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Characterization of Novel Bacterial Species Identified by Undergraduate Students in a General Microbiology Course.

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Abstract

Background: While surveying the microbial diversity in local freshwater creeks as part of an Undergraduate Microbiology course, the 16S rRNA sequences several isolates suggested that they may be novel species within the genera *Chryseobacterium*, *Kaistella*, *Comamonas*, and *Micrococcus*.

Methods: Subsequent polyphasic characterization and comparison to related Type species included morphological studies, Biolog GenIII Microplate analysis, API test panels, Fatty Acid Methyl Ester (FAME) analysis, a variety of differential and selective media, and multi-locus sequence analysis.

Results: The phenotypes and protein coding sequences of *Chryseobacterium* sp. BLS98 were sufficiently similar to *Chryseobacterium oranimense* that DNA/DNA hybridization or whole genome sequencing may be required to conclusively demonstrate its novelty. Strain KM showed clear phenotypic and genomic differences from its closest relatives, *Chryseobacterium luteum*, C. *shigense* and C. *vrystaatense.* Characteristics of strain JJC are very distinct from most Chryseobacteria but cluster with *Chryseobacterium* (formerly *Kaistella*) *koreensis* and *Chryseobacterium haifense*. The fatty acid composition and sequences of strain SJM are significantly different from its closest relatives, *Comamonas aquatica*, *C. kerstersii*, *C. testosteroni*, and *C. terrigena*. Strains LYO1 and LYE1 appear to be atypically pigmented members of the species *Micrococcus luteus*, but will require DNA-DNA hybridization or genomic sequence to draw conclusions.

Conclusions: Several strains are clearly distinct and the names *Chryseobacterium angstadti* KM, *Kaistella zaccaria* JJC, and *Comamonas franzi* SJM are proposed.

Methods

- **Environmental unknowns cultured & characterized in Microbiology course**
- **Colony PCR of 16S rDNA with primers 27f & 1492r, 1 Sanger seq reaction**
- **Compare sequence to validly published type strains (Eztaxon.org)**

<99.0% identical

>99.0% identical

Species identification assigned

- **Fully sequence both strands of nearly complete 16S rDNA**
  - **Submit Sequence to GenBank**
  - **Clustal W Alignment, Neighbor Joining Tree to infer phylogenetic relationships**
- **Morphological/Metabolic characterization w/standard tests**
  - **Colony morphology, color, Gram stain, wet mount**
  - **Temperature, O_2 , pH, NaCl requirements**
  - **Carbohydrate & Nitrogen metabolism**
  - **Exoenzymes, differential and selective medium**
- **API Test Strips (50 CH, 20E/NE, ZYM)**
- **Fatty Acid Methyl Ester (FAME) Analysis (MIDI)**
- **Biolog GenIII metabolic profile**
- **Family-specific tests such as Multi Locus Sequence Analysis, pigment analysis, respiratory quinones, polar lipids, cell wall amino acids.**
- **Deposit Strains in Culture Collections (e.g. ATCC, DSMZ, CCUG, JCM, ARS/NRRL)**

Publish new species in IJSEM

Comamonas franzi SJM

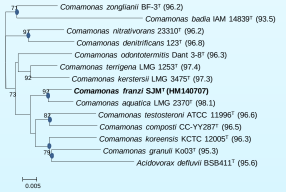
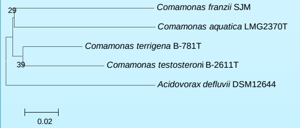
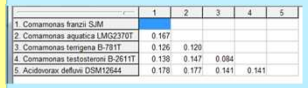


Fig C1. Neighbor Joining Tree to Illustrate Phylogenetic Relationships based on 16S rRNA Sequence. Numbers in parentheses correspond to pairwise similarity to *C. franzi.*



<u>Figure & Table C1</u>. Estimates of Evolutionary Divergence between *Comamonas recA* Sequences.
Neighbor-Joining tree (above) and distance table (below) based on the number of base substitutions per site from analysis between sequences is shown . All results are based on the pairwise analysis of 5 sequences. Analyses were conducted using the Maximum Composite Likelihood method in MEGA4 [1, 2]. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 444 positions in the final dataset.



	1	2	3	4	5
1. Comamonas franzii SJM					
2. Comamonas aquatica LMG2370T	0.167				
3. Comamonas terrigena B-781T	0.126	0.120			
4. Comamonas testosteroni B-2611T	0.138	0.147	0.084		
5. Acidovorax defluvii DSM12644	0.178	0.177	0.141	0.141	

abbrev	Cfran	Caqu	Cterr	Ckers	Cnit	Ctest
strain	SJM	LMG 2370T	B-781T	LMG 3475T	23310	B-2611T
<-- test Genus species	Comamonas franzi	Comamonas aquatica	Comamonas terrigena	Comamonas kerstersii	Comamonas nitrativorans	Comamonas testosteroni
pH 5	-	+	-	-	-	-
1% NaCl	w	+	+	+	+	+
D-fucose	-	+	-	-	-	-
L-fucose	-	+	-	-	-	-
1% Na-lactate	-	+	+	+	+	+
glycerol	-	-	+	-	-	-
D-aspartic acid	-	+	+	-	-	+
troleandomycin	-	-	+	-	-	+
L-alanine	-	-	+	-	-	-
L-arginine	-	+	-	-	-	-
L-aspartic acid	w	+	-	+	w	+
L-histidine	-	-	-	-	-	+
L-pyroglutamic acid	-	+	+	+	-	+
L-serine	-	-	+	-	-	-
lincomycin	-	+	+	+	+	+
guanidine HCl	-	+	-	+	+	w
D-gluconic acid	w	+	+	-	-	+
mucic acid	-	-	-	-	-	+
D-saccharic acid	-	-	+	-	-	+
vancomycin	+	+	+	+	+	+
tetrazolium violet	-	-	+	-	w	+
p-hydroxy-phenylacetic acid	-	-	-	-	w	+
α-keto-glutaric acid	+	+	+	+	-	+
D-malic acid	+	+	+	+	-	+
L-malic acid	+	+	-	+	+	+
bromo-succinic acid	+	+	-	+	-	+
LiCl	-	+	-	+	-	w
tween-40	-	w	-	-	-	+
α-hydroxy-butyric acid	+	+	+	+	-	+
acetoacetic acid	-	+	-	w	w	-
propionic acid	+	+	+	+	-	+
acetic acid	+	+	+	+	-	+
formic acid	-	w	+	+	-	+
aztreonam	w	+	+	+	-	w
Na-butyrate	-	w	-	+	w	+
C8 esterase lipase	w	+	-	+	w	+
valine arylamidase	-	-	-	-	+	-
acid phosphatase	-	w	-	-	-	w
α-mannosidase	-	-	-	w	w	-
nitrate reduction	+	+	+	+	denit	+
indole production	-	+	-	+	+	+
gluconate assimilation	-	-	-	-	-	+
caprate assimilation	-	-	-	-	-	+
phenylacetate assim	-	-	-	-	+	-
44C	-	+	-	+	nd	-
EG minimal medium	-	-	-	-	nd	+

Table C3. Differential Characteristics among *Comamonas* species.
nd = not determined, w = weak,
+ = positive response, - = negative response

Table C2. Cellular fatty acid profiles (%) of strain SJM^T and most closely related Comamonas species grown on TSBA at 30˚C for 24 hrs. Strains: 1, SJM^T; 2, Comamonas aquatica LMG2370^T; 3, Comamonas terrigena B781^T; 4, Comamonas kerstersi LMG3475^T; 5, Comamonas nitrativorans, St23310^T; 6, Comamonas testosteroni B2611^T. tr = trace (<1%). Fatty acids amounting to <1% of the total fatty acids in the six strains are not shown. All data from this study.

Fatty acid	1	2	3	4	5	6
10:0 3OH	3.5	4.4	4.7	4.1	4.7	4.1
12:0	3.0	3.3	3.3	3.1	3.0	2.7
14:0	4.6	3.9	4.3	3.3	3.7	tr
SF3 - 16:1 w6c/w7c	15.7	34.9	37.5	23.5	45.3	33.9
16:0	29.7	27.4	30.5	25.2	24.2	32.2
17:0 cyclo	28.2	4.4	7.9	6.5	tr	6.2
SF8 18:1 w6c/w7c	13.6	19.4	10.2	31.8	18.6	14.0

Conclusions

Strain SJM is sufficiently distinct at the phenotypic and molecular levels to merit description as a novel species. The name *Comamonas franzi* sp. nov will be proposed.

3 *Flavobacteriaceae*

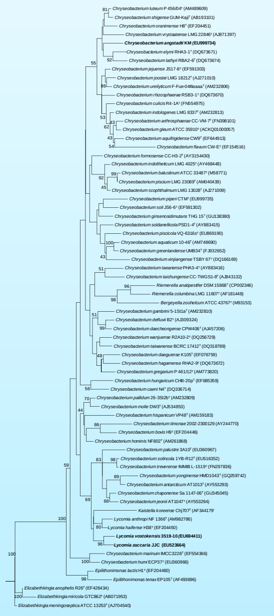


Fig F1. Neighbor Joining Tree to Illustrate Phylogenetic Relationships based on 16S rRNA Sequence.

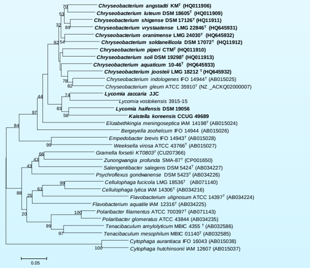


Fig F2. Neighbor Joining Tree to Illustrate Phylogenetic Relationships based on gyrB Sequence.
An ~700 bp fragment of the gyrB gene was amplified by PCR, sequenced by Beckman Coulter Genomics,

TSA for 36 hrs @ 30°C
C = *Chryseobacterium angstadti* KM^T
h = *Chryseobacterium aquaticum* KCTC 12483^T
r = *Chryseobacterium greenlandense* UMB 34^T
y = *Chryseobacterium jejuense* DSM 19399^T
s = *Chryseobacterium joosteii* DSM 18212^T
e = *Chryseobacterium luteum* DSM 18606^T
o = *Chryseobacterium oranimense* DSM 24030^T
b = *Chryseobacterium piperi* CTM^T
a = *Chryseobacterium shigense* DSM 17126^T
c = *Chryseobacterium soldanellicola* DSM 17072^T
t = *Chryseobacterium soli* DSM 19298^T
e = *Chryseobacterium vrystaatense* LMG 22846^T

After spraying with 40% KOH solution
red color = + for flexirubins

Ly = *Lycomia haifensis* DSM 19056^T
co = *Lycomia vostokensis* 3915-10^T
mia = *Lycomia zaccaria* JJC^T

Kaistella = *Kaistella koreensis* CCUG 49689^T

Fig F3. Variation in Pigment Production

Table F1. Fatty Acid Composition of *Chryseobacterium* species and related genera.
Note high percentage of 15:0 anteiso among non-*Chryseobacterium* strains

	strain	KM	DSM 18605	DSM 17126	LMG 22846	LMG 24030	BLS98	DSM 19399	LMG 18212	CTM	DSM 17072	DSM 19298	KCTC 12483	UMB 34	CCUG 49689	DSM 19056	3915-10	JJC
location/ form	<-- test Genus species	Chryseobacterium angstadti	Chryseobacterium luteum	Chryseobacterium shigense	Chryseobacterium vrystaatense	Chryseobacterium oranimense	Chryseobacterium diehli	Chryseobacterium jejuense	Chryseobacterium joostei	Chryseobacterium piperi	Chryseobacterium soldanellicola	Chryseobacterium soli	Chryseobacterium aquaticum	Chryseobacterium greenlandense	Kaistella koreensis	Lycomia haifense	Lycomia vostokensis	Lycomia zaccaria
ECL	fatty acid																	
12.623	13:0 iso	1.2	1.8	1.5	1.1	0.9	0.9	1.3	1.0	1.1	1.0	1.1	4.4	6.3	3.4	2.2	1.1	2.6
12.718	13:0 anteiso	0.3													0.4	0.2	0.3	0.3
12.957	13:1 at 12-13							0.2					2.2	1.2				
13.616	14:0 iso			1.6	1.2										2.8		1.0	
14.000	14:0		0.4	0.3	0.3	0.4	0.3	0.4	0.3	0.3	0.5	0.3	1.9	1.1	0.3	0.3	0.1	0.2
14.632	15:0 iso	43.9	41.8	42.2	39.5	38.0	39.1	37.1	40.5	36.6	43.9	38.1	48.2	51.1	42.8	36.0	23.9	36.0
14.725	15:0 anteiso	0.3	3.6	0.6	0.5	0.6	0.6	0.4	0.5	0.4	1.4	2.9	3.4	5.2	18.8	15.4	28.8	13.2
14.935	15:1 w5c				3.3													
15.634	16:0 iso	0.1		0.2	0.3		0.1			0.1	0.2	0.2	0.2	0.3	1.9	0.3	2.9	0.9
15.880	SF3 16:1 w6c/w7c	9.4	5.5	8.5	6.4	11.1	9.3	11.4	8.1	12.6	10.5	12.0	10.7	7.8	2.1	4.8	1.8	2.6
16.001	16:0	1.4	1.6	1.6	1.5	1.8	1.7	1.5	1.3	1.1	1.7	1.6	6.3	3.7	0.7	0.9		
16.163	15:0 iso 3OH	3.1	2.9	2.8	2.8	3.0	3.0	3.3	2.6	4.2	2.5	2.6	1.8	2.1	2.1	3.2	1.0	2.9
16.259	15:0 2OH		0.5		0.2							0.3	0.2	0.2	1.0	0.8	1.4	0.8
16.444	SF9 17:1 iso w9c/ 10 me 16:0	19.8	18.1	18.4	16.8	20.8	19.7	20.2	22.8	22.0	21.0	19.0	6.0	4.8	8.4	21.6	14.0	22.3
16.637	17:0 iso	1.3	0.6	0.9	1.1	1.7	2.0	1.3	1.4	0.6	0.6	1.1	0.8	1.1	0.2	0.1	0.6	0.3
17.173	16:0 iso 3OH	1.1	0.4	0.4	0.6	0.3	0.2	0.8	0.3	0.3	0.9	0.8	1.2	2.0	3.6	1.3	3.5	1.9
17.550	16:0 3OH	0.8	1.0	1.1	1.2	0.9	0.4	1.3	1.0	1.8	1.0	1.2		1.9	0.5	0.1	0.2	0.3
17.798	18:1 w9c	0.4	0.3	0.4	0.6	0.4	0.4	0.4	0.5	0.3	0.3	0.4	0.3	0.3	0.2	0.4	0.3	0.3
18.194	17:0 iso 3OH	15.5	18.7	17.9	22.0	19.6	19.1	19.3	19.0	17.9	13.0	17.0	9.1	9.2	8.2	9.3	10.6	11.5
18.297	17:0 2OH	0.1	1.5	0.3	0.3	0.3	0.3	0.2	0.2	0.2	0.3	1.0		0.4	1.2	1.8	5.9	2.0

strain	KM	DSM 18605	DSM 17126	LMG 22846	LMG 24030	BLS98	DSM 19399	LMG 18212	CTM	DSM 17072	DSM 19298	KCTC 12483	UMB 34	CCUG 49689	DSM 19056	3915-10	JJC
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dextrin	w	-	+	-	-	-	-	w	+	-	+	-	-	w	+	-	+
D-maltose	w	w	+	-	w	+	+	+	+	+	+	+	+	-	+	+	+
D-trehalose	-	w	+	+	+	+	+	-	+	w	+	w	+	-	-	w	-
gentiobiose	w	+	+	+	+	+	+	+	+	+	+	+	+	-	-	w	w
sucrose	-	w	+	-	-	-	-	-	+	-	+	+	+	-	-	-	-
D-raffinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	w	-
D-melibiose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
D-salicin	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
α-D-glucose	-	-	+	+	-	+	+	+	+	-	+	w	+	-	+	w	w
D-mannose	w	-	+	+	+	+	+	+	+	+	+	-	-	-	-	+	-
D-fructose	w	-	+	w	+	+	+	+	+	+	+	+	-	-	+	w	-
L-rhamnose	-	-	-	-	-	-	-	-	w	+	-	+	-	-	-	w	-
1% Na-lactate	+	+	-	w	+	+	+	+	+	w	+	+	+	+	+	-	+
D-serine	-	-	-	-	-	-	+	-	w	-	-	-	-	-	-	-	-
D-mannitol	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	w	-
glycerol	-	-	+	-	-	+	w	w	+	-	+	-	-	-	-	w	-
D-glucose-6-PO4	-	-	-	-	-	w	w	-	+	w	-	-	-	-	+	w	+
D-fructose-6-PO4	-	-	-	-	-	+	+	-	+	w	-	w	-	-	-	+	w
D-aspartic acid	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-
D-serine	-	-	-	-	-	-	w	-	-	-	-	-	-	-	w	-	+
troleandomycin	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
rifamycin SV	-	-	-	+	+	+	+	w	+	+	-	-	-	-	-	-	-
gelatin	w	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
glycyl-L-proline	+	w	+	+	+	+	+	+	+	w	+	+	+	+	+	+	+
L-alanine	-	-	w	-	-	-	+	w	w	-	-	-	-	-	-	w	w
L-arginine	-	-	-	-	-	-	w	-	-	-	-	+	-	-	-	w	w
L-aspartic acid	+	-	+	w	+	+	+	w	+	w	+	+	-	+	+	-	+
L-pyroglutamic acid	-	-	-	-	-	w	w	-	+	-	-	-	-	-	-	w	w
L-serine	w	-	+	+	+	+	+	+	+	-	+	-	-	-	w	w	+
lincomycin	+	-	-	+	+	+	+	+	w	+	-	-	-	-	-	-	-
guanidine HCl	w	-	-	-	w	w	-	w	w	-	-	-	-	-	-	-	-
pectin	-	+	+	-	+	+	w	+	+	w	+	+	+	-	+	w	+
D-galacturonic acid	-	-	+	+	-	w	+	-	+	+	+	+	+	w	w	+	+
D-glucuronic acidi	-	-	-	-	-	w	w	-	+	w	-	-	-	-	w	+	w
glucuronamide	-	-	-	-	-	w	w	-	-	w	-	-	-	w	w	+	w
tetrazolium violet	+	-	+	+	-	-	+	+	+	-	-	+	-	-	-	w	-
tetrazolium blue	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	-	w
methyl pyruvate	-	-	+	+	-	+	-	-	-	-	w	-	-	-	-	-	+
citric acid	w	-	-	-	+	+	+	-	+	-	-	w	-	-	w	-	w
K-tellurite	-	-	-	-	-	-	-	-	w	w	-	+	-	+	+	-	-
tween-40	+	-	+	+	+	+	+	+	+	-	+	+	-	-	+	-	+
acetoacetic acid	+	+	+	+	+	+	+	+	+	-	w	+	+	+	+	+	+
formic acid	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-
aztreonam	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	w	+
Na-butyrate	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
cystine arylamidase	-	w	-	-	-	-	-	-	w	-	-	-	nd	w	+	w	w
trypsin	w	-	w	-	-	-	+	w	-	w	-	w	nd	-	-	-	-
β-galactosidase	-	-	-	-	-	-	-	-	-	-	-	-	nd	-	+	-	-
α-glucosidase	w	+	+	+	+	w	+	w	+	+	+	w	nd	w	w	w	w
β-glucosidase	-	+	+	-	+	-	-	-	-	-	-	-	nd	-	-	-	-
N-acetyl-β-glucosaminidase	+	+	+	w	+	+	w	w	+	+	+	w	nd	-	-	-	-
nitrate reduction	-	-	+	-	-	-	-	-	-	-	-	-	nd	-	-	-	-
indole production	w	-	w	w	+	w	+	-	+	w	w	-	nd	+	+	+	+
urease	-	-	-	+	-	+	+	+	+	+	-	-	nd	-	-	-	-
esculin (β-glucosidase)	w	+	+	w	+	+	+	+	w	+	+	+	nd	w	-	-	+
gelatinase	w	-	+	+	+	+	+	+	+	+	+	+	nd	+	+	w	+
β-galactosidase	+	+	-	-	-	-	-	-	+	-	-	-	nd	-	+	-	-
starch accum	+	+	+	+	-	+	+	+	w	+	+	-	-	nd	-	nd	+
amylase	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+	-
caseinase	+	-	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
DNase	w	+	-	+	w	w	-	w	+	w	+	+	+	nd	-	nd	+
hemolysis	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Tween 80	+	w	+	+	+	+	+	+	+	-	+	w	w	nd	-	nd	-
bile esculin	-	-	-	g+bw	w	-	+	+	+	+	-	-	-	-	-	-	b
Biolog Universal Yeast (BUY)	+	+	+	+	+	+	+	+	+	+	+	w	w	nd	w	nd	w
cetrimide	+	+	+	+	+	+	+	+	+	+	+	w	w	nd	w	nd	w
EMB	+	+	+	+	+	+	+	+	+	+	w	-	-	-	-	-	-
Endo	+	+	+	+	+	+	+	+	+	w	w	-	-	-	-	-	-
amoxicillin (30 μg)	R	R	R	R	R	R	nd	nd	R	nd	nd	nd	nd	S	S	S	I
ampicillin (10 μg)	R	R	R	R	R	R	nd	nd	R	nd	nd	nd	nd	S	S	S	I
carbenicillin (100 μg)	R	R	R	R	R	R	nd	nd	R	nd	nd	nd	nd	S	S	S	I
chloramphenicol (30 μg)	I	I	I	I	I	I	nd	nd	I	nd	nd	nd	nd	S	S	S	S
chlortetracycline (30 μg)	R	R	R	R	I	I	nd	nd	I	nd	nd	nd	nd	I	I	I	S
penicillin (10 units)	R	I	R	R	R	R	nd	nd	R	nd	nd	nd	nd	S	S	S	I
spectinomycin (100 μg)	I	I	I	I	I	S	nd	nd	R	nd	nd	nd	nd	S	S	I	I
tetracycline (30 μg)	R	I	I	R	I	I	nd	nd	I	nd	nd	nd	nd	S	I	I	S

Table F2. Differential Characteristics among *Chryseobacterium* species and related genera.
R = resistant, I = intermediate resistance, S = sensitive, nd = not determined,
w = weak, + = positive response, - = negative response

Conclusions

- **Strain KM is sufficiently distinct at the molecular and phenotypic levels and merits description as a novel species for which the name *Chryseobacterium angstadti* is proposed.**
- **Strain BLS98 is sufficiently similar to Chryseobacterium oranimense that DNA-DNA hybridization will probably be necessary to determine whether it can be described as a novel species.**
- **Strain JJC and several others are sufficiently different from other *Chryseobacterium* strains to merit description as a new genus, and the name *Lycomia zaccaria* gen. nov, sp nov. is proposed.**

Micrococcus strains

Figure M1. Variation in Pigments among Micrococcus strains.
M = *Micrococcus luteus* B-287^T
i = *Micrococcus* sp. AF
c = *Micrococcus* sp. SSX
r = *Micrococcus* sp. TMG
o = *Micrococcus* sp. LYLL
c = *Micrococcus* sp. LYG1
o = *Micrococcus* sp. LYO1
c = *Micrococcus* sp. LYE1
c = *Micrococcus* sp. D7
us = *Micrococcus yunnanensis* DSM 21948^T

test/ characteristic	Micrococcus luteus	Micrococcus sp.	Micrococcus sp.	Micrococcus sp.	Micrococcus sp.	Micrococcus sp.	Micrococcus sp.	Micrococcus sp.	Micrococcus sp.	Micrococcus yunnanensis
strain	B-287	AF	SSX	TMG	LYLL	LYG1	LYO1	LYE1	D7	DSM 21948
11:0_									4.3	
13:0 anteiso		1.1	1.0	1.3	0.6	1.0		1.0		1.1
14:0 iso	1.5	3.0	3.8	2.7	1.7	1.7	1.7	2.8		3.9
14:0_	3.8	0.7	0.5	0.5	0.6	0.7		0.5		1.7
15:0 iso	21.1	14.2	9.6	10.8	18.1	15.3	10.8	8.7	10.0	13.9
15:0 anteiso	49.4	74.1	77.7	72.4	70.2	74.7	80.0	80.1	55.9	69.3
15:0_				4.4		0.5	1.4			
16:1 w7c alcohol									2.5	
16:0 iso	4.7	2.1	2.4	1.8	3.1	1.2	1.5	2.0	2.7	4.7
16:1 w7c/16:1 w6c	2.0								0.5	0.92
16:0_	3.7				0.6					0.84
15:0 iso 3OH									1.5	
15:0 2OH		0.6		0.6					1.4	
17:0 anteiso	8.4	1.2	1.3	1.3	2.5	1.1	1.2	1.3	0.8	2.6
20:1 w7c									1.2	

Table M1. Fatty Acid Composition of *Micrococcus* strains.

test/ characteristic	Micrococcus luteus	Micrococcus sp.	Micrococcus sp.	Micrococcus sp.	Micrococcus sp.	Micrococcus sp.	Micrococcus sp.	Micrococcus sp.	Micrococcus sp.	Micrococcus yunnanensis
strain	B-287	AF	SSX	TMG	LYLL	LYG1	LYO1	LYE1	D7	DSM 21948
neg control	-	-	-	-	-	-	-	-	-	-
dextrin	w	w	+	+	+	+	+	+	+	+
D-maltose	-	-	+	+	+	+	+	+	+	+
D-trehalose	-	-	+	+	+	+	+	+	+	+
D-cellobiose	-	-	+	+	+	+	-	+	w	-
gentiobiose	-	-	-	-	+	+	+	+	-	+
sucrose	-	-	+	+	+	+	+	+	+	+
D-turanose	-	-	+	+	+	+	+	+	+	+
stachyose	-	-	+	-	-	-	-	-	-	-
pos control	+	+	+	+	+	+	+	+	+	+
pH 6	+	+	+	+	+	+	+	+	+	+
pH 5	-	-	-	-	-	-	-	-	-	-
D-raffinose	-	-	+	+	+	+	+	+	+	-
α-D-lactose	-	-	-	-	-	-	-	-	-	-
D-melibiose	-	w	+	+	+	+	+	w	-	-
β-methyl-D-glucoside	-	-	-	-	+	+	-	w	-	-
D-salicin	-	-	-	-	-	+	-	+	-	-
N-acetyl-D-glucosamine	-	-	+	-	-	+	-	-	-	-
N-acetyl-β-D-mannosamine	-	-	+	-	-	+	-	+	-	+
N-acetyl-D-galactosamine	-	-	+	+	+	+	-	+	-	-
N-acetyl neuraminic acid	-	-	+	+	+	+	+	+	-	-
1% NaCl	+	+	+	+	+	+	+	+	+	+
4% NaCl	+	+	+	+	+	+	+	+	+	+
8% NaCl	-	w	w	+	+	+	+	+	+	w
α-D-glucose	-	-	+	+	+	+	+	+	w	+
D-mannose	-	+	+	+	+	+	+	+	+	+
D-fructose	-	-	+	+	+	+	+	+	w	-
D-galactose	-	+	+	+	+	+	+	+	w	-
3-methyl glucose	-	+	+	+	+	+	+	+	+	-
D-fucose	-	+	+	+	+	+	+	+	+	+
L-fucose	-	+	+	+	+	+	+	+	+	+
L-rhamnose	-	+	+	+	+	+	+	+	w	-
inosine	-	-	+	-	-	+	-	-	-	-
1% Na-lactate	-	+	+	+	+	+	+	+	+	-
fusidic acid	-	-	-	-	-	-	-	-	-	-
D-serine	-	w	+	+	+	+	+	+	+	-
D-sorbitol	-	-	-	-	-	-	-	-	w	-
D-mannitol	-	-	+	-	-	+	-	+	+	-
D-arabitol	-	-	+	+	+	+	-	+	w	-
myo-inositol	-	-	+	+	+	+	-	+	w	-
glycerol	-	-	+	+	+	+	+	+	+	+
D-glucose-6-PO4	-	-	+	+	+	+	-	+	+	+
D-fructose-6-PO4	-	+	+	+	+	+	+	+	+	+
D-aspartic acid	-	-	+	+	+	-	-	-	-	+
D-serine	-	-	+	+	+	+	-	+	+	+
troleandomycin	-	-	-	-	-	-	-	-	-	-
rifamycin SV	-	-	-	-	-	-	-	-	-	-
minocycline	-	-	-	-	-	-	-	-	-	-
gelatin	-	-	+	+	+	+	-	+	+	+
glycyl-L-proline	-	-	+	+	+	+	-	w	w	+
L-alanine	-	-	+	+	+	+	-	-	-	+
L-arginine	-	-	+	+	+	+	-	+	w	-
L-aspartic acid	-	-	+	+	+	+	-	+	+	+
L-glutamic acid	-	-	+	+	+	+	-	+	+	+
L-histidine	-	-	+	+	+	+	-	+	+	+
L-pyroglutamic acid	-	-	+	+	+	+	-	+	+	+
L-serine	-	-	+	+	+	+	-	+	+	+
lincomycin	-	-	-	-	-	-	-	-	-	-
guanidine HCl	-	-	-	-	-	-	-	-	-	-
niaproof 4	-	-	-	-	-	-	-	-	-	-
pectin	+	-	+	+	+	+	+	+	w	+
D-galacturonic acid	-	+	+	+	+	+	+	+	+	+
L-galacturonic acid lactone	-	-	+	-	-	-	-	-	-	-
D-gluconic acid	-	-	+	+	+	+	-	w	w	+
D-glucuronic acidi	-	+	+	+	+	+	+	w	w	+
glucuronamide	+	+	+	+	+	-	+	-	w	+
mucic acid	-	-	+	+	+	+	+	+	+	+
quinic acid	-	-	+	+	+	+	-	w	w	+
D-saccharic acid	-	-	+	+	+	+	-	w	w	+
vancomycin	-	-	-	-	-	-	-	-	-	-
tetrazolium violet	-	-	-	-	-	-	-	-	-	-
tetrazolium blue	-	-	-	-	-	-	-	-	-	-
p-hydroxy-phenylacetic acid	-	-	+	+	+	+	+	+	+	+
methyl pyruvate	-	-	-	-	-	-	-	-	w	-
D-lactic acid methyl ester	-	-	+	+	+	+	-	+	+	+
L-lactic acid	-	+	+	+	+	+	+	+	+	+
citric acid	-	-	+	+	+	+	-	-	w	+
α-keto-glutaric acid	-	-	+	+	+	+	-	+	w	-
D-malic acid	-	-	+	+	+	+	-	+	+	+
L-malic acid	-	-	+	+	+	+	-	+	w	-
bromo-succinic acid	-	-	-	-	-	-	-	-	-	-
nalidixic acid	+	+	+	+	+	+	+	+	+	+
LiCl	+	+	+	+	+	+	+	+	+	+
K-tellurite	+	+	+	+	+	+	+	+	+	+
tween-40	-	+	+	+	+	+	-	+	+	+
γ-amino-butyric acid	-	-	+	-	+	+	-	-	w	+
α-hydroxy-butyric acid	-	+	-	+	-	+	-	+	+	+
β-hydroxy-D,L-butyric acid	-	-	+	+	+	+	-	+	+	+
α-keto-butyric acid	-	+	+	+	+	+	+	+	+	+
acetoacetic acid	+	+	+	+	+	+	+	+	+	+
propionic acid	-	+	+	+	+	+	-	+	+	+
acetic acid	-	+	+	+	+	+	-	+	+	+
formic acid	-	-	-	-	+	-	-	-	-	-
aztreonam	+	-	+	+	+	+	+	+	+	-
Na-butyrate	+	+	+	+	+	+	+	+	+	+
Na bromate	+	+	+	+	+	+	+	+	+	+

Table M2. Biolog GenIII response among *Micrococcus* strains
w = weak, + = positive response, - = negative response

Conclusions

- **<1% difference in 16S rRNA separates the 8 strains described here and 6 different type strains.**
- **Protein coding genes must be sequenced to confidently assign these strains to particular species**

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