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Skolkovo Institute of Science and Technology

Thesis Changes Log

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PhD Program: Life Sciences

Title of Thesis: phazolicin — a novel azole-modified peptide antibiotic: structure, mechanisms of action, transport, and biosynthesis.

Supervisor: Prof. Konstantin Severinov

The thesis document includes the following changes in answer to the external review process.

1. "prokaryotic translation" substituted with "bacterial translation" throughout the text
2. "adopted from" substituted with "adapted from" throughout the text
3. "Alfa" substituted with "Alpha" throughout the text
4. "RIPPER" corrected to "RiPPER" throughout the text
5. Colour scheme of the figures 2.1.1, 2.1.3, and 2.1.5. was changed to avoid simultaneous use of red and green. Legends were corrected accordingly.
6. Fig. 2.1.2 C_α and C_β atoms were labelled
7. Fig. 2.1.3 was supplemented with three additional examples of tailoring modifications of lasso-peptides. References for each compound were added to the legend. Typos in the words "oxime", "cinnamycin", and "dehydrobutyrine" were corrected. Z-isomer is now shown for dehydrobutyrine. Stereochemistry is drawn for all modifications.
8. Fig. 2.1.5 Molecular drawing settings were adjusted for the compounds shown. References for each compound are added to the figure legend.
9. Fig. 2.1.7B PDB ID and the reference are provided for the structure shown
10. Rpm values are substituted with g for all centrifugation descriptions
11. All sentences starting with numbers are rephrased.
12. Sentences starting with "to confirm" (pp. 85, 87, 104) are rephrased.
13. Spaces are added before all °C symbols
14. Double spaces are removed throughout the text
15. p. 19 "Another obvious obstacle on the way from the BGC in the genome to the bioactive molecule discovery is the search for the biological activity of the metabolite, as it can be proposed in advance only based on the close homology to the clusters of known compounds or on the presence of specific self-immunity determinants. In many cases, the compounds are characterized structurally, but remain without a function they can play in the natural microbial community." substituted with "Another obvious obstacle on the way from the BGC in the genome to the bioactive molecule discovery is the search for the biological activity of the metabolite, as it can be proposed in advance only based on the close homology with the clusters of known compounds or, in rare cases, on the presence of specific self-immunity determinants [16]. Often the compounds are characterized structurally, but the function they can play in the natural microbial community remains unknown."
16. p. 26 "only a handful of RiPPs was shown to be synthesized by archaea [49]." was removed
17. p. 27 typo in the word "heterocycles" was corrected
18. p. 35 "Azoline dehydrogenases can either recognize the precursor peptide themselves (e.g., ThcOx from the biosynthetic pathway of cyanobactin cyanothecamide [97]) or form a ternary complex with YcaO and its partner." Instead of "To recognize the precursor peptide, azoline dehydrogenases

can either have their own RRE (e.g., ThcOx from the biosynthetic pathway of cyanobactin cyanothecamide [81]) or form a ternary complex with YcaO and its partner."

19. p. 36 "Second, YcaO enzymes install thioamide bonds (Fig. 2.1.3B) in several groups of RiPPs (thiopeptins [101], saalfelduracin [79], and thioamitides [57]) as well as in some proteins encoded by archaea (methyl-coenzyme M reductase [102]) and, probably, bacteria (ribosomal protein uL16 [103]). The activity of a partner protein TfuA is required for this reaction in several cases studied: it catalyzes the hydrolysis of thiocarboxylated ThiS, a proteinaceous donor of sulfur (Fig. 2.1.6D) [95]." instead of "Second, YcaO enzymes install thioamide bonds (Fig. 2.1.3B) in several groups of RiPPs (thiopeptins [85], saalfelduracin [61], and thioamitides [86]) as well as in some proteins encoded by bacteria (ribosomal protein uL16 [87]) and archaea (methyl-coenzyme M reductase [88]). The activity of a partner protein TfuA is required for this reaction: it catalyzes the hydrolysis of thiocarboxylated ThiS, a proteinaceous donor of sulfur (Fig. 2.1.6D) [79]."
20. p. 38 "probably" added before "cell membrane"
21. p. 42 Reference #95 is added after the words "TfuA described earlier"
22. p. 69 "Since the initial hmmer search was performed with relatively permissive parameters, for some of the hits RODEO proposed a profile HMM other than those for YcaOs (TIGR03549, TIGR03604, or PF02624) as the most probable. We removed such genomic regions from further analysis." Instead of "Initial search was very sensitive and false positive results were obtained. Thus, we removed predicted YcaO proteins that were not annotated with TIGR03549, TIGR03604, or PF02624 domains in the RODEO output."
23. p. 72 "This protein was selected, as it is involved in the modification of all azol(in)e-containing RiPPs catalyzing the first step in the formation of heterocycles (see section 2.1.2)." instead of "This protein was selected, as it is the class-defining enzyme for all azole-containing RiPPs catalyzing the first step in the formation of azole heterocycles (see section 2.1.2)."
24. p. 77 "The obtained values of 2362.8679, 2433.9052, and 2534.9524 are all within 1.5 ppm of the corresponding masses calculated based on empirical formulas (Fig. 5.2.3)." instead of "The obtained values of 2362.8679, 2433.9052, and 2534.9524 are all within 1.5 ppm of the corresponding masses calculated based on the brutto-formulas (Fig. 5.2.3)."
25. p. 78 "The proteolytic degradation of PHZ by the aminopeptidases is apparently not possible, as the carbonyl of Ala1 (Ala29 of the precursor) is involved in the formation of the first thiazole cycle, which protects the peptide bond from the cleavage." Is substituted with "The proteolytic degradation of PHZ by aminopeptidases may include only the removal of the N-terminal Ala1 (Ala29 of the precursor), since the carbonyl of Thr2 is involved in the formation of the first thiazole cycle, which protects the peptide bond from the cleavage."
26. p. 86 "As was already reviewed above (see section 2.1.5), known LAPs can have diverse mechanisms of action." instead of "As was already reviewed above (see section 2.1.5), known LAPs display unprecedented diversity of the mechanisms of action."
27. p. 89 Spelling of "thermophiles" was corrected.
28. p. 92 "face-to-face stacking" substituted with "displaced face-to-face stacking"
29. p. 95 "drug" was substituted by "compound"
30. p. 102 word "non-specific" was removed
31. p. 119 "cyclodehydrogenase" corrected to "cyclodehydratase"
32. p. 128 "Residues converted into azoles in PHZ and those matching them are blue, positively charged residues are red" was changed to "Residues converted into azoles in PHZ and those matching them are red, positively charged residues are blue".
33. p. 128 and in Supp. Table 2. DSRed changed to DsRed
34. p. 134 "and absorption on inorganic clay and silica particles" added after "organic polymers"
35. References were updated