Plant Pathogenic Bacteria Characterisation & Identification

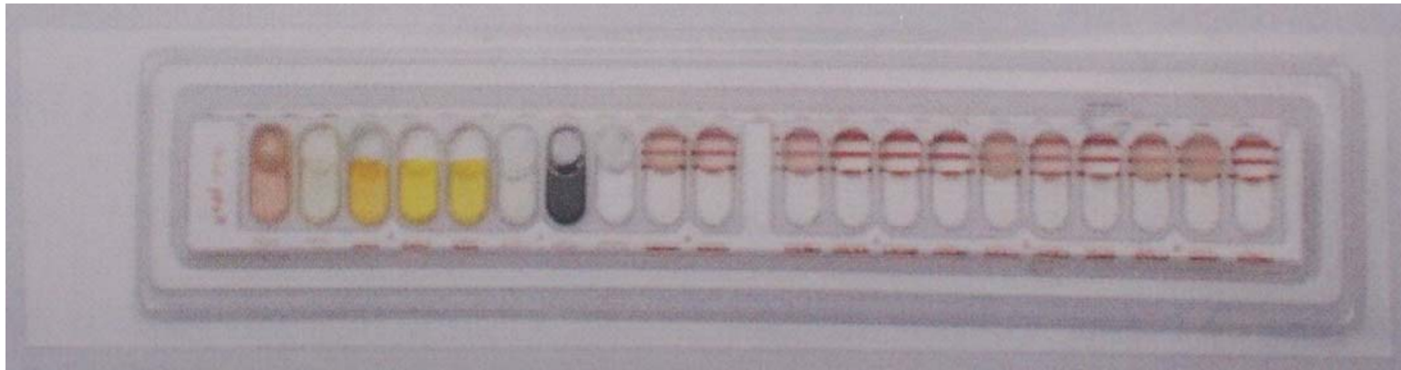
By Julian Smith

Biochemical formatted platforms

Biochemical formatted platforms

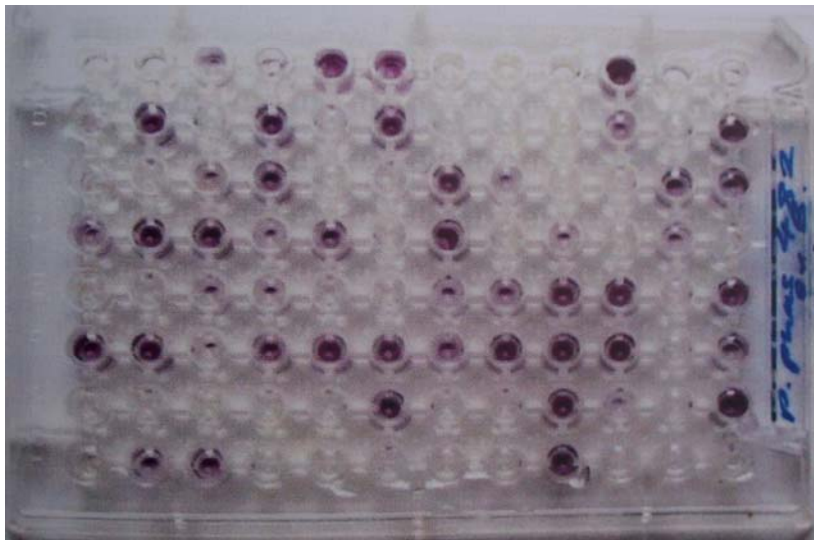
- Takes the biochemical tests and places them on a more convenient format
- Two main commercial products
  - API strips – [http://industry.biomerieux-usa.com/industry/food/api/index.htm]
  - Biolog – [http://www.biolog.com/main.html]
- Results achieved within 48hrs
- Results [+ & -ve data] fed into library of described strains
- Similarity values on most likely identification
- Requires judgement over identifications presented

The API system



- Each well contains a different substrate
- Results are recorded as either a substrate colour change or as growth

The Biolog system



A1 Water	A2 α-cyclodextrin	A3 dextrin	A4 glycogen	A5 tween 40	A6 tween 80	A7 N-acetyl-D-galactosamine	A8 N-acetyl-D-glucosamine	A9 adonitol	A10 L-arabinose	A11 D-arabitol	A12 cellobiose
B1 i-erythritol	B2 D-fructose	B3 L-fucose	B4 D-galactose	B5 gentiobiose	B6 α-D-glucose	B7 m-inositol	B8 α-D-lactose	B9 lactulose	B10 maltose	B11 D-mannitol	B12 D-mannose
C1 D-melibiose	C2 β-methyl D-glucoside	C3 D-psicose	C4 D-raffinose	C5 L-rhamnose	C6 D-sorbitol	C7 sucrose	C8 D-trehalose	C9 turanose	C10 xylitol	C11 methyl pyruvate	C12 mono-methyl succinate
D1 acetic acid	D2 cis-aconitic acid	D3 citric acid	D4 formic acid	D5 D-galactonic acid lactone	D6 D-galacturonic acid	D7 D-gluconic acid	D8 D-glusaminic acid	D9 D-glucuronic acid	D10 α-hydroxy butyric acid	D11 β-hydroxy butyric acid	D12 γ-hydroxy butyric acid
E1 p-hydroxy phenylacetic acid	E2 itaconic acid	E3 α-keto butyric acid	E4 α-keto glutaric acid	E5 α-keto valeric acid	E6 D, L-lactic acid	E7 malonic acid	E8 propionic acid	E9 quinic acid	E10 D-saccharic acid	E11 sebacic acid	E12 succinic acid
F1 bromo succinic acid	F2 succinamic acid	F3 glucunoramide	F4 alaninamide	F5 D-alanine	F6 L-alanine	F7 L-alanyl -glycine	F8 L-asparagine	F9 L-aspartic acid	F10 L-glutamic acid	F11 glycyl L-aspartic acid	F12 glycyl L-glutamic acid
G1 L-histidine	G2 hydroxy L-proline	G3 L-leucine	G4 L-ornithine	G5 L-phenylalanine	G6 L-proline	G7 L-pyroglutamic acid	G8 D-serine	G9 L-serine	G10 L-threonine	G11 D, L-carnitine	G12 γ-amino butyric acid
H1 urocanic acid	H2 inosine	H3 uridine	H4 thymidine	H5 phenyl ethylamine	H6 putrescine	H7 2-amino ethanol	H8 2,3-butanediol	H9 glycerol	H10 D, L-α-glycerol phosphate	H11 glucose-1-phosphate	H12 glucose-6-phosphate

- The Biolog system presents an extended array of biochemical tests
- A positive result is seen as a purple colour change
- The plate can be read by eye or by a plate reader

Biochemical formatted platforms [Biolog]

- Advantages
  - Is not requiring of expensive equipment
  - System is quick, reproducible and easy to perform
  - Data can be shared between laboratories
  - Can provide a reasonable identification to the genus and species level
- Limitations
  - Requires investment [access] to the library
  - Has limitation in resolving below species level [pathovar separation]
  - Library stronger on human microbials than plant pathogenic bacteria

Fatty acid analysis – the Midi system

Fatty acids

- Gram-negatives
  - Unique hydroxy patterns
  - Some cyclopropanes
  - Few branched acids

- Gram positives
  - Many branched acids
  - Very few hydroxy and cyclopropane acids.

The Midi system

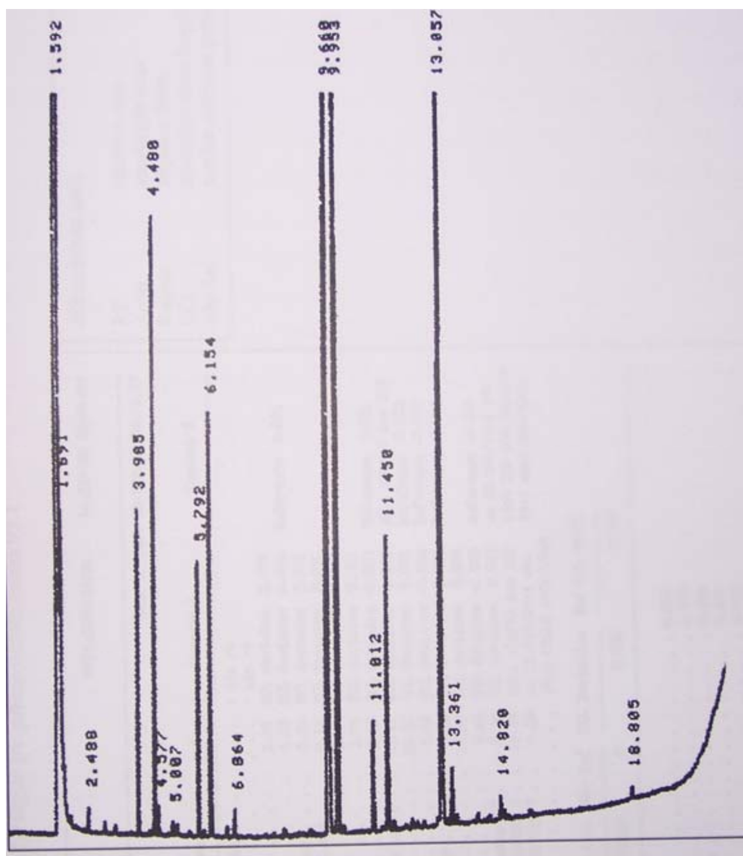
- Commercial and / or lab produced – [http://www.midi-inc.com/]
- Based on comparisons of types and amounts of acids
- Interrogates library for identification
- Typical "return":

NCPPB Rev 3.0	Agrobacterium biovar 1 ………. 0.814
	Agrobacterium biovar 2 ………. 0.567
	Agrobacterium biovar 3 [vitis] … 0.316

Fatty acid extraction process

- Culture Cells [i.e. 24hr on TSBA]
- Harvest Cells
- Saponify Lipids
- Methylate Fatty Acids [FAMEs]
- Extract and Purify
- GC analysis
- Comparison to library

MIDI system print outs



Sherlock Version: 3.10 DATA:E00915545A 16-SEP-00 09:06:00

ID: 2032 NM-P. PHAS. 95.1 Date of run: 16-SEP-00 08:35:27
Bottle: 41 SAMPLE [TSBA40]

RT	Area	Ar/Ht	Respon	ECL	Name	%	Comment 1	Comment 2
1.604	309664512	0.031	. . .	7.021	SOLVENT PEAK	. . .	< min rt	
2.497	656	0.026	. . .	8.942	. . . .	. . .	< min rt	
3.995	10872	0.032	1.053	11.424	10:0 3OH	2.41	ECL deviates 0.002	
4.490	23184	0.035	1.031	12.000	12:0	5.03	ECL deviates 0.000	Reference 0.001
4.587	1112	0.034	1.028	12.090	11:0 ISO 3OH	0.24	ECL deviates 0.001	
5.015	600	0.037	1.015	12.485	unknown 12.484 . . .	0.13	ECL deviates 0.001	
5.803	11480	0.038	0.996	13.177	12:0 2OH	2.40	ECL deviates 0.000	
6.164	18088	0.039	0.989	13.455	12:0 3OH	3.76	ECL deviates 0.001	
6.872	1216	0.038	0.978	14.000	14:0	0.25	ECL deviates -0.000	Reference 0.001
9.670	165296	0.046	0.954	15.819	Sum In Feature 3 . .	33.17	ECL deviates -0.003	16:1 w7c/15 iso 2OH
9.963	141496	0.046	0.953	16.001	16:0	28.35	ECL deviates 0.001	Reference 0.002
11.021	6136	0.049	0.949	16.628	17:0 ISO	1.22	ECL deviates -0.002	Reference -0.001
11.460	16376	0.049	0.948	16.889	17:0 CYCLO	3.26	ECL deviates 0.001	Reference 0.001
13.068	94856	0.050	0.946	17.824	18:1 w7c	18.87	ECL deviates 0.001	
13.372	3184	0.050	0.946	18.000	18:0	0.63	ECL deviates -0.000	Reference -0.001
14.830	1384	0.051	0.945	18.848	Sum In Feature 7 . .	0.28	ECL deviates 0.002	un 18.846/19:1 w6c
*******	165296	. . .	. . .	. . .	SUMMED FEATURE 3 . .	33.17	16:1 w7c/15 iso 2OH	15:0 ISO 2OH/16:1w7c
*******	1384	. . .	. . .	. . .	SUMMED FEATURE 7 . .	0.28	un 18.846/19:1 w6c	19:1 w6c/.846/19cy
*******	. . .	. . .	. . .	. . .	. . . .	. . .	19:0 CYCLO w10c/19w6	

Solvent Ar	Total Area	Named Area	% Named	Total Amnt	Nbr Ref	ECL Deviation	Ref ECL Shift
309664512	495280	495280	100.00	475532	6	0.001	0.001

IMIBAC [Rev 1.0] * NO MATCH *
TSBA40 [Rev 4.10] Pseudomonas 0.899
P. syringae 0.899
P. s. phaseolicola 0.899
P. s. syringae 0.841
P. s. glycinea 0.820

- GC trace [left]
- Library analysis [above]

Key acids from 4 genera

Acid	*Acidovorax*	*Ralstonia*	*Pseudomonas*	*Burkholderia*
10:0 3OH	+		+	+
12:0 2OH			+	
12:0 3OH			+	
14:0 3OH		+	+	+
16:0 2OH		+		+
16:0 3OH			+	+
16:1 2OH		+		
18:1 2OH		+		+

Fatty acid analysis

- Advantages
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DNA approaches

DNA sequencing

- 16s rDNA sequencing
  - One example: 27F and 1492R primers amplification followed by 518F and 800R primers
  - Stringent annealing conditions
  - BLAST search for nearest relatives
  - Assemblage of closest relatives

Sequencing of other genes

- Whilst 16S rDNA is the normal target for sequencing, for some bacteria insufficient variation may be present to allow differentiation below the species level [pathovar level]
- For these bacteria different target sequences can be used which present more variation
  - Examples include:
    - Hrp genes
    - Gyrase gene
    - 16-23S rDNA interspacer region

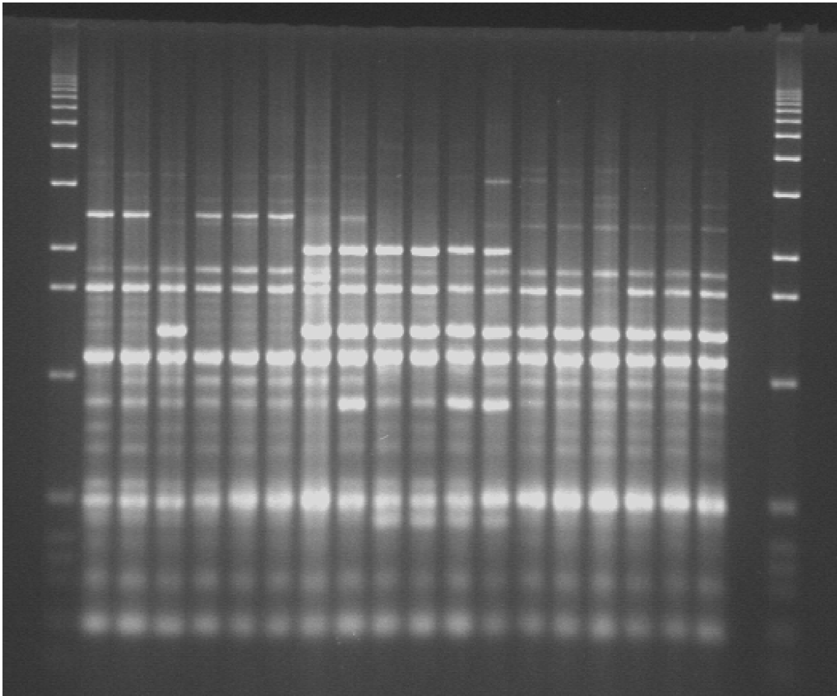
Sequence alignment

0.1

- Gyrase sequence data for *Xanthomonas* sp.
- Sequences increasingly available on GeneBank
- Provides pathovar level differentiation

x-camp pv musacearum W9616
x-camp pv musacearum 392224
x-camp pv musacearum NCPPB2005
x-camp pv musacearum W9615
x-camp pv musacearum W9624
x-vas. pv vascolurum NCPPB889
x-vas. pv vascolurum NCPPB206
x-vas. pv vascolurum NCPPB895
x-vas. pv vascolurum NCPPB702
x-camp pv musacearum W9623
x-camp pv musacearum CABI392966
x-camp pv musacearum 014/LUW/05
x-camp pv musacearum 392223
91%
x-camp pv musacearum KY44
96%
x-vas. pv holcicola NCPPB2417
x-vas. pv holcicola NCPPB1060
x-ory pv oryzae –AY055110
100%
100%
x-camp-vascatoria 85-10
X. axono. pv citri
X. aboricola pv celebensis NCPPB1832
100%
x. camp. pv campestris NCPPB528
x. camp. pv campestris 33913
x. camp. pv campestris 8004
Streptomyces roseochromogenes subs. oscitans

DNA fingerprinting



- By comparing DNA fingerprint of unknown to known strains an identification can be achieved
- Is particularly appropriate for pathovar level identifications
- Require access to known strains [genetic resource collection]

DNA fingerprinting: rep-PCR

REP-PCR

20 40 60 80 100

REP-PCR

Pseudomonas	cichorii		NCPPB 3179	Pepper	.
Pseudomonas	cichorii		NCPPB 1512	Chrysanthemum	.
Pseudomonas	tolaasii		NCPPB 741	Mushroom	.
Pseudomonas	tolaasii		NCPPB 1116	Mushroom	.
Pseudomonas	tolaasii		NCPPB 2194	Mushroom	.
Pseudomonas	tolaasii		NCPPB 2192	Mushroom	.
Pseudomonas	syringae	dendropanacis	NCPPB 3464		.
Pseudomonas	syringae	morsprunorum	NCPPB 2787	Cherry	.
Pseudomonas	syringae	morsprunorum	NCPPB 2787	Cherry	.
Pseudomonas	syringae	morsprunorum	NCPPB 2755	Sour Cherry	.
Pseudomonas	syringae	morsprunorum	NCPPB 560		.
Pseudomonas	syringae	aesculi	NCPPB 3681		.
Pseudomonas	savastanoi	fraxinii	NCPPB 1006	Ash	.
Pseudomonas	savastanoi	fraxinii	NCPPB 3471	Ash	.
Pseudomonas	savastanoi	fraxinii	NCPPB 2716	Ash	.
Pseudomonas	savastanoi	fraxinii	NCPPB 1464	Ash	.
Pseudomonas	cichorii		NCPPB 2474	Mexican Poppy	.
Pseudomonas	cichorii		NCPPB 3466	Chrysanthemum	.
Pseudomonas	syringae		NCPPB 3116	Coriander	.
Pseudomonas	syringae		NCPPB 3319	Coriander	.
Pseudomonas	cichorii		NCPPB 3153	Lettuce	.
Pseudomonas	amygdali		NCPPB 2608	Almond	.
Pseudomonas	amygdali		NCPPB 2607T	Almond	.
Pseudomonas	amygdali		NCPPB 2608		.
Pseudomonas	cichorii		NCPPB 943	Endive	.
Pseudomonas	tremae		NCPPB 3465		.
Pseudomonas	meliae		NCPPB 3033		.
Pseudomonas	syringae	photiniae	NCPPB 3688	Photinia	.
Pseudomonas	syringae	photiniae	NCPPB 3689	Photinia	.
Pseudomonas	cichorii		NCPPB 1513	Celery	.
Pseudomonas	avellanae		NCPPB 3489	Hazel	.
Pseudomonas	avellanae		NCPPB 3488	Hazel	.
Pseudomonas	avellanae		NCPPB 3490	Hazel	.
Pseudomonas	avellanae		NCPPB 3487T	Hazel	.
Pseudomonas	syringae	rhaphiolepidis	NCPPB 3618T	Rhaphiolepis	.
Pseudomonas	savastanoi	savastanoi	NCPPB 639	Olive	.
Pseudomonas	syringae	eriobotryae	NCPPB 2801	Loquat	.
Pseudomonas	savastanoi	savastanoi	NCPPB 3471	Jasmine	.
Pseudomonas	savastanoi	nerii	NCPPB 3278	Oleander	.
Pseudomonas	savastanoi	nerii	NCPPB 640	Oleander	.
Pseudomonas	savastanoi	savastanoi	NCPPB 1506	Olive	.
Pseudomonas	savastanoi	nerii	NCPPB 1343	Oleander	.
Pseudomonas	savastanoi	nerii	NCPPB 1343	Oleander	.
Pseudomonas	savastanoi	nerii	NCPPB 3334	Oleander	.
Pseudomonas	savastanoi	nerii	NCPPB 3606	Oleander	.
Pseudomonas	savastanoi	savastanoi	NCPPB 1344	Olive	.
Pseudomonas	savastanoi	savastanoi	NCPPB 1342	Olive	.
Pseudomonas	savastanoi	savastanoi	NCPPB 2328		.
Pseudomonas	savastanoi	fraxinii	NCPPB 3474	Ash	.
Pseudomonas	savastanoi	savastanoi	NCPPB 64	Olive	.
Pseudomonas	viridiflava		NCPPB 1252	Pear	.
Pseudomonas	caricapapa.		NCPPB 1873	Papaya	.
Pseudomonas	caricapapa.		NCPPB 1873	Papaya	.
Pseudomonas	syringae	lachrymans	NCPPB 1425	Cucumber	.
Pseudomonas	syringae	lachrymans	NCPPB 1428	Cucumber	.
Pseudomonas	syringae	lachrymans	NCPPB 1097	Cucumber	.
Pseudomonas	syringae	lachrymans	NCPPB 277	Cucumber	.
Pseudomonas	syringae	lachrymans	NCPPB 467	Cucumber	.
Pseudomonas	syringae	lachrymans	NCPPB 541	Cucumber	.
Pseudomonas	syringae	lachrymans	NCPPB 542	Cucumber	.
Pseudomonas	syringae	lachrymans	NCPPB 543	Cucumber	.
Pseudomonas	syringae	lachrymans	NCPPB 1436	Cucumber	.
Pseudomonas	syringae	lachrymans	NCPPB 2754	Cucumber	.

- CSL has done extensive studies using rep-PCR for pathovar level differentiation
- With extensive data sets, sophisticated software is required to analyse the data

CSL®

DNA approaches to identification

- Advantages
  - Commercial services available for sequencing
  - Data can be shared between laboratories
  - By a combination of approaches identification to the genus, species and pathovar level can be achieved
- Limitations
  - Requires investment in PCR and gel equipment
  - Cost of molecular consumables is high
  - Technically demanding; PCR is notorious for ‘random’ problems

What is the best approach for East Africa

Thank you