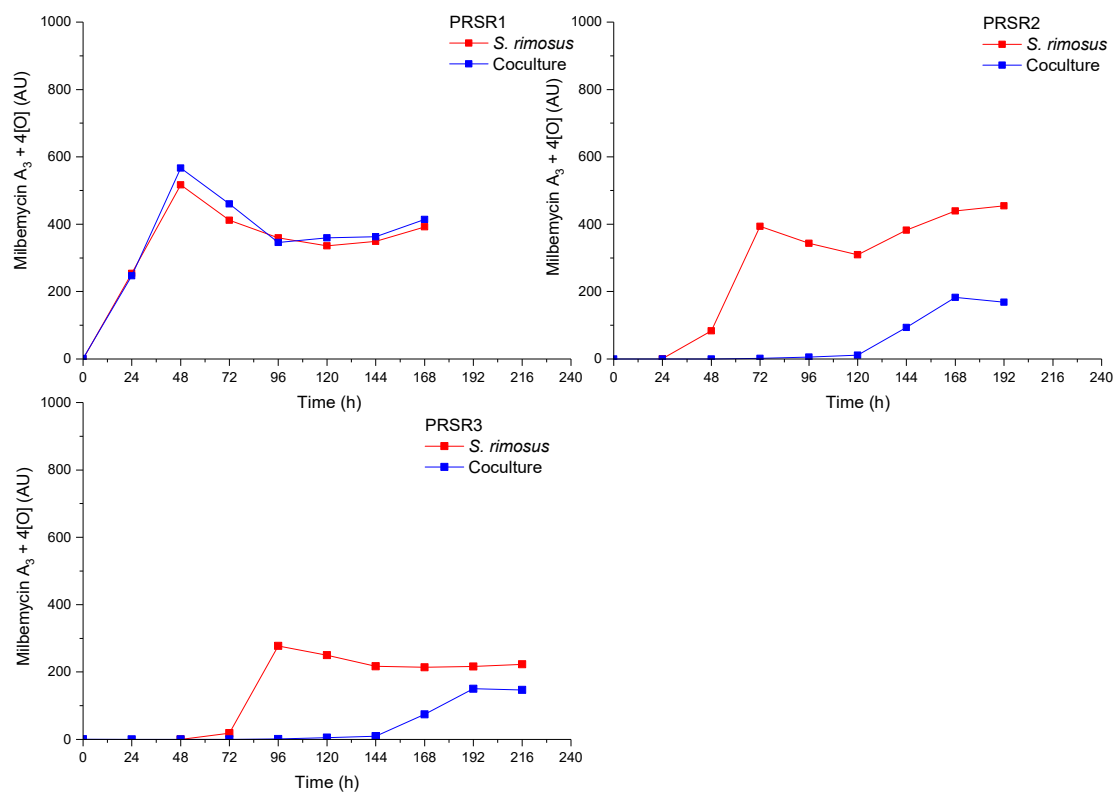


Figure S34 PR[®]R1, PR[®]R2 and PR[®]R3 profiles of milbemycin A₃+4[O] levels in the “*S. rimosus* vs. *P. rubens*” cocultures and the corresponding monocultures of *S. rimosus*. AU-auxiliary units. The metabolite was tentatively identified on the basis of M[®] data, however, its identity was not confirmed due to a lack of analytical standard.

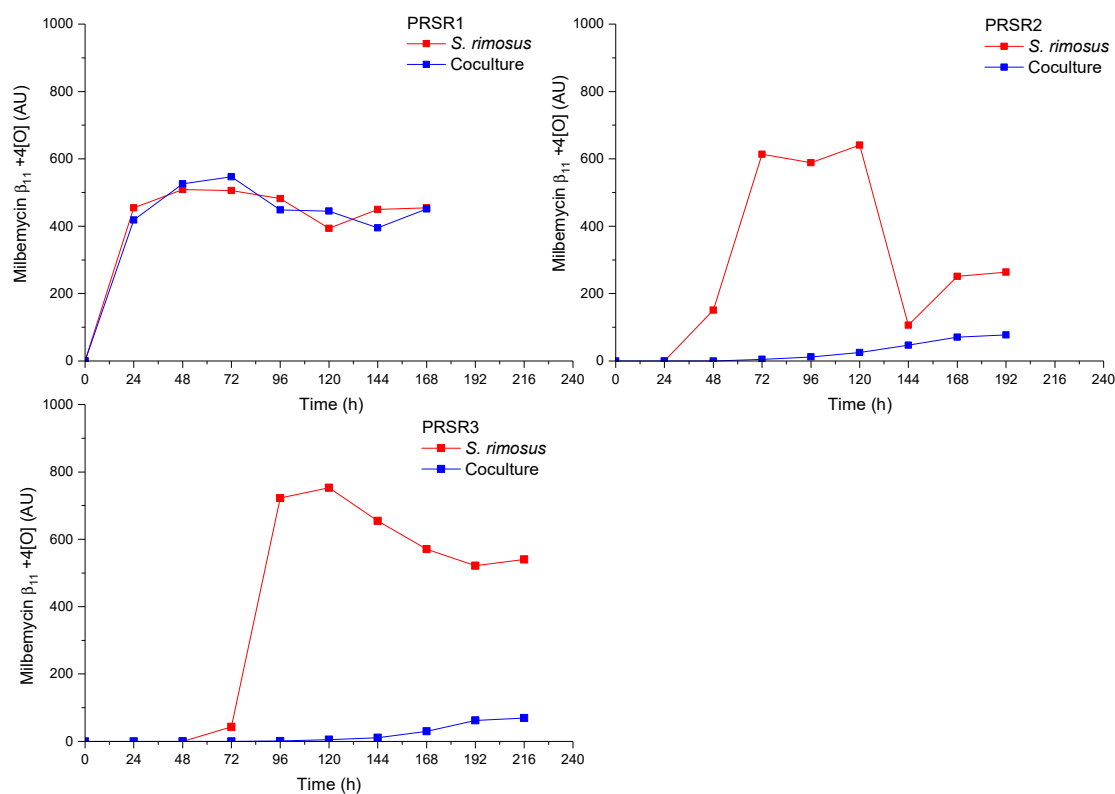


Figure S35 PR[®]R1, PR[®]R2 and PR[®]R3 profiles of milbemycin $\beta_{11} + 4[O]$ levels in the “*S. rimosus* vs. *P. rubens*” cocultures and the corresponding monocultures of *S. rimosus*. AU-auxiliary units. The metabolite was tentatively identified on the basis of M[®] data, however, its identity was not confirmed due to a lack of analytical standard.

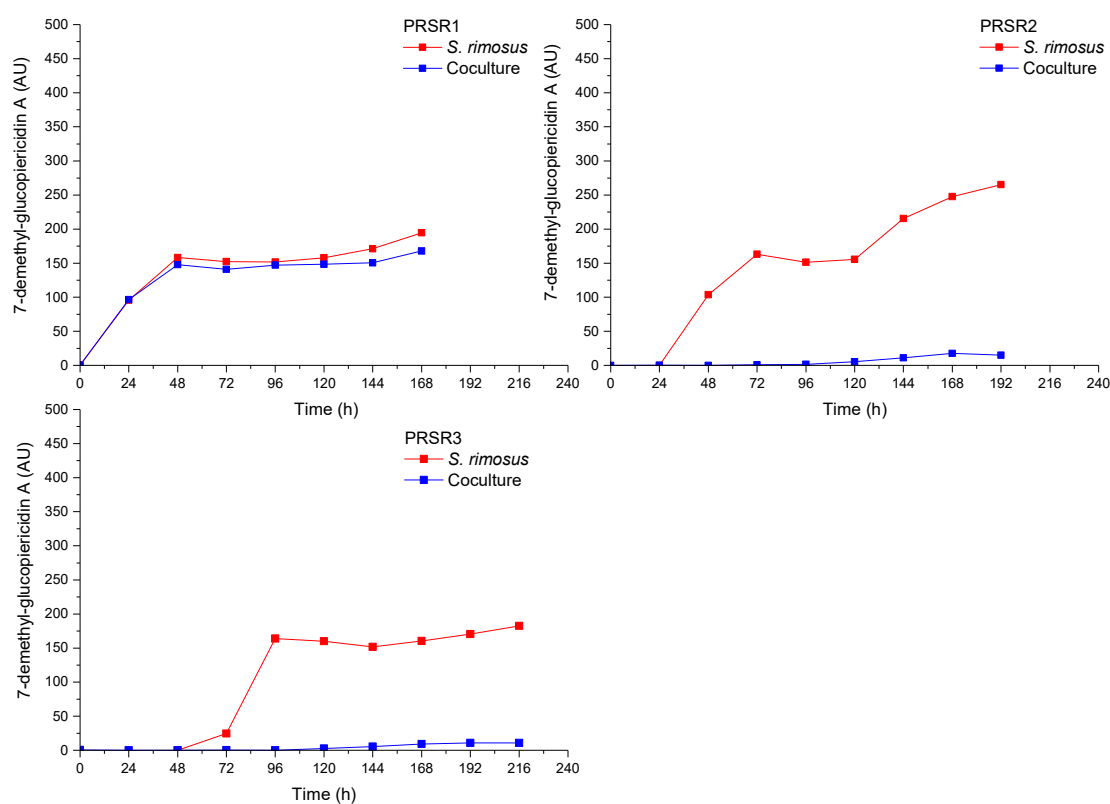


Figure S36 PR[®]R1, PR[®]R2 and PR[®]R3 profiles of 7-demethyl-glucopiericidin A levels in the “*S. rimosus* vs. *P. rubens*” cocultures and the corresponding monocultures of *S. rimosus*. AU-auxiliary units. The metabolite was tentatively identified on the basis of M[®] data, however, its identity was not confirmed due to a lack of analytical standard.

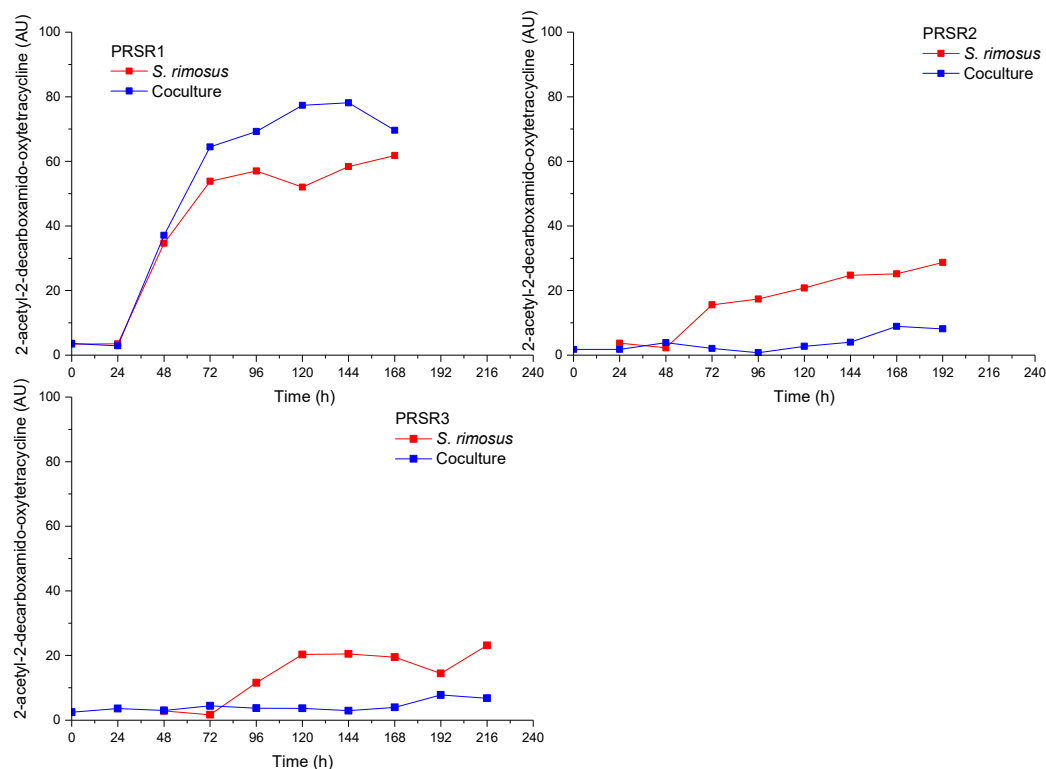


Figure S37 PR[®]R1, PR[®]R2 and PR[®]R3 profiles of 2-acetyl-2-decarboxamido-oxytetracycline (ADOTC) levels in the “*S. rimosus* vs. *P. rubens*” cocultures and the corresponding monocultures of *S. rimosus*. AU-auxiliary units. The metabolite was tentatively identified on the basis of M⁺ data, however, its identity was not confirmed due to a lack of analytical standard.

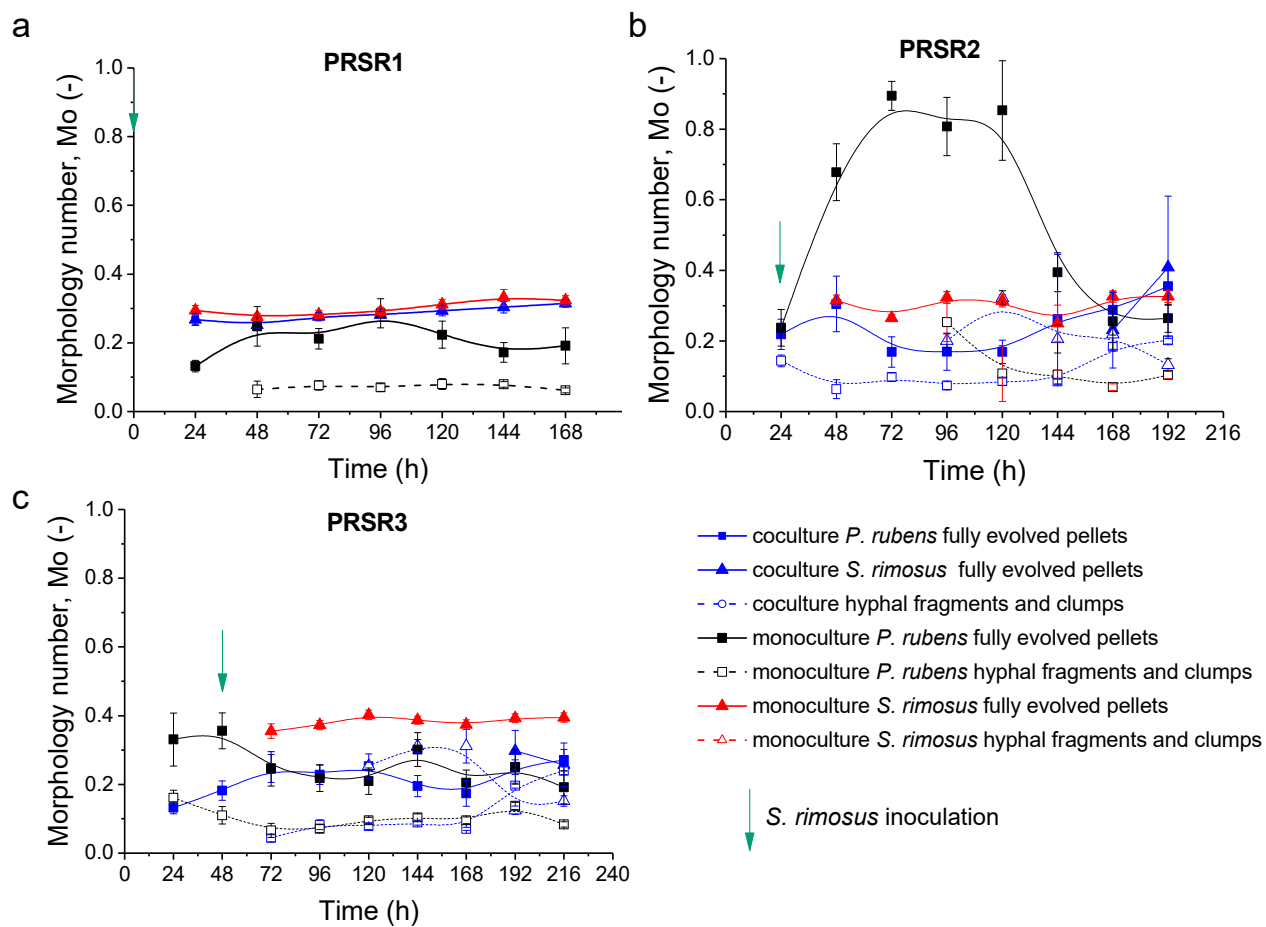


Figure S38 The values of morphology number (Mo) recorded over the course of PRSR1 (a), PRSR2 (b) and PRSR3 (c) experiments for the cocultures, monocultures of *P. rubens* and monocultures of *S. rimosus*.

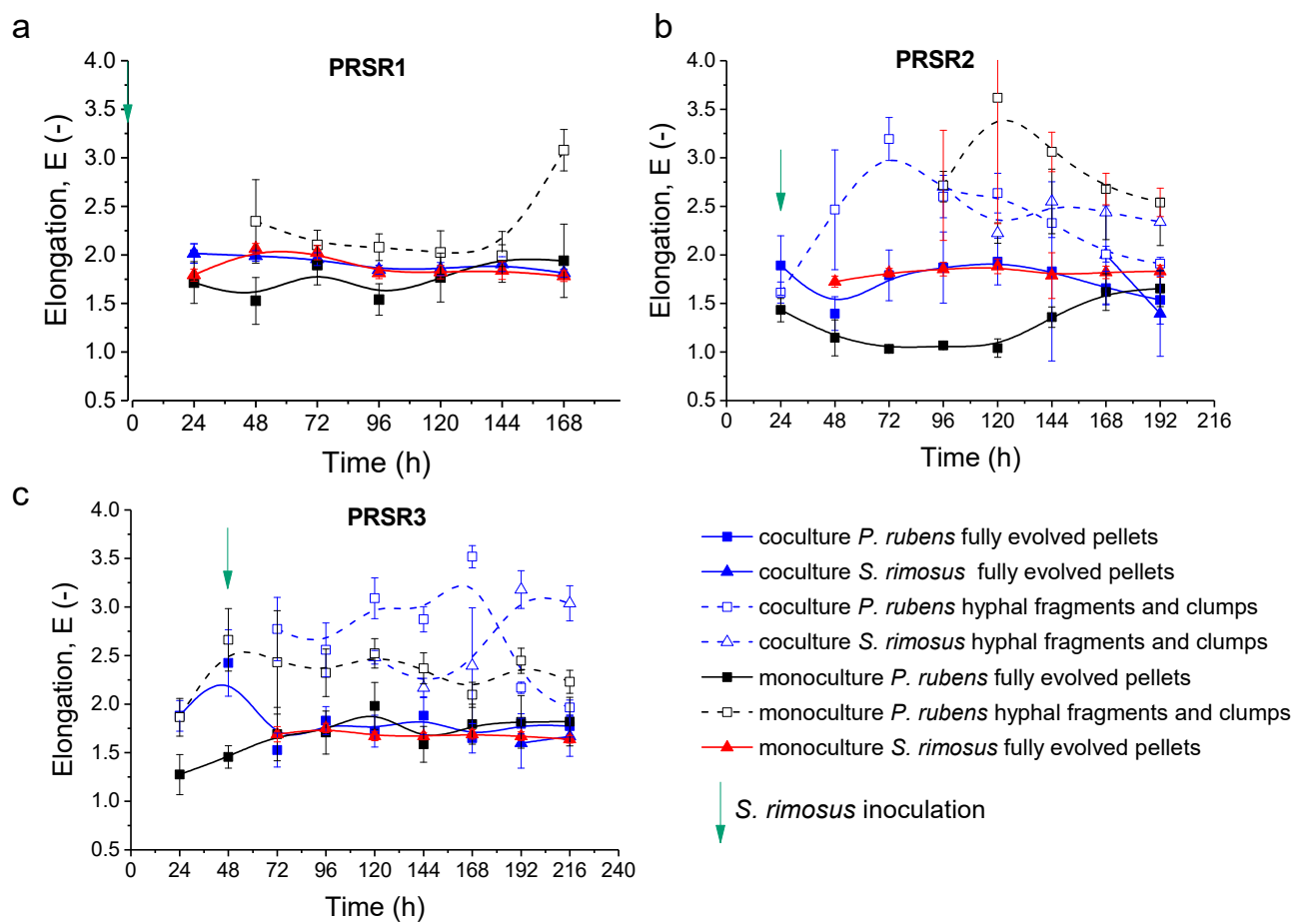


Figure S39 The values of elongation (E) recorded over the course of PR[®]R1 (a), PR[®]R2 (b) and PR[®]R3 (c) experiments for the cocultures, monocultures of *P. rubens* and monocultures of *S. rimosus*.

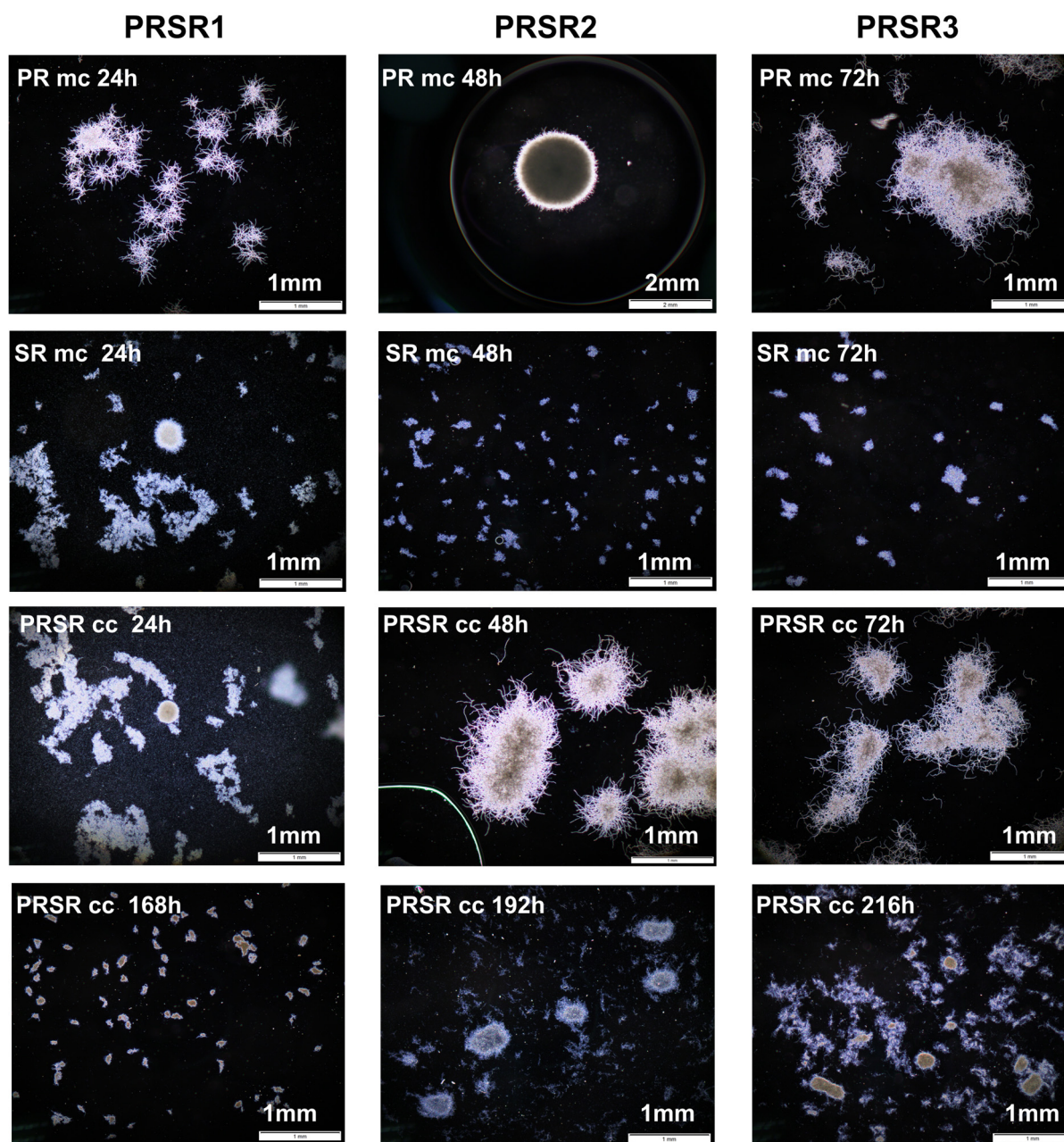


Figure S40 Selected microscopic images corresponding to the PRSR1, PRSR2, and PRSR3 experiments. The samples were drawn at the indicated time points. PR – *P. rubens*; SR mc – monoculture of *S. rimosus*; PR mc – monoculture of *P. rubens*; PRSR cc – coculture of *P. rubens* and *S. rimosus*.