

Assessment of toxin producing *Pithomyces* sp.
By Random Amplified Polymorphic DNA analysis

Manaaki Whenua
Landcare Research
NEW ZEALAND LTD.

Duckchul Park
Stephanie Parkes
Ross Beever

Introduction

- *Pithomyces* sp. Produce sporadesmin toxin that cause liver damage in sheep to make Facial Eczema disease.
- Losses due to moderate Facial Eczema challenge
 - Sheep \$ 150 m
 - Dairy \$ 120 m
- It is very difficult to distinguish toxin-producing strain and toxin-nonproducing strain by conventional method.
- We have to make more fast, reliable and accurate toxin producing strain detection method.

Summary of Method

1. Collection of *Pithomyces* sp. Strain
2. Isolation of Chromosomal DNA
3. Preliminary PCR Reaction typical *Pithomyces* strain with large number of primer
4. Select a few good primer
5. PCR Reaction good primer with all of collected *Pithomyces* sp.
6. RAPD pattern analysis

1. Collection of *Pithomyces* sp. Strain

Total 76 strain Collected

	Locality	Toxin	Number
<i>Pithomyces chartarum</i>	New Zealand	+	14
		-	19
		?	6
	Brazil	+	0
		-	4
	Colombia	+	0
		-	3
	South Africa	+	3
		-	3
	France	+	2
		-	3
	Uruguay	+	5
		-	2
	Australia	+	3
-		2	
<i>Pithomyces funiculosa</i>	Swaziland	?	1
<i>Pithomyces graminicola</i>	Australia	-	1
<i>Pithomyces karooi</i>	South Africa	-	1
<i>Pithomyces maydicus</i>	?	?	1
<i>Pithomyces quadratus</i>	ATCC32855	-	1
<i>Pithomyces sacchari</i>	South Africa	-	1
<i>Pithomyces valparadisiacus</i>	Spain	?	1
<i>Total No. -chartarum</i>		+	27
		-	36
		?	6
<i>other sp.</i>		+/-	7

Strain Chart

Order: Strain No.

NO.	Strain No.	Strain Name	Locality	Toxin	RC No.	spr/plt	Spd/spr
1	616- 1	<i>Pithomyces chartarum</i>	Brazil	-	56	4.3	0.0
2	618- 1	<i>Pithomyces chartarum</i>	Brazil	-	57	4.6	0.0
3	620- 1	<i>Pithomyces chartarum</i>	Brazil	-	58	2.5	0.0
4	621- 1	<i>Pithomyces chartarum</i>	Brazil	-			
5	625- 1	<i>Pithomyces chartarum</i>	Colombia	-	59	3.4	0.0
6	627- 1	<i>Pithomyces chartarum</i>	Colombia	-	60	6.8	0.0
7	633- 1	<i>Pithomyces chartarum</i>	NZ, WI, Palmersto	+			
8	634- 1	<i>Pithomyces chartarum</i>	NZ, AU	-	65	6.5	0.0
9	641- 1	<i>Pithomyces chartarum</i>	NZ	+			
10	646- 1	<i>Pithomyces chartarum</i>	NZ	+			
11	720- 1	<i>Pithomyces chartarum</i>	S. Africa	+			
12	722- 1	<i>Pithomyces chartarum</i>	S. Africa	+			
13	723- 1	<i>Pithomyces chartarum</i>	S. Africa	+			
14	725- 1	<i>Pithomyces chartarum</i>	S. Africa	-	67	0.0	0.0
15	728- 1	<i>Pithomyces chartarum</i>	S. Africa	-	66	?	0.0
16	742- 1	<i>Pithomyces chartarum</i>	NZ, CO, Thames	+	43	9.5	0.5
17	743- 1	<i>Pithomyces funiculosa</i>	S. Africa	-	46	11.9	0.0
18	744- 1	<i>Pithomyces chartarum</i>	Columbia	-	61	?	0.0
19	754- 1	<i>Pithomyces funiculosa</i>	Swaziland	?	54	?	?
20	755- 1	<i>Pithomyces graminicola</i>	Australia	-	52	20.5	0.0
21	756- 1	<i>Pithomyces karooi</i>	S. Africa	-	50	4.8	0.0
22	758- 1	<i>Pithomyces quadratus</i>	ATCC32855	-	53	0.5	0.0
23	759- 1	<i>Pithomyces sacchari</i>	S. Africa	-	51	32.4	0.0
24	760- 1	<i>Pithomyces valparadisiacus</i>	Spain	?	55	?	?
25	762- 1	<i>Pithomyces chartarum</i>	NZ, AU, Mt Albert	?			
26	765- 1	<i>Pithomyces chartarum</i>	NZ, AU, Mt Albert	?			
27	769- 1	<i>Pithomyces chartarum</i>	NZ, AU, Mt Albert	?			
28	772- 1	<i>Pithomyces chartarum</i>	NZ, AU, Mt Albert	?			
29	775- 1	<i>Pithomyces chartarum</i>	NZ, AU, Mt Albert	?			
30	777- 1	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	+	1	30.0	0.8
31	777- 2	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	+	2	15.4	1.4
32	777- 3	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-	3	16.8	0.0
33	777- 4	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	?	4	?	?
34	777- 5	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	+	5	28.3	1.3
35	779- 1	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-	9	15.6	0.0
36	779- 2	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-	10	14.5	0.0
37	783- 1	<i>Pithomyces chartarum</i>	France	-	68	0.5	0.0
38	783- 2	<i>Pithomyces chartarum</i>	France	-	69	?	0.0
39	783- 3	<i>Pithomyces chartarum</i>	France	-	70	1.8	0.0
40	783- 4	<i>Pithomyces chartarum</i>	France	+	71	7.4	0.0
41	783- 5	<i>Pithomyces chartarum</i>	France	+	72	5.3	0.8

42	783- 6	<i>Pithomyces chartarum</i>	Uruguay	+	41	4.9	0.3
43	783- 7	<i>Pithomyces chartarum</i>	Uruguay	+	42	15.2	0.5
44	784- 1	<i>Pithomyces chartarum</i>	NZ, TO, Te Kopia	+			
45	785- 1	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-	12	11.6	0.0
46	785- 2	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-	13	4.0	0.0
47	785- 3	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-	14	22.3	0.0
48	785- 4	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-	15	17.9	0.0
49	785- 5	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-	16	12.1	0.0
50	785- 6	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-	17	10.8	0.0
51	787- 1	<i>Pithomyces maydicus</i>	?	?			
52	795- 1	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-	33	15.9	0.0
53	795- 2	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-	34	14.0	0.0
54	795- 3	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-	36	15.0	0.0
55	795- 4	<i>Pithomyces chartarum</i>	Australia, Victoria	+	74	2.7	4.7
56	795- 5	<i>Pithomyces chartarum</i>	Australia	+	82	22.8	1.3
57	795- 6	<i>Pithomyces chartarum</i>	Australia, Victoria	+	89	1.9	0.6
58	795- 8	<i>Pithomyces chartarum</i>	Australia	-	90	5.2	0.0
59	795- 9	<i>Pithomyces chartarum</i>	Australia	-	95	13.0	0.0
60	795-10	<i>Pithomyces chartarum</i>	Uruguay	+			
61	795-11	<i>Pithomyces chartarum</i>	Uruguay, Colonia	+			
62	795-12	<i>Pithomyces chartarum</i>	Uruguay	+			
63	795-13	<i>Pithomyces chartarum</i>	Uruguay, Paysand	-			
64	795-14	<i>Pithomyces chartarum</i>	Uruguay, Soriano	-			
65	809- 1	<i>Pithomyces chartarum</i>	NZ, Nelson	+	268	3.9	3.0
66	809- 2	<i>Pithomyces chartarum</i>	NZ, Nelson	-	271	0.8	0.0
67	809- 3	<i>Pithomyces chartarum</i>	NZ, Nelson	-	273	7.5	0.0
68	809- 5	<i>Pithomyces chartarum</i>	NZ, Nelson	-	278	5.2	0.0
69	809- 6	<i>Pithomyces chartarum</i>	NZ, Gisborne	+	281		
70	809- 7	<i>Pithomyces chartarum</i>	NZ, Gisborne	+	287	16.9	1.2
71	809- 8	<i>Pithomyces chartarum</i>	NZ, Gisborne	-	289	5.1	0.0
72	809- 9	<i>Pithomyces chartarum</i>	NZ, Gisborne	-	290	12.0	0.0
73	809-10	<i>Pithomyces chartarum</i>	NZ, Gisborne	-	291	9.0	0.0
74	809-11	<i>Pithomyces chartarum</i>	NZ, Gisborne	+	313		
75	809-12	<i>Pithomyces chartarum</i>	NZ, Northland	+	311	14.2	1.4
76	809-13	<i>Pithomyces chartarum</i>	NZ, Northland	+	312	9.1	1.2

Strain Chart (OPB08)

NO.	Strain No.	Strain Name	Locality	Toxin
1	633- 1	<i>Pithomyces chartarum</i>	NZ, WI, Palmerston	+
2	641- 1	<i>Pithomyces chartarum</i>	NZ	+
3	646- 1	<i>Pithomyces chartarum</i>	NZ	+
4	720- 1	<i>Pithomyces chartarum</i>	S. Africa	+
5	722- 1	<i>Pithomyces chartarum</i>	S. Africa	+
6	742- 1	<i>Pithomyces chartarum</i>	NZ, CO, Thames	+
7	777- 1	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	+
8	777- 2	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	+
9	777- 4	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	?
10	777- 5	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	+
11	784- 1	<i>Pithomyces chartarum</i>	NZ, TO, Te Kopia	+
12	762- 1	<i>Pithomyces chartarum</i>	NZ, AU, Mt Albert	?
13	765- 1	<i>Pithomyces chartarum</i>	NZ, AU, Mt Albert	?
14	769- 1	<i>Pithomyces chartarum</i>	NZ, AU, Mt Albert	?
15	772- 1	<i>Pithomyces chartarum</i>	NZ, AU, Mt Albert	?
16	775- 1	<i>Pithomyces chartarum</i>	NZ, AU, Mt Albert	?
17	777- 3	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
18	779- 1	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
19	779- 2	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
20	785- 1	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
21	785- 2	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
22	785- 3	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
23	785- 4	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
24	785- 5	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
25	785- 6	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
26	795- 1	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
27	795- 2	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
28	795- 3	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
29	616- 1	<i>Pithomyces chartarum</i>	Brazil	-
30	618- 1	<i>Pithomyces chartarum</i>	Brazil	-
31	620- 1	<i>Pithomyces chartarum</i>	Brazil	-
32	621- 1	<i>Pithomyces chartarum</i>	Brazil	-
33	625- 1	<i>Pithomyces chartarum</i>	Colombia	-
34	627- 1	<i>Pithomyces chartarum</i>	Colombia	-
35	725- 1	<i>Pithomyces chartarum</i>	S. Africa	-
36	783- 1	<i>Pithomyces chartarum</i>	France	-
37	783- 2	<i>Pithomyces chartarum</i>	France	-
38	783- 3	<i>Pithomyces chartarum</i>	France	-

39	795- 8	<i>Pithomyces chartarum</i>	Australia	-
40	795- 9	<i>Pithomyces chartarum</i>	Australia	-
41	795-13	<i>Pithomyces chartarum</i>	Uruguay, Paysandu	-
42	795-14	<i>Pithomyces chartarum</i>	Uruguay, Soriano	-
43	723- 1	<i>Pithomyces chartarum</i>	S. Africa	+
44	783- 4	<i>Pithomyces chartarum</i>	France	+
45	783- 5	<i>Pithomyces chartarum</i>	France	+
46	783- 6	<i>Pithomyces chartarum</i>	Uruguay	+
47	783- 7	<i>Pithomyces chartarum</i>	Uruguay	+
48	795- 4	<i>Pithomyces chartarum</i>	Australia, Victoria	+
49	795- 5	<i>Pithomyces chartarum</i>	Australia	+
50	795- 6	<i>Pithomyces chartarum</i>	Australia, Victoria	+
51	795-10	<i>Pithomyces chartarum</i>	Uruguay	+
52	795-11	<i>Pithomyces chartarum</i>	Uruguay, Colonia	+
53	795-12	<i>Pithomyces chartarum</i>	Uruguay	+
54	743- 1	<i>Pithomyces chartarum</i>	S. Africa	-
55	744- 1	<i>Pithomyces chartarum</i>	Columbia	-

Strain Chart (OPB11)

NO.	Strain No.	Strain Name	Locality	Toxin
1	633- 1	<i>Pithomyces chartarum</i>	NZ, WI, Palmerston	+
2	641- 1	<i>Pithomyces chartarum</i>	NZ	+
3	646- 1	<i>Pithomyces chartarum</i>	NZ	+
4	742- 1	<i>Pithomyces chartarum</i>	NZ, CO, Thames	+
5	777- 1	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	+
6	777- 2	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	+
7	777- 4	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	?
8	777- 5	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	+
9	784- 1	<i>Pithomyces chartarum</i>	NZ, TO, Te Kopia	+
10	634- 1	<i>Pithomyces chartarum</i>	NZ, AU	-
11	762- 1	<i>Pithomyces chartarum</i>	NZ, AU, Mt Albert	?
12	765- 1	<i>Pithomyces chartarum</i>	NZ, AU, Mt Albert	?
13	769- 1	<i>Pithomyces chartarum</i>	NZ, AU, Mt Albert	?
14	772- 1	<i>Pithomyces chartarum</i>	NZ, AU, Mt Albert	?
15	775- 1	<i>Pithomyces chartarum</i>	NZ, AU, Mt Albert	?
16	777- 3	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
17	779- 1	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
18	779- 2	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
19	785- 1	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
20	785- 2	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
21	785- 3	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
22	785- 4	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
23	785- 5	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
24	785- 6	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
25	795- 1	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
26	795- 2	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
27	795- 3	<i>Pithomyces chartarum</i>	NZ, WO, Ruakura	-
28	616- 1	<i>Pithomyces chartarum</i>	Brazil	-
29	618- 1	<i>Pithomyces chartarum</i>	Brazil	-
30	620- 1	<i>Pithomyces chartarum</i>	Brazil	-
31	621- 1	<i>Pithomyces chartarum</i>	Brazil	-
32	625- 1	<i>Pithomyces chartarum</i>	Colombia	-
33	627- 1	<i>Pithomyces chartarum</i>	Colombia	-
34	725- 1	<i>Pithomyces chartarum</i>	S. Africa	-
35	728- 1	<i>Pithomyces chartarum</i>	S. Africa	-
36	783- 1	<i>Pithomyces chartarum</i>	France	-
37	783- 2	<i>Pithomyces chartarum</i>	France	-
38	783- 3	<i>Pithomyces chartarum</i>	France	-

39	795- 8	<i>Pithomyces chartarum</i>	Australia	-
40	795- 9	<i>Pithomyces chartarum</i>	Australia	-
41	795-13	<i>Pithomyces chartarum</i>	Uruguay, Paysandu	-
42	795-14	<i>Pithomyces chartarum</i>	Uruguay, Soriano	-
43	720- 1	<i>Pithomyces chartarum</i>	S. Africa	+
44	723- 1	<i>Pithomyces chartarum</i>	S. Africa	+
45	783- 4	<i>Pithomyces chartarum</i>	France	+
46	783- 5	<i>Pithomyces chartarum</i>	France	+
47	783- 6	<i>Pithomyces chartarum</i>	Uruguay	+
48	783- 7	<i>Pithomyces chartarum</i>	Uruguay	+
49	795- 4	<i>Pithomyces chartarum</i>	Australia, Victoria	+
50	795- 5	<i>Pithomyces chartarum</i>	Australia	+
51	795- 6	<i>Pithomyces chartarum</i>	Australia, Victoria	+
52	795-10	<i>Pithomyces chartarum</i>	Uruguay	+
53	795-11	<i>Pithomyces chartarum</i>	Uruguay, Colonia	+
54	795-12	<i>Pithomyces chartarum</i>	Uruguay	+
55	743- 1	<i>Pithomyces chartarum</i>	S. Africa	-
56	744- 1	<i>Pithomyces chartarum</i>	Columbia	-

2. Isolation of Chromosomal DNA

100 ml liquid culture(static) - 1 - 2 weeks
or two of 10ml petridish culture(static) - 4 - 7 days



Freeze drying



Grinding in Liquid Nitrogen



SDS / Potassium acetate



Ethanol Precipitation



Spool or centrifuge



Rnase / Protenase K treatment



Phenol / Chloroform treatment



Ethanol Precipitation



Dissolve in distilled water
(ready for PCR)

3. Preliminary PCR Reaction typical pithomyces strain with large number of primer

PCR Reaction condition:

1X 92(1min), 40X (94(1min), 34(1min), 72(3min)), 1X 68(7min)

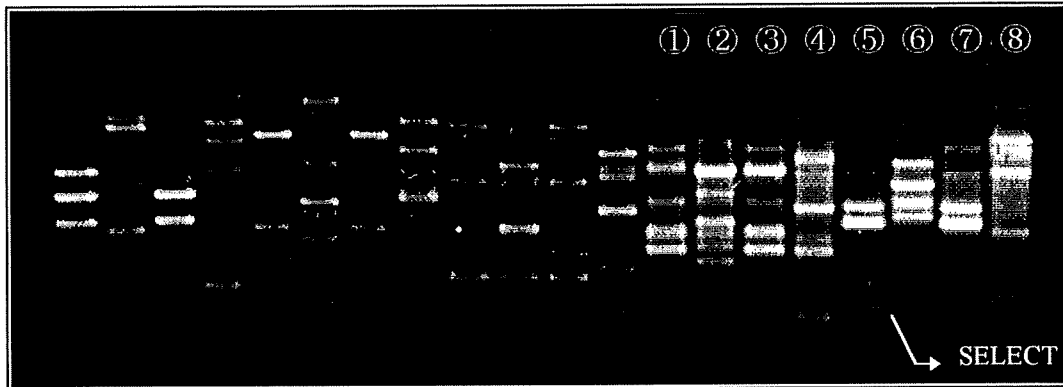
PCR Machine:

Techne Thermocycler 96well plate

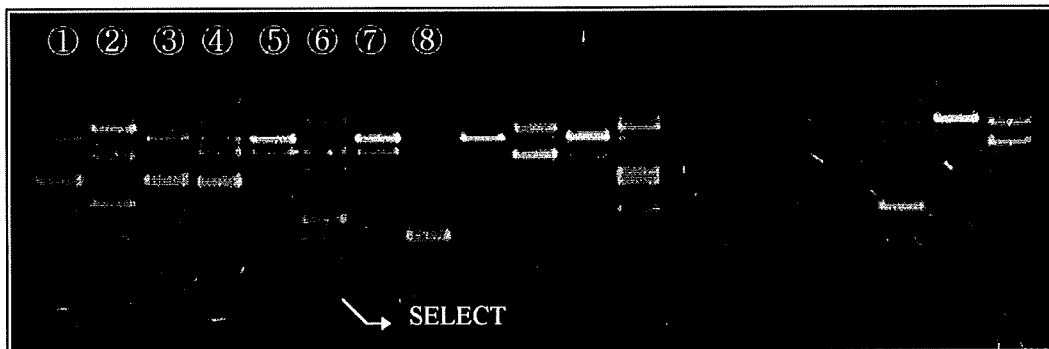
Primer: Operon Tech. OPA01 - 20
OPB01 - 20
OPAP01 - 20
OPAE01 - 20
OPAW01 - 20
OPAX01 - 20
Total 120 Primer

Strain: *Pithomyces chartarum* 742-1 (NZ positive)
Pithomyces chartarum 779-2 (NZ negative)
Pithomyces chartarum 795-10 (Uruguay positive)
Other strain 1166-3

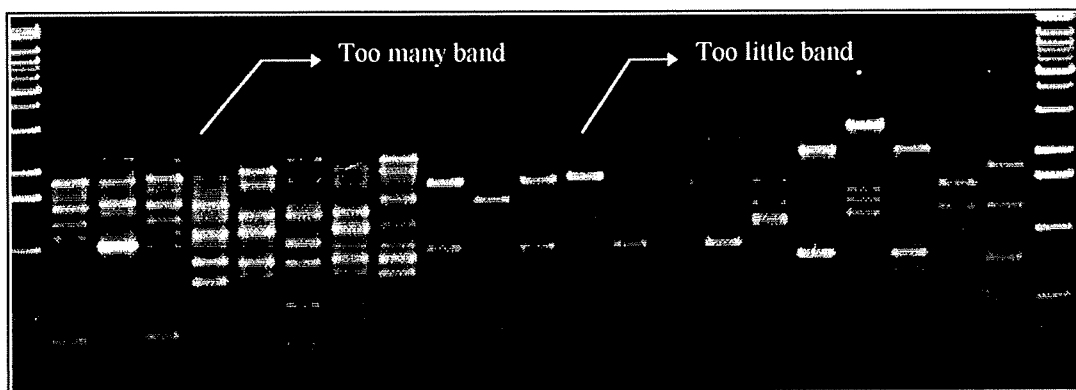
4. Select a few good primer



- | | | | |
|------------------------------|---------|------------------------|---------|
| ① 742-1 (NZ, positive) | + OPB07 | ② 779-2 (NZ, negative) | + OPB07 |
| ③ 795-10 (Uruguay, positive) | + OPB07 | ④ 1166-3 | + OPB07 |
| ⑤ 742-1 (NZ, positive) | + OPB08 | ⑥ 779-2 (NZ, negative) | + OPB08 |
| ⑦ 795-10 (Uruguay, positive) | + OPB08 | ⑧ 1166-3 | + OPB08 |

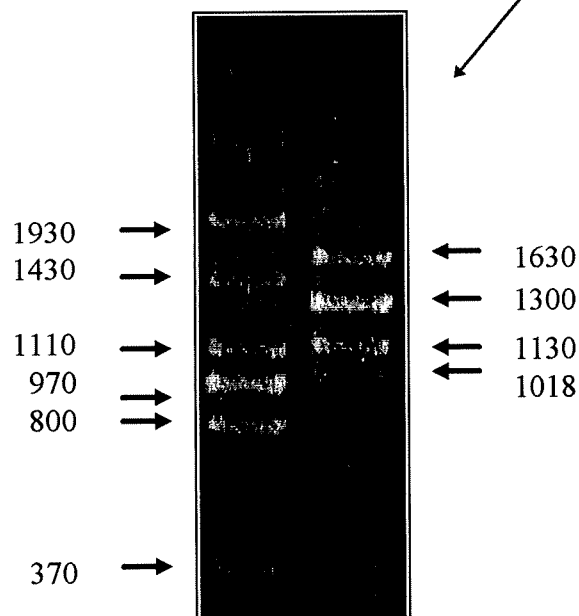
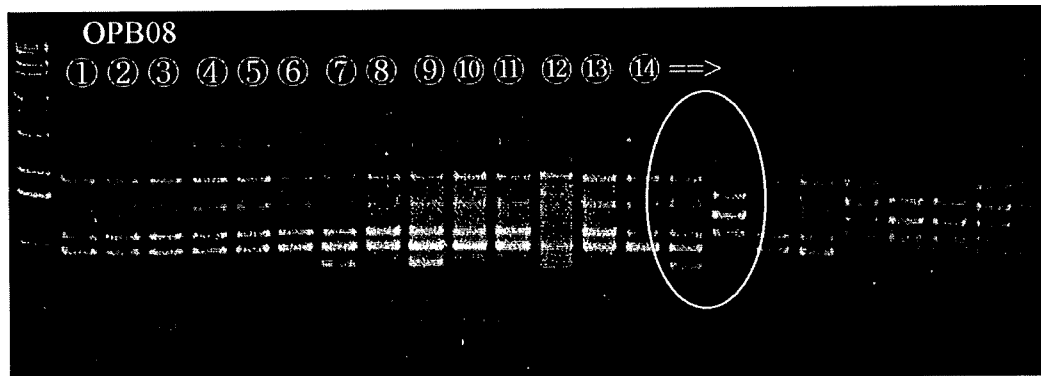


- | | | | |
|------------------------------|---------|------------------------|---------|
| ① 742-1 (NZ, positive) | + OPB10 | ② 779-2 (NZ, negative) | + OPB10 |
| ③ 795-10 (Uruguay, positive) | + OPB10 | ④ 1166-3 | + OPB10 |
| ⑤ 742-1 (NZ, positive) | + OPB11 | ⑥ 779-2 (NZ, negative) | + OPB11 |
| ⑦ 795-10 (Uruguay, positive) | + OPB11 | ⑧ 1166-3 | + OPB11 |



5. PCR Reaction good primer with all of collected *Pithomyces* sp.

Select 2 primer (OPB08, OPB11)



1. 2900 (base)
2. 1930
3. 1630
4. 1590
5. 1430
6. 1300
7. 1130
8. 1110
9. 1018
10. 990
11. 970
12. 800
13. 520
14. 370

769-1	0	1	0	0	1	0	0	1	0	0	1	1	0	1
772-1	0	0	1	0	0	1	1	0	1	0	0	0	0	0

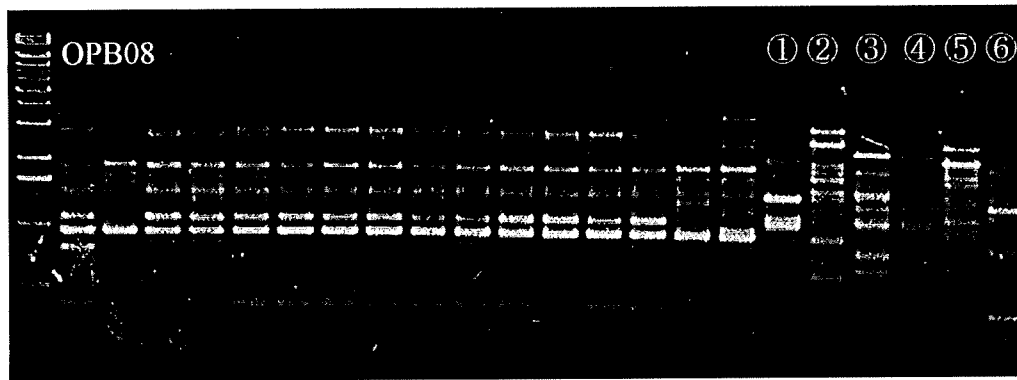
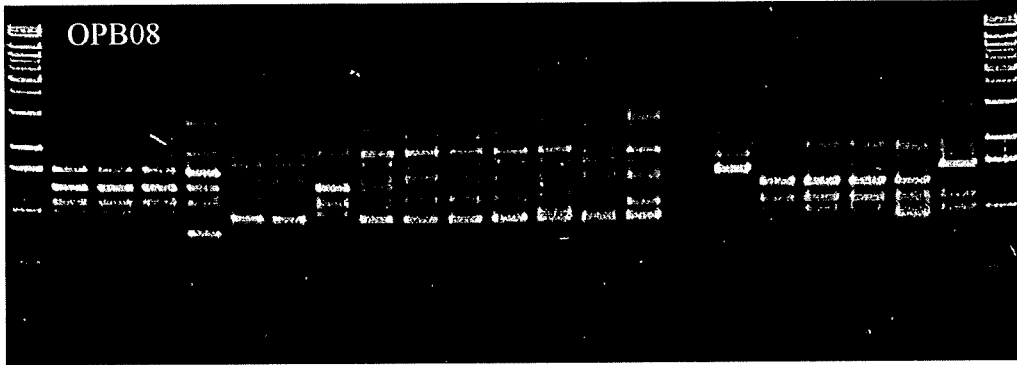
Primer: OPB11

Strain	Band Number																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
633-1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
641-1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
646-1	0	0	1	0	0	1	0	1	0	0	1	0	0	0	1	0	0	0	0	0
742-1	0	0	1	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0
777-1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0
777-2	0	0	1	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0
777-4	0	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0
777-5	0	0	1	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0
784-1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0
634-1	0	1	0	1	0	1	1	1	0	1	0	1	0	0	0	0	0	0	0	1
762-1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0
765-1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0
769-1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0
772-1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0
775-1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0
777-3	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
779-1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1	0	1	0	0
779-2	0	0	1	1	0	0	0	0	1	0	1	0	0	0	0	0	1	1	0	0
785-1	0	0	1	1	0	0	0	0	1	0	1	0	0	0	0	0	1	1	0	0
785-2	1	0	1	1	0	0	0	0	0	1	1	0	0	0	0	1	0	1	0	0
785-3	0	0	1	1	1	0	0	0	1	0	1	0	0	0	0	0	1	1	0	0
785-4	0	0	1	1	1	0	0	0	1	0	1	0	0	0	0	0	1	1	0	0
785-5	0	0	1	1	0	0	0	0	1	0	1	0	0	0	0	0	1	1	0	0
785-6	0	0	1	1	1	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0
795-1	0	0	0	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1
795-2	0	0	0	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1
795-3	0	0	1	1	0	0	0	0	1	0	1	0	0	0	0	0	1	1	0	0
616-1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
618-1	0	0	0	1	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0
620-1	0	0	0	1	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	1
621-1	0	1	0	1	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0
625-1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
627-1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
725-1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
728-1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
783-1	0	0	1	1	0	0	0	0	1	0	1	0	0	0	0	0	1	1	0	0
783-2	0	0	1	1	0	0	0	0	1	0	1	0	0	0	0	0	1	1	0	0
783-3	0	0	1	1	0	0	0	0	1	0	1	0	0	0	0	0	1	1	0	0
795-8	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
795-9	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
795-13	0	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0
795-14	0	0	1	1	0	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0
720-1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0
723-1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0
783-4	0	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0
783-5	0	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0
783-6	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
783-7	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
795-4	0	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0
795-5	0	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0
795-6	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
795-10	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0
795-11	0	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0
795-12	0	0	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0
743-1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
744-1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1

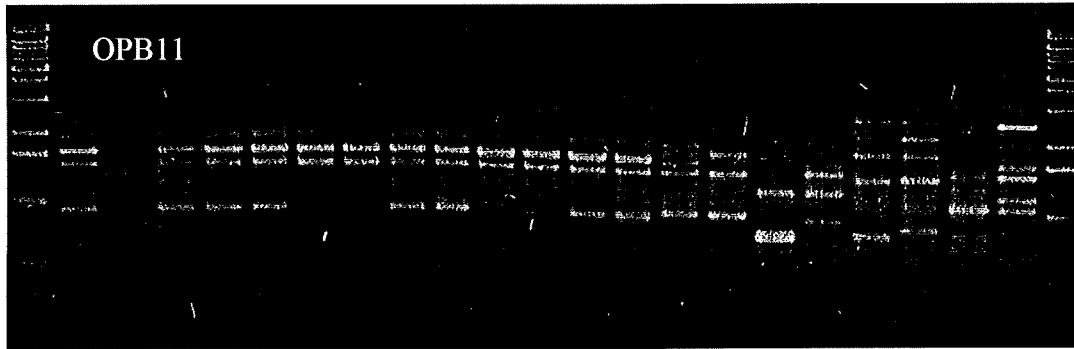
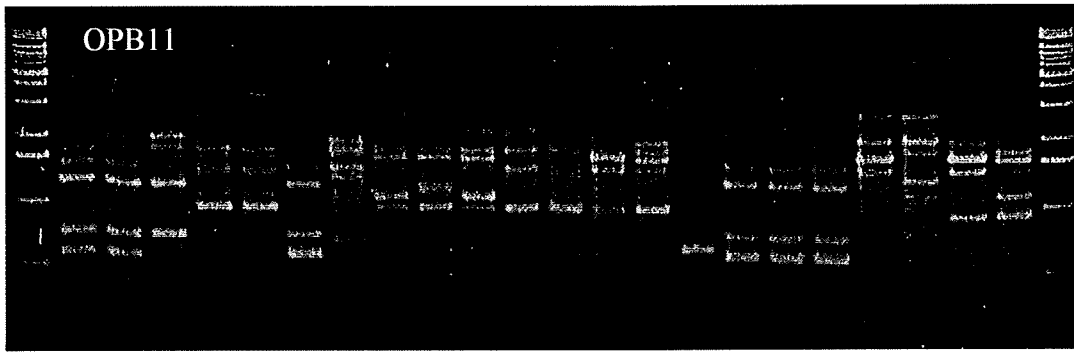
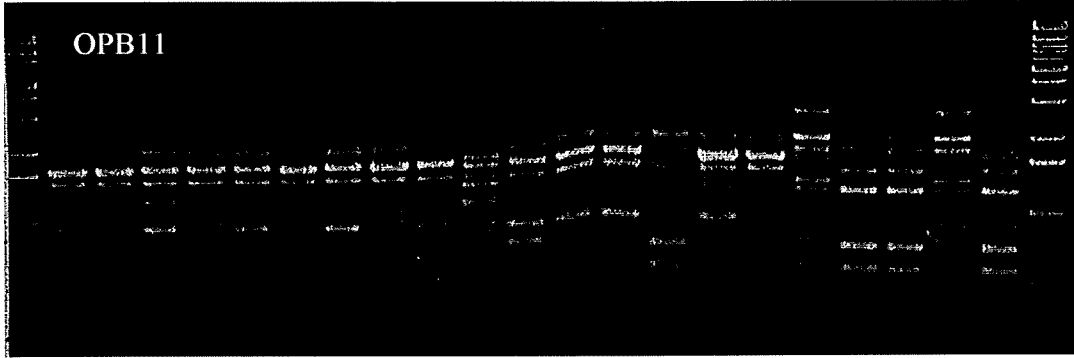
Primer: OPB08

Band Number

Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14
633-1	1	1	0	0	1	0	0	1	0	0	1	0	0	1
641-1	1	1	0	0	1	0	0	1	0	0	1	0	0	1
646-1	1	1	0	0	1	0	0	1	0	0	1	0	0	1
720-1	1	1	0	0	1	0	0	1	0	0	1	0	0	0
722-1	1	1	0	0	1	0	0	1	0	0	1	0	0	0
742-1	1	1	0	0	1	0	0	1	0	0	1	0	0	1
777-1	1	1	0	0	1	0	0	1	0	0	1	1	0	1
777-2	1	1	0	0	1	0	0	1	0	0	1	0	0	1
777-4	1	1	0	0	1	0	0	1	0	0	1	1	0	1
777-5	1	1	0	0	1	0	0	1	0	0	1	0	0	1
784-1	1	1	0	0	1	0	0	1	0	0	1	0	0	1
762-1	1	1	0	0	1	0	0	1	0	0	1	0	1	0
765-1	1	1	0	0	1	0	0	1	0	0	1	0	0	1
769-1	0	1	0	0	1	0	0	1	0	0	1	1	0	1
772-1	0	0	1	0	0	1	1	0	1	0	0	0	0	0
775-1	0	1	0	0	1	0	0	1	0	0	1	0	0	0
777-3	0	1	0	0	1	0	0	1	0	0	1	0	0	0
779-1	0	1	0	1	0	1	1	0	1	0	0	0	0	0
779-2	0	0	1	0	0	1	0	1	1	0	0	0	0	0
785-1	0	0	1	0	0	1	0	1	1	0	0	0	0	0
785-2	0	1	0	1	0	1	1	0	1	0	0	0	0	0
785-3	0	0	1	0	0	1	0	1	1	0	0	0	0	0
785-4	0	0	1	0	0	1	0	1	1	0	0	0	0	0
785-5	0	0	1	0	0	1	0	1	1	0	0	0	0	0
785-6	0	0	0	1	1	0	0	1	1	0	1	0	0	0
795-1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
795-2	0	0	0	0	0	0	0	0	0	0	0	1	0	0
795-3	0	0	0	0	0	1	0	1	1	0	0	0	0	0
616-1	0	1	0	0	0	0	0	0	0	0	0	1	0	0
618-1	0	1	0	0	0	0	0	0	0	0	0	1	0	0
620-1	0	1	0	0	0	0	0	0	0	0	0	1	0	0
621-1	0	1	0	0	0	0	0	0	0	0	0	1	0	0
625-1	0	1	0	0	0	0	0	0	0	0	0	1	0	0
627-1	0	1	0	0	0	0	0	0	0	0	0	1	0	0
725-1	1	1	0	0	1	0	0	1	0	0	1	0	0	0
783-1	0	0	0	0	0	1	0	1	1	0	0	0	0	0
783-2	0	0	0	0	0	1	0	1	1	0	0	0	0	0
783-3	0	0	0	0	0	1	0	1	1	0	0	0	0	0
795-8	0	0	0	0	0	1	0	1	1	1	0	0	0	0
795-9	0	0	0	1	0	0	0	1	1	0	0	0	0	0
795-13	1	1	0	0	1	0	0	1	0	0	1	1	0	1
795-14	0	1	0	0	0	0	0	0	0	0	1	0	0	0
723-1	1	1	0	0	1	0	0	1	0	0	1	0	0	0
783-4	1	1	0	0	1	0	0	1	0	0	1	0	0	1
783-5	1	1	0	0	1	0	0	1	0	0	1	0	0	1
783-6	1	1	0	0	1	0	0	1	0	0	1	0	0	1
783-7	1	1	0	0	1	0	0	1	0	0	1	0	0	1
795-4	1	1	0	0	1	0	0	1	0	0	1	0	0	1
795-5	1	1	0	0	1	0	0	1	0	0	1	0	0	1
795-6	1	1	0	0	1	0	0	1	0	0	1	0	0	1
795-10	1	1	0	0	1	0	0	1	0	0	1	0	0	0
795-11	1	1	0	0	1	0	0	1	0	0	1	0	0	1
795-12	1	1	0	0	1	0	0	1	0	0	1	0	0	1
743-1	0	1	0	0	1	0	0	0	0	0	0	1	0	0
744-1	0	1	0	0	1	0	0	0	0	0	0	1	0	0

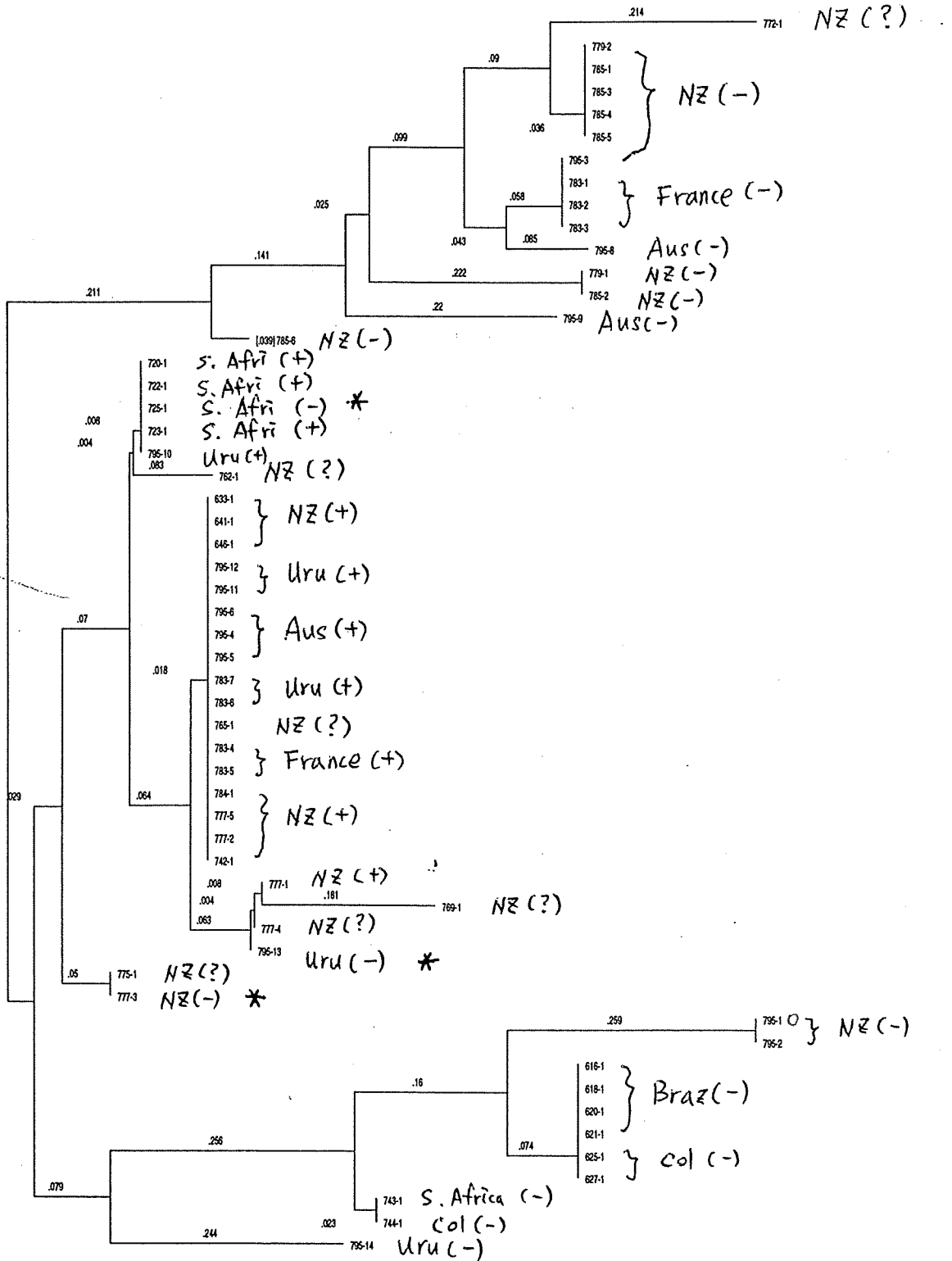


- ① *Pithomyces funiculosa*
- ② *Pithomyces graminicola*
- ③ *Pithomyces karooi*
- ④ *Pithomyces quadratus*
- ⑤ *Pithomyces sacchari*
- ⑥ *Pithomyces valparadisiacus*



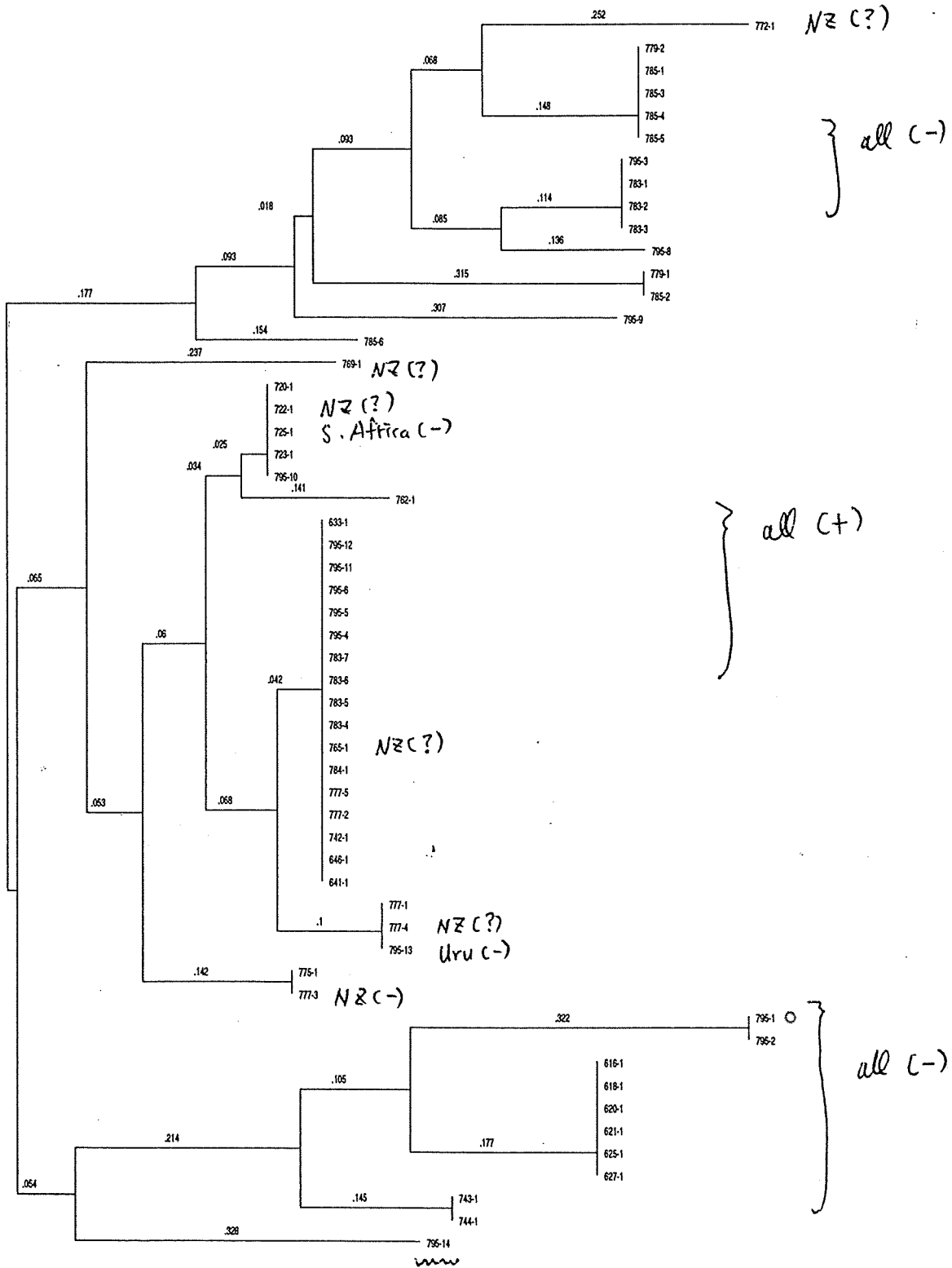
Primer: OPB08

Dice method



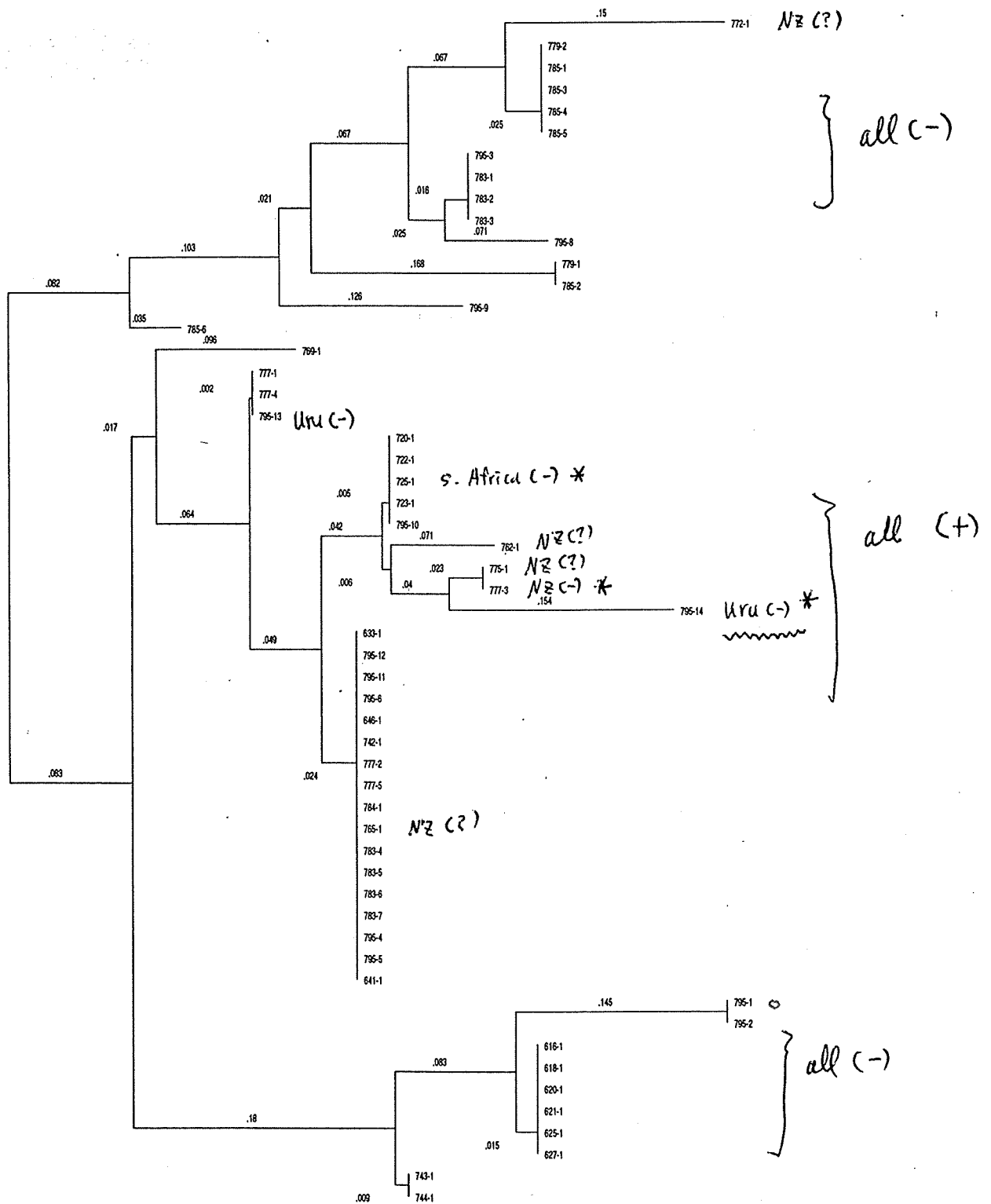
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Jaccard method



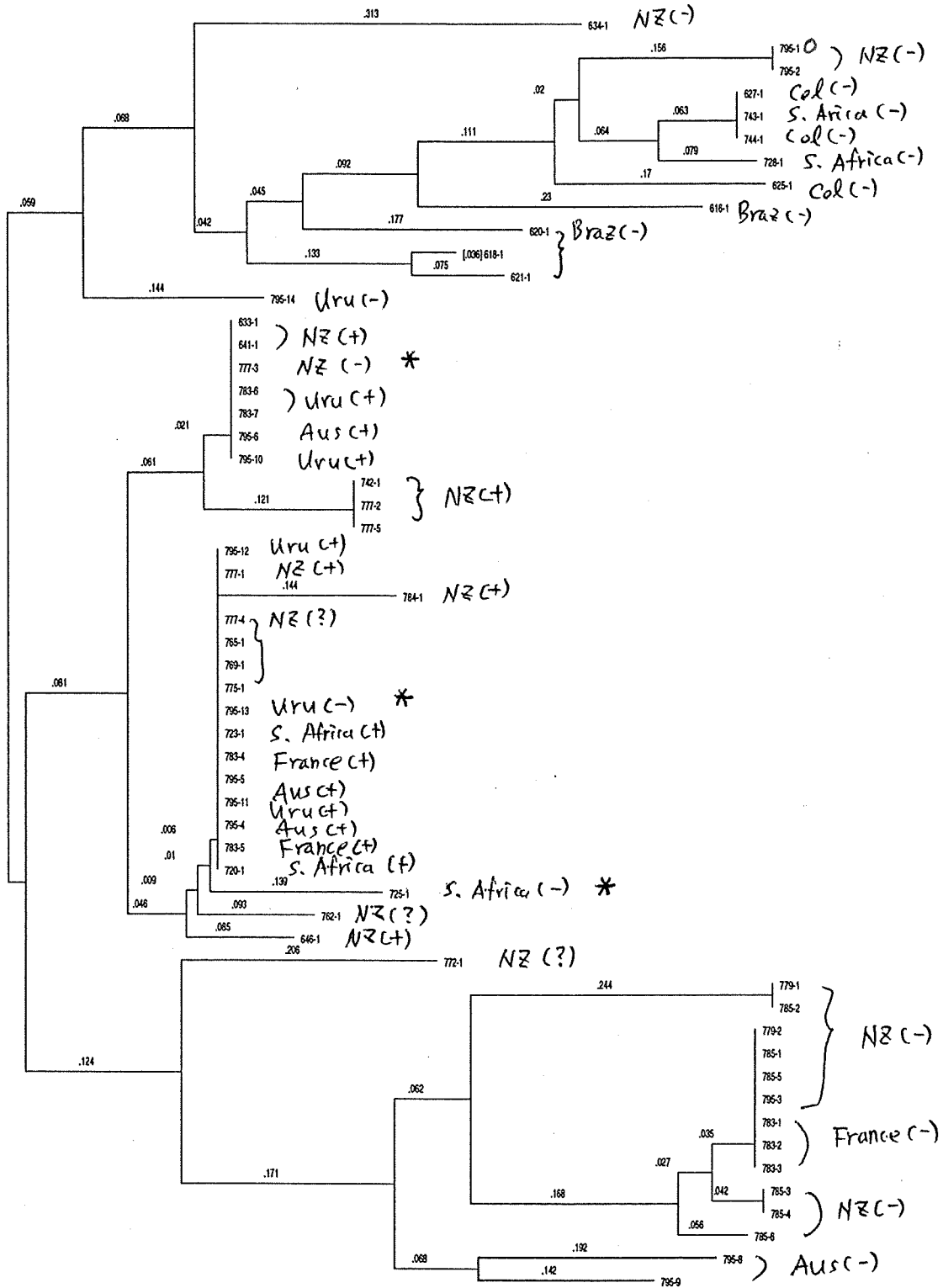
Primer: oPB08

Pearsons Phi



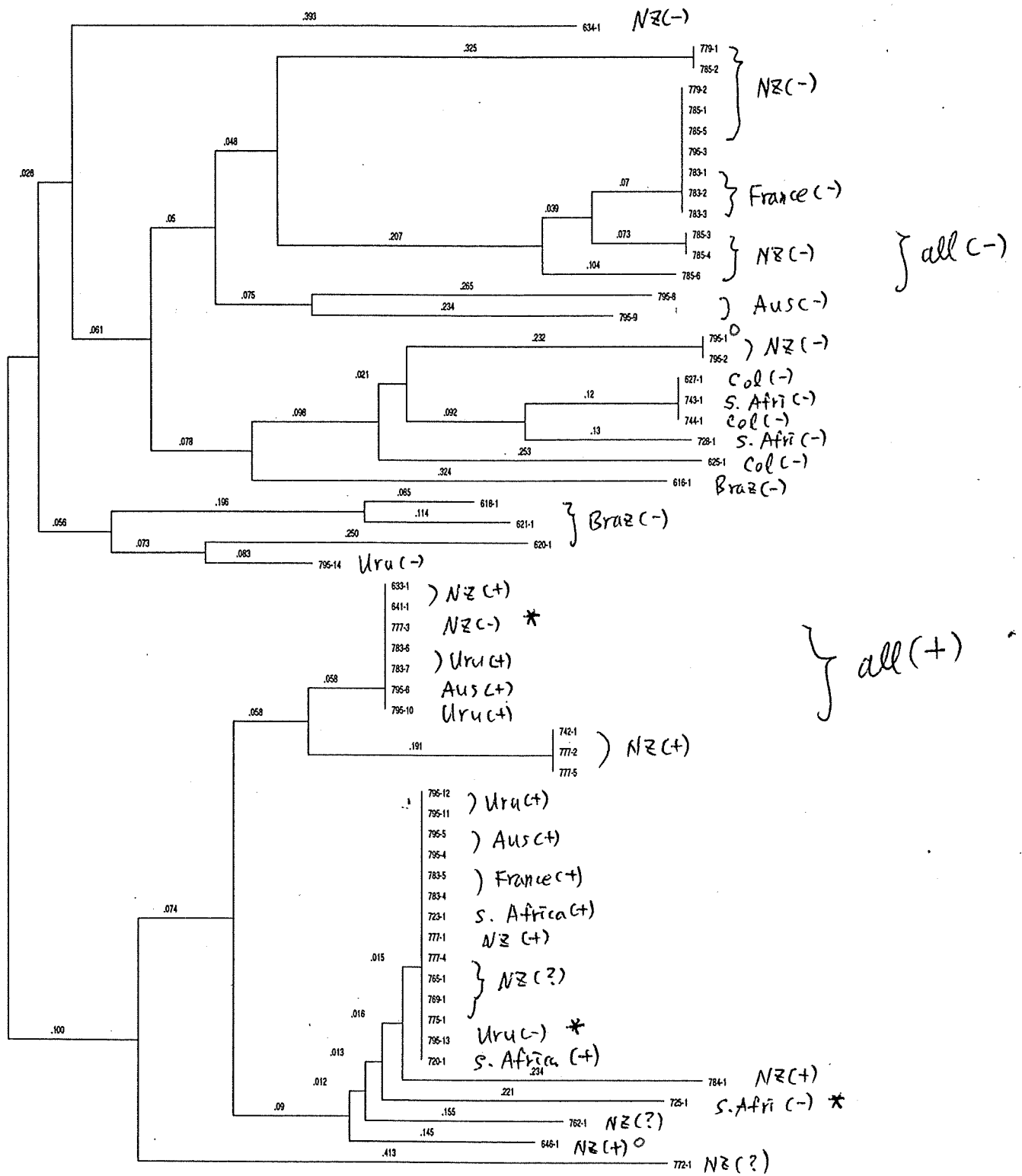
Primer: OPB11

Dice method



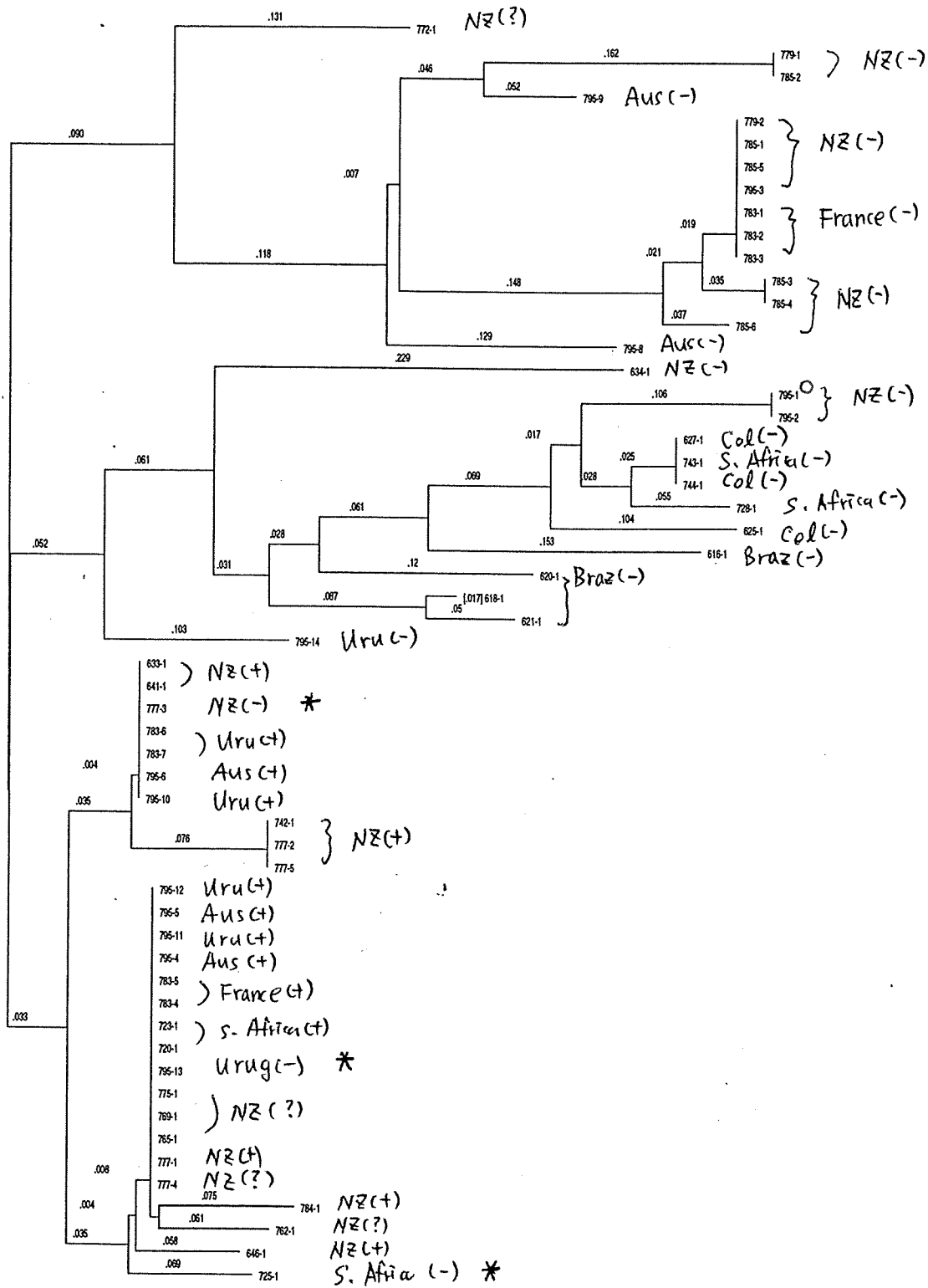
OPB II

Jaccard method



Primer: OPB11

Pearsons Phi



Further Research

- Recheck toxin(sporadesmin)
- Reproductivity check
 - Different DNA preps
 - Different Machine
- More PCR reaction with 2-4 another primer
- Mathematical Analysis
 - Compare with different algorithm, select best algorithm
- Biological property test
 - Any difference(spore, optimum temp. Growth rate) between the group
- Identify bands specific to toxin plus strain.