

Supplemental Material 1

Automatic reconstruction of metabolic pathways from identified biosynthetic gene clusters

Snorre Sulheim, Fredrik A. Fossheim, Alexander Wentzel and Eivind Almaas

Tailoring reactions

We surveyed gene clusters in MIBiG [1] to explore how specific smCOG gene annotations could be used to determine the tailoring reactions associated with the biosynthesis of a polyketide or non-ribosomal peptide synthetase: we compared the presence of specific secondary metabolism Clusters of Orthologous Groups (smCOGs) [2] annotations with the presence of different tailoring reactions as described in the literature. This led to three fairly robust heuristic that were implemented into the BiGMeC pipeline (Table S1). The data that these heuristics are based on are further described in separate sections below.

Table S1: Boolean logic used to determine tailoring reactions from smCOG gene annotation. The peptide or polyketide that is modified by the tailoring reactions is denoted by X_n , both prior and subsequent to the tailoring. Other abbreviations used in the table: bpg: 1,3-biphosphoglycerate; P_i : phosphate; P_{ii} : diphosphate; CoA: Coenzyme A.

smCOG logic	Reaction
1256 and 1084	$bpg + NADH + H^+ + X_n \rightarrow X_n + 2 P_i + NAD^+$
1002 and 1109	$glycine + succinyl-CoA + ATP + X_n \rightarrow X_n + AMP + P_{ii} + CO_2 + H_2O + CoA$
1062	$glucose\ 6-phosphate + X_n \rightarrow X_n + P_i + H^+$

Addition of glycerate

Tailoring by addition of glycerate from 1,3-biphosphoglycerate was identified from the presence of the smCOGs 1256 (FkbH like protein) and 1084 (3-oxoacyl-(acyl carrier protein) synthase III). Across all BGCs in MIBiG, this heuristic provides a correct tailoring reaction in 10 out of 11 cases (Table S2). The single error derives from BGC0000082 which still incorporates a glycerate unit, but lacking the smCOG 1084 annotation.

Incorporation of 2-Amino-3-hydroxycyclopent-2-enone

The tailoring reaction that incorporate 2-Amino-3-hydroxycyclopent-2-enone synthesized from succinyl-CoA and glycine [12], was identified from the

Table S2: Listing of BGCs in MIBiG used to determine when the addition of glycerate is used to tailor the polyketide or peptide. These are all BGCs in MIBiG containing the FkbH domain and adds glycerate. Of these 11 BGCs, 10 are annotated with the smCOG 1084.

MIBiG ID	Product	1084 present	Reference
BGC0000001	Abyssomycin	Yes	[3]
BGC0000036	Chlorothricin	Yes	[4]
BGC0000082	Kijanimicin	No	[5]
BGC0000133	Quartromicin	Yes	[6]
BGC0000140	4H3H2HMe2HF5	Yes	[6]
BGC0000162	Tetrocarcin	Yes	[7]
BGC0000164	Tetronomycin	Yes	[8]
BGC0001004	Lobophorin B	Yes	[9]
BGC0001183	Lobophorin A	Yes	[9]
BGC0001204	Versipelostatin	Yes	[10]
BGC0001288	Maklamicin	Yes	[11]

presence of the smCOGs of 1109 (8-amino-7-oxononanoate synthase) and a neighboring 1002 (AMP-dependent ligase and synthetase). This pair of smCOG annotations were present in 8 BGCs in MIBiG, of which 5 features a tailoring reaction that incorporates 2-Amino-3-hydroxycyclopent-2-enone (Table S3). For one of the BGCs (BGC0001420) the available literature was not sufficient to make a clear decision about the compound tailoring. The remaining two BGCs (BGC0000091 and BGC0001063) feature a similar tailoring reaction where the succinyl-CoA precursor is replaced by several malonyl-CoA units [13, 14].

Glycosylation

Glycosylation of the peptide or polyketide is identified by the presence of smCOG 1062 (glycosyltransferase), and as one might expect the number of incorporated sugar monomers increase with increasing number of smCOG 1062 annotations (Table S4). Based on 40 BGC synthesis pathways that incorporate sugar monomers (we consider Komodoquinone B as an outlier), we find a significant correlation between the number of incorporated sugar monomers and the number of smCOG 1062 Pearson $\rho = 0.78$, $P = 3e - 9$. Based on this result the BiGMeC pipeline therefore assumes that all glycosyltransferases are active, i.e. a 1 to 1 relationship between the number of smCOG 1062 and active glycosyltransferase tailoring reactions.

Table S3: Listing of BGCs in MIBiG used to determine when the peptide or polyketide is tailored by the incorporation of 2-Amino-3-hydroxycyclopent-2-enone synthesized from succinyl-CoA and glycine [12]. All of these BGCs are annotated with the smCOG 1002 and 1109 on adjacent genes. The "Correct" column indicate if these synthesis of the molecules associated with each BGC incorporates 2-Amino-3-hydroxycyclopent-2-enone.

MIBiG ID	Product	Correct	Reference
BGC0000028	Bafilomycin	Yes	[15]
BGC0000091	Marineosin	No	[13]
BGC0000187	Asukamycin	Yes	[16]
BGC0000213	Colabomycin	Yes	[17]
BGC0001063	Undecylprodigiosin	No	[14]
BGC0001298	Annimycin	Yes	[18]
BGC0001420	Myxochromide	Unknown	[19]
BGC0001740	Phthoxazolin	Yes	[20]

Table S4: Listing of BGCs in MIBiG that incorporates sugar monomers by glycosyltransferase in one of the tailoring steps. The ”# in BGC” and ”# in pathway” columns display the number of smCOG 1062 annotations in the BGC and the number of active glycosyltransferases in the synthesis of the associated compound, respectively.

MIBiG ID	Product	# in BGC	# in pathway	Reference
BGC0000002	Aculeximycin	5	8	[21]
BGC0000021	Apoptolidin	3	2	[22]
BGC0000033	Calicheamicin	4	4	[23]
BGC0000034	Candicidin	1	1	[24]
BGC0000035	Chalcomycin	2	1	[25]
BGC0000036	Chlorothricin	2	2	[4]
BGC0000042	Creminycin	1	1	[26]
BGC0000052	ECO-02301	1	1	[27]
BGC0000054	Erythromycin B	2	2	[28]
BGC0000078	Inecidine	2	3	[29]
BGC0000081	Kedarcidin	2	2	[30]
BGC0000082	Kijanamicin	5	5	[5]
BGC0000085	Lankamycin	2	3	[31]
BGC0000092	Megalomicins	3	3	[32]
BGC0000096	Midecamycin	2	1	[33]
BGC0000102	Mycinamicin II	2	1	[34]
BGC0000105	Nanchangmycin	1	1	[35]
BGC0000108	Natamycin	1	1	[36]
BGC0000115	Nystatin A1	1	1	[37]
BGC0000136	Rifamycin	1	1	[38]
BGC0000141	Rubradirin	1	2	[39]
BGC0000148	A83543A	2	2	[40]
BGC0000151	Stambomycin A	1	1	[41]
BGC0000162	Tetrocarcin A	5	4	[7]
BGC0000165	Tiacumicin B	2	2	[42]
BGC0000167	Vicenistatin	1	1	[43]
BGC0000197	Aranciamycin	1	1	[44]
BGC0000198	Arenimycin A/B/C	2	2	[45]
BGC0000199	ArimetamycinA	2	3	[46]
BGC0000199	ArimetamycinB	1	3	[46]
BGC0000199	ArimetamycinC	1	3	[46]
BGC0000200	Arixanthomycin A	1	2	[47]
BGC0000203	BE-7585A	3	1	[48]
BGC0000208	Chelocardin	0	1	[49]
BGC0000210	Chromomycin A3	5	4	[50]
BGC0001183	Lobophorin	3	4	[9]
BGC0001452	Sipanmycin	2	4	[51]
BGC0001522	Auroramycin	2	4	[52]
BGC0001619	Ibomycin	6	7	[53]
BGC0001851	Komodoquinone B	0	5	[54]
BGC0002033	Spiramycin	3	4	[55]

Rare extender units

The smCOG gene annotations were also leveraged to determine if the enzymes responsible for the synthesis of the rare polyketide precursor methoxymalonyl-CoA were encoded in the BGC. This synthesis pathway was identified by the presence of smCOG 1256 (FkbH like protein) and smCOG 1095 (3-hydroxybutyryl-CoA dehydrogenase), see Table S5. This heuristic predicts the correct extender unit in 16 of the 24 relevant BGCs. The relevant BGC are selected from the MIBiG database based those that contains a gene with the smCOG 1245 and incorporates one or more rare extender unit. Seven of the 8 incorrect predictions are due to BGCs incorporating hydroxymalonyl-ACP and not methoxymalonyl-ACP, and the last error derives from BGC0000090 which incorporates methoxymalonyl-ACP, but lacks the smCOG 1095 annotation. Because we are currently not able to discriminate the synthesis of hydroxymalonyl-CoA from the synthesis of methoxymalonyl-ACP based on smCOG annotations, the BiGMeC pipeline assumes that the rare extender unit is methoxymalonyl-ACP. While this determines the incorporation of the reactions synthesising methoxymalonyl-ACP [56], the incorporation of methoxymalonyl-ACP as an extender unit only occurs if this requirement is fulfilled and methoxymalonyl-ACP is the extender unit as suggested by antiSMASH [57].

Table S5: List of BGCs in MIBiG that contain a gene with the smCOG 1245 annotation and incorporates a rare extender unit.

MIBiG ID	Product	Substrate	1095 present	Reference
BGC0000020	Actinosynnema	Hydroxymalonyl-ACP	Yes	[58]
BGC0000021	Apoptolidin	Methoxymalonyl-ACP	Yes	[22]
BGC0000028	Bafilomycin	Methoxymalonyl-ACP	Yes	[15]
BGC0000040	Concanamycin A	Methoxymalonyl-ACP	Yes	[59]
BGC0000065	Rustmicin	Methoxymalonyl-ACP	Yes	[60]
BGC0000066	Geldanamycin	Methoxymalonyl-ACP	Yes	[61]
BGC0000074	Herbimycin A	Methoxymalonyl-ACP	Yes	[38]
BGC0000078	Incednine	Methoxymalonyl-ACP	Yes	[29]
BGC0000090	Macbecin	Methoxymalonyl-ACP	No	[62]
BGC0000096	Midecamycin	Methoxymalonyl-ACP	Yes	[33]
BGC0000159	Tautomycin	Methoxymalonyl-ACP	Yes	[63]
BGC0000970	Chondrochloren A	Methoxymalonyl-ACP	Yes	[64]
BGC0001034	Pellasoren	Methoxymalonyl-ACP	Yes	[65]
BGC0001054	Xenocoumacin	Hydroxymalonyl-ACP	Yes	[66]
BGC0001059	Zwittermycin A	Hydroxymalonyl-ACP	Yes	[67]
BGC0001106	Oxazolomycin B	Methoxymalonyl-ACP	Yes	[68]
BGC0001348	JBIR-100	Methoxymalonyl-ACP	Yes	[69]
BGC0001511	Ansamitocin P-3	Methoxymalonyl-ACP	Yes	[70]
BGC0001537	Butyrolactol A	Hydroxymalonyl-ACP	Yes	[71]
BGC0001902	Bengamide	Hydroxymalonyl-ACP	Yes	[72]
BGC0001956	Miharamycin A	Hydroxymalonyl-ACP	Yes	[73]
BGC0001957	Amipurimycin	Hydroxymalonyl-ACP	Yes	[73]
BGC0002011	Ansacarbamitocin A	Methoxymalonyl-ACP	Yes	[74]
BGC0002033	Spiramycin	Methoxymalonyl-ACP	Yes	[55]

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