

Supplementary material

# Biosynthetic Gene Clusters and Liquid Chromatography Coupled to Mass Spectrometry Analysis of Aryl Polyene Pigments from *Chryseobacterium* sp. kr6 and *Lysobacter* sp. A03

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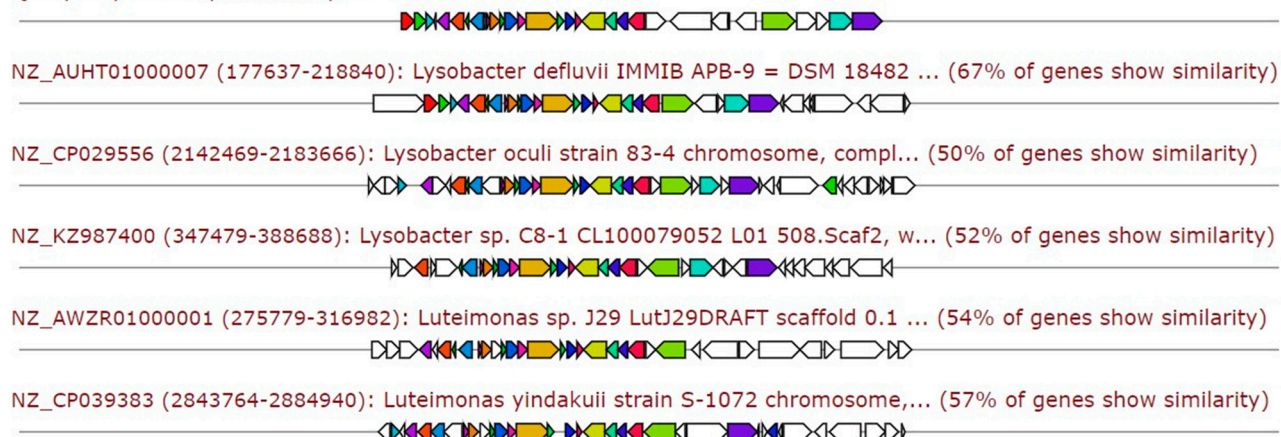
Supplementary Figure S1



**Figure S1.** Gene clusters for biosynthesis of aryl polyenes (APE) and resorcinol, retrieved from antiSMASH 7, as secondary metabolites, found in the genome of *Chryseobacterium* sp. kr6 (**A**) and *Lysobacter* sp. A03 (**B**).

## Supplementary Figure S2

Query sequence: *Lysobacter* sp. A03



**Figure S2.** APE gene cluster of *Lysobacter* sp. A03 and ClusterBlast showing the regions from the antiSMASH database that are similar to the selected region. Genes marked with the same color are interrelated, colorless genes have no relationship.

**A**

Predicted Secondary structure  
Query Sequence  
Template Sequence  
Template Known Secondary structure  
Template Predicted Secondary structure

3 10 20 30 40 50 60 63 70 80 90 100 110 120

Predicted Secondary structure  
Query Sequence  
Template Sequence  
Template Known Secondary structure  
Template Predicted Secondary structure

63 70 80 90 100 110 120

Predicted Secondary structure  
Query Sequence  
Template Sequence  
Template Known Secondary structure  
Template Predicted Secondary structure

123 130 140 150 160

**B**

FabA\_A03  
FabZ\_Kr6

3 10 20 30 40 50 60 63 70 80 90 100 110 120

**C**

FabZ\_A03  
FabZ\_Kr6

3 10 20 30 40 50 60 63 70 80 90 100 110 120

**Figure S3.** (A) Alignment between the *Lysobacter* A03 FabA sequences with that of *P. aeruginosa*. Alpha-helices are represented by green spirals and beta-sheets are represented by blue arrows. (B) Alignment between *Lysobacter* A03 FabA and *Chryseobacterium* kr6 FabZ sequences. Asterisks over residues indicate points of correspondence between sequences. (C) Alignment between the sequences of the FabZ of *Lysobacter* A03 and the FabZ of *Chryseobacterium* kr6. Asterisks over residues indicate points of correspondence between the sequences.

**Supplementary Table S1**
**Table S1.** Putative proteins encoded in bacterial APE BGCs of strains kr6, A03 and other relevant strains.

APE gene	<i>Chryseobacterium</i> sp. kr6 (This study)	<i>Lysobacter</i> sp. A03 (This study)	<i>Xenorhabdus doucetiae</i> DSM 17909T [14]	<i>E. coli</i> CFT073 [12]	<i>Cytophaga hutchinsonii</i> ATCC 33406 [7]
A	AMP binding enzyme (phenylacetate CoA ligase family)	Acyl carrier protein (ACP1)	Methyltransferase	Lipoprotein	Predicted exporter contains acyl/methyltransferase domains
B	Hypothetical protein	Beta-ketoacyl synthase	Beta-ketoacyl synthase CLF	SAM dependent methyltransferase	Glycosyl transferase
C	Tryptophan 7-halogenase	(3R)-hydroxymyristoyl ACP dehydratase FabZ	Acyl transferase	3-ketoacyl ACP synthase	Dehydratase
D	Histidine ammonia lyase	Lysophospholipid acyltransferase	Acyl carrier protein (85 aminoacids)	Lysophospholipid acyl transferase	Lipoprotein carrier protein
E	3-oxoacyl ACP reductase FabG	LolA-related protein	Acyl carrier protein (83 aminoacids)	Acyl carrier protein	Polysaccharide deacetylase
F	Beta-ketoacyl ACP synthase family protein	MMPL family transporter	Acyl CoA synthetase	Acyl carrier protein	3-oxoacyl ACP synthase
G	Acyl carrier protein	Phosphotransferase	Hydroxymyristol ACP dehydratase	COG 4648 membrane protein	Acyl carrier protein
H	Lipid A biosynthesis acyltransferase	3-hydroxydecanoyl ACP dehydratase	Glycosyl transferase	Acyl ACP synthetase	Thioester super family protein
I	NAD(P)/FAD-dependent oxidoreductase	3-oxoacyl ACP reductase FabG	Acyl CoA thioesterase	3-hydroxyacyl ACP dehydratase	Methyltransferase
J	C45 family autoproteolytic acyltransferase/hydrolase	Hypothetical protein	LolA carrier protein	Acyltransferase	Lipid biosynthesis acyltransferase
K	Hypothetical protein	Tryptophan 7-halogenase	MMPL Family transporter	Thioesterase	Short chain dehydrogenase/reductase

L	Alpha/beta fold hydrolase	Glycosyl transferase 2	DUF 3261 domain containing protein	Outer membrane lipoprotein carrier protein LolA	Histidine ammonia lyase
M	T9SS-dependent M36 family metallopeptidase	Beta-ketoacyl synthase chain length factor	Beta-ketoacyl ACP synthase	Transporter	Acyl CoA ligase
N	DUF2062 domain-containing protein	3-oxoacyl ACP synthase FabV like	Beta-hydroxy ACP dehydratase FabA-Z	Duf 3261 membrane protein	Holo-ACP synthase
O	Hypothetical protein		3-ketoacyl ACP reductase FabG2	Beta-ketoacyl ACP synthase	Hydrolase superfamily
P	3-hydroxyacyl ACP dehydratase		3-oxoacyl ACP synthase	Beta-hydroxy ACP dehydratase	Reductase
Q	Hypothetical protein		Beta-ketoacyl ACP synthase	3-oxoacyl ACP reductase	Tryptophan halogenase
R	Outer membrane lipoprotein carrier protein LolA				DAR aromatase dialkylrecorsinol condensing protein
S	Polysaccharide deacetylase family protein				
T	Beta-ketoacyl synthase N-terminal- like domain-containing protein CLF				
U	Beta-ketoacyl ACP synthase family protein				
V	Acyl carrier protein				
W	3-oxoacyl-ACP synthase				
X	Beta-ketoacyl synthase N-terminal-like domain-containing protein				
Y	4- hydroxybenzoyl CoA thioesterase				

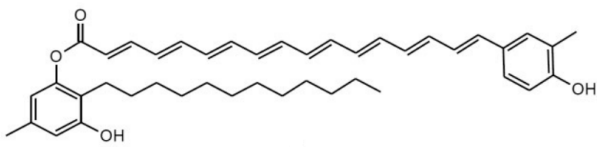
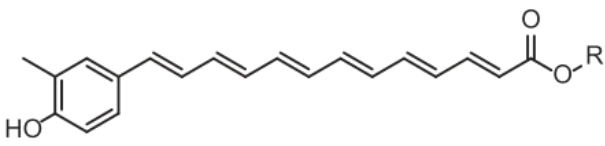
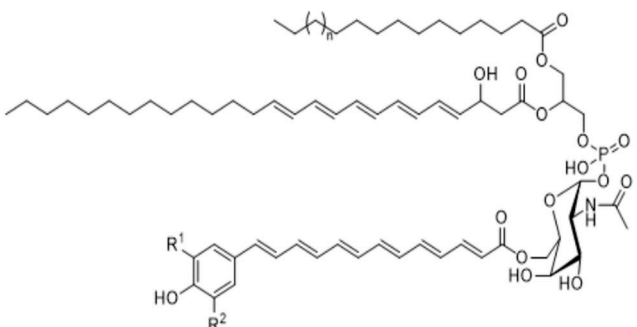
Z	ABC transporter permease				
Aa	ABC transporter ATP-binding protein				
Bb	BtrH N-terminal domain-containing protein				
Cc	Hypothetical protein				
Dd	Insulinase family protein				
Ee	LysM peptidoglycan-binding domain-containing protein				
Ff	ABC transporter permease				
Gg	Hypothetical protein				
Hh	Beta-ketoacyl ACP synthase III				
Ii	Dialkylresorcinol condensing enzyme DarA				
Jj	Glycerol acyltransferase				
Kk	Hypothetical protein				
Ll	EpsG family protein				
Mm	T9SS type A sorting domain-containing protein				
Nn	Type I DNA topoisomerase				
Oo	Formimidoylglutamase				
Pp	Glycosyltransferase 2				
Qq	MraY family glycosyltransferase				
Rr	Polysaccharide biosynthesis/export family protein				



## Supplementary Table S2

Table S2. Proposed chemical structures of aryl polyene pigments derived from different bacteria.

Pigment description <sup>1</sup>	Chemical structure
Xanthomonadin, Xanthomonadaceae family. Derivatives with different levels of bromine, methyl and chain length are described [1,2]	
Esters I and II of the xanthomonadins from <i>Xanthomonas campestris</i> ATCC 11329 [3]. R = H or Br; X = -OH or -O-CH <sub>3</sub>	
Hybrid xanthomonadin DAR pigment named arcuflavin from <i>Azoarcus</i> sp. BH72 [4]	
Flexirubin from <i>Flavobacterium johnsoniae</i> [5]	
Flexurubin from <i>Chryseobacterium</i> sp. UTM-3T [6]	
Flexirubins from <i>Cytophaga hutchinsonii</i> . [7]. R1 and R2 in ring B indicate alkyl chains with different lengths and structures; R3 in ring A indicates H or CH <sub>3</sub> ; n indicates the length of polyene units (6 to 8).	

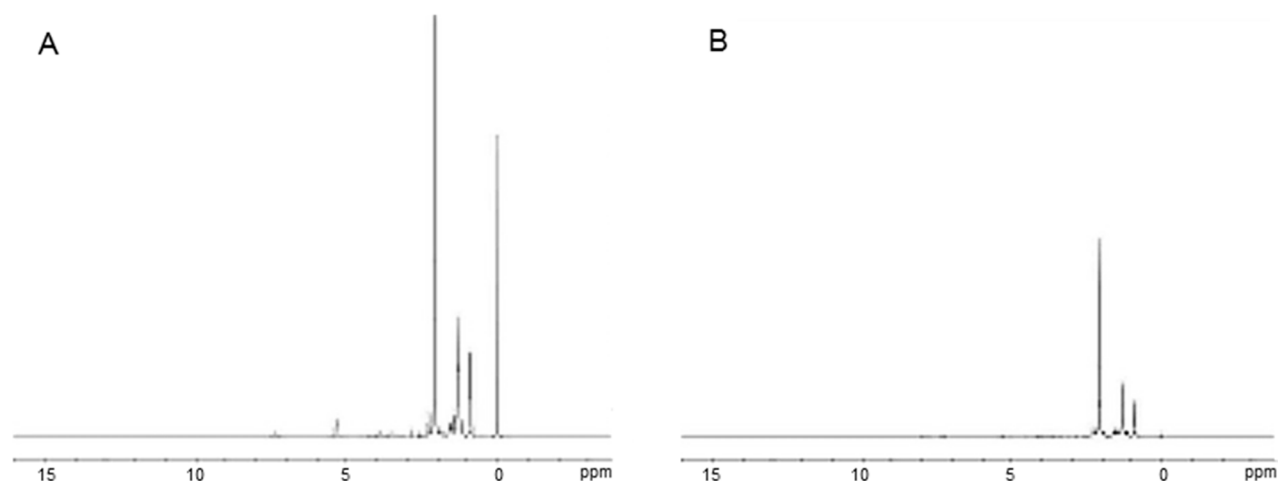
Flexirubin from <i>Chitinophaga filiformes</i> and <i>Chitinophaga pinensis</i> [8]	
Aryl polyene APEEc from uropathogenic <i>E. coli</i> [9]	
Aryl polyene APELs from <i>X. doucetiae</i> [10]	

<sup>1</sup> References:

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**Supplementary Figure S4**



**Supplementary Figure S4.** <sup>1</sup>H-NMR spectra of the pigments extracted from (A) *Lysobacter* A03 and (B) *Chryseobacterium* kr6. The dried pigment extracts were solubilized in deuterated acetone, and the analysis was carried out on a Bruker Avance III 400 MHz instrument (Bruker Daltonics, Billerica, MA, USA) using one-dimensional <sup>1</sup>H-NMR spectroscopy.