Table 1. Refference sequences of the genus *Fusarium* used in this study

Species in traditional taxonomic system[a]	Collection No.	GenBank accession No.					
		18S rDNA	ITS1	5.8S rDNA	28S rDNA	*β-tub*	*lys2*
F. acuminatum	CBS[d] 485.94	AB586907	AB587001	AB587001	AB587001	AB587049	AB586952
	MAFF[e] 236716	AB586908	AB587002	AB587002	AB587002	AB587050	AB586953
F. avenaceum	ATCC 200255[f] (Type strain)	AB586921	AB587015	AB587015	AB587015	AB587063	AB586964
	MAFF 239206	AB586922	AB587016	AB587016	AB587016	AB587064	AB586965
F. decemcellulare	MAFF 238421	AB586923	AB587017	AB587017	AB587017	AB587065	AB586966
	MAFF 238422	AB586924	AB587018	AB587018	AB587018	AB587066	AB586967
F. dimerum	CBS 632.76 (Neotype strain)	AB586901	AB586995	AB586995	AB586995	AB587043	AB586947
	MAFF 237465	AB586902	AB586996	AB586996	AB586996	AB587044	AB586948
F. equiseti[b]	MAFF 236434	AB586905	AB586999	AB586999	AB586999	AB587047	AB586950
	MAFF 236723	AB586906	AB587000	AB587000	AB587000	AB587048	AB586951
F. graminearum	MAFF 240264	AB586898	AB586991	AB586991	AB586991	AB587039	AB586943
	MAFF 240270	AB586992	AB586992	AB586992	AB586992	AB587040	AB586944
F. larvarum	CBS 169.30	AB586891	AB586984	AB586984	AB586984	AB587032	—
	CBS 638.76 (Isotype strain)	AB586892	AB586985	AB586985	AB586985	AB587033	—
F. lateritium	MAFF 235344	AB586910	AB587004	AB587004	AB587004	AB587052	AB586954
	MAFF 840045	AB586911	AB587005	AB587005	AB587005	AB587053	AB586955
F. moniliforme[c]	CBS 576.78 (Epitype strain)	AB586916	AB587010	AB587010	AB587010	AB587058	AB586960
	CBS 100312	AB586917	AB587011	AB587011	AB587011	AB587059	AB586961
F. merismoides	CBS 634.76 (Type strain)	AB586903	AB586997	AB586997	AB586997	AB587045	AB586949
	MAFF 236504	AB586904	AB586998	AB586998	AB586998	AB587046	—
F. nivale	CBS 116205 (Isotype strain)	AB586893	AB586986	AB586986	AB586986	AB587034	AB586938
	MAFF 236681	AB586894	AB586987	AB586987	AB586987	AB587035	AB586939
F. oxysporum	MAFF 240304	AB586899	AB586993	AB586993	AB586993	AB587041	AB586945
	MAFF 240321	AB586900	AB586994	AB586994	AB586994	AB587042	AB586946
F. poae	FRC T-0796	AB586930	AB586983	AB586983	AB586983	AB587072	AB586973
	MAFF 305947	AB586931	AB587024	AB587024	AB587024	AB587073	AB586974
F. proliferatum	CBS 216.76 (Type strain)	AB586912	AB587006	AB587006	AB587006	AB587054	AB586956
	MAFF 238035	AB586913	AB587007	AB587007	AB587007	AB587055	AB586957
	MAFF 237651	AB725617	AB725616	AB725616	AB725616	AB725614	AB725615
F. semitectum	MAFF 236521	AB586895	AB586988	AB586988	AB586988	AB587036	AB586940
F. solani	MAFF 238538	AB586919	AB587013	AB587013	AB587013	AB587061	AB586963
	MAFF 239038	AB586920	AB587014	AB587014	AB587014	AB587062	—
	NBRC[g] 8505	AB674259	AB674260	AB674260	AB674260	AB674261	AB586981
F. sporotrichioides	ATCC 34914	AB586932	AB587025	AB587025	AB587025	AB587074	AB586975
	CBS 119839	AB586933	AB587026	AB587026	AB587026	AB587075	AB586976
	MAFF 236639	AB586934	AB587027	AB587027	AB587027	AB587076	AB586977
F. subglutinans[c]	ATCC 38016	AB586914	AB587008	AB587008	AB587008	AB587056	AB586958
	MAFF 235376	AB586915	AB587009	AB587009	AB587009	AB587057	AB586959
F. tricinctum	ATCC 38183 (Type strain)	AB586935	AB587028	AB587028	AB587028	AB587077	AB586978
	MAFF 235551	AB586937	AB587030	AB587030	AB587030	AB587079	AB586980
F. kyushuense	MAFF 237645 (Ex holotype strain)	AB586925	AB587019	AB587019	AB587019	AB587067	AB586968
	NRRL[h] 6490 (Type strain)	AB586926	AB587020	AB587020	AB587020	AB587068	AB586969
F. langsethiae	CBS 113234 (Holotype strain)	AB586927	AB587021	AB587021	AB587021	AB587069	AB586970
	FRC[i] T-1000	AB586929	AB587023	AB587023	AB587023	AB587071	AB586972

[a] Nelson, Toussoun, and Marasas, 1983. *Fusarium* species—An Illustrated Manual for Identification.
[b] This species belongs to *F. incarnatum*/*F. equiseti* species complex.
[c] This species belongs to *Gibberella fujikuroi* species complex.
[d] Centraalbureau voor Schimmelcultures.
[e] Ministry of Agriculture, Forestry and Fisheries.
[f] American Type Culture Collection.
[g] National Institute of Technology and Evaluation, Biological Resource Center.
[h] Agricultural Research Service Culture Collection in United states Department of Agriculture.
[i] *Fusarium* Research Center in The Pennsylvania State University.

行った．5.8S rDNA，ITS1領域，5.8S rDNA，28S rDNA D1/D2領域，*β-tub*および*lys2*でそれぞれ493，136，159，466，586，560塩基長の塩基配列を解析に用いた．マルチプルアライメントおよびホモロジー検索は，Mega ver. 4.1[8]を用いて行った．

結果および考察

形態学的指標および塩基配列のホモロジー検索の結果に基づく食品由来分離株の同定結果をTable 3に示した．本表では，遺伝子ごとに各分離株に対してのホモロジーが高かったリファレンス菌株をリストアップした．本表を見ると，必ずしも上位から1菌種が並ぶとはかぎらず，時には複数種が入れ子状に並ぶ結果となった．このような現象は，*Fusarium*属菌の遺伝子塩基配列ホモロジー検索を行う場合にしばしば見られる．分子系統学的な解析結果によると，多くの*Fusarium*属菌が，単系

Table 2. The sources of isolates used in this study

Source	Strain No.
Apple	Ap-1
Banana	Ba-1, Ba-2, Ba-3, Ba-4
Cherry	Ch-1
Corn	Co-1, Co-2
Eggplant	Eg-1
Grain mix	Gmi-1
Grape	Gr-1
Green soybean	So-1
Japanese pear	Jp-1
Oats	Oa-1, Oa-2
Potato	Po-1
Satsuma mandarin	Ma-1, Ma-2
Tomato	To-1
Total isolate No.	19

統群ではなく側系統群または多系統群となる[1,5,11]ことが知られている．このことから，正確に同定されているリファレンス菌株のみが収録されているデータベースを用いているかぎりにおいては，入れ子状のホモロジー検索結果を示す原因は，菌種間の系統関係が原因の一つであると考えられる．そこで本研究では，各分離株において，最も上部にリストアップされたリファレンス菌株のみを参照し同定を行うこととした．

18S rDNA，5.8S rDNAでは，供試した分離株のうち，*F. nivale*または*F. solani*と同定されたBa-1，Gmi-1およびSo-1については同定が可能であったが，これら3菌株以外の16菌株において，100.0％のホモロジーを示したリファレンス菌株が複数の菌種にわたり，同定が不可能であった(Table 3)．両遺伝子座は，解析対象とした6遺伝子座の中では塩基置換速度が遅いことが以前筆者らが報告した結果において示されており[10]，本研究の結果はこれと一致した．

28S rDNA D1/D2領域では，上述の18S rDNAおよび5.8S rDNAで同定可能であった3菌株に加えて，菌株Ap-1，Ba-1，Ba-2，Co-1，Co-2，Oa-1，Po-1およびTo-1については同定が可能であった (Table 3)．しかし，その他の9分離株については，100.0％のホモロジーを示したリファレンス菌株が複数の菌種にわたり，同定が不可能であった．同定が可能であった菌種の内訳を見ると，*F. nivale* または *F. solani* のほかに，*F. oxysporum*，*F. subglutinans* および *F. verticillioides* であれば必ず同定できていたが，*F. proliferatum* は1菌株のみで同定が可能であり，*F. equiseti*，*F. semitectum*，*F. tricinctum* および *F. sporotrichioides* は1菌株も同定できていなかった．これら同定が不可能であった菌種はspecies complexを形成する菌種の一つであることが示唆されており[3,5,11]，菌種の区別が困難な互いにごく近縁な菌種が存在する．本遺伝子座は，species complexに属するすべての菌種間の差異を十分に識別できるほどの配列の特異性が蓄積していなかったものと考えられた．このことは，本遺伝子座の塩基置換速度が比較的遅いという以前筆者らが報告した結果と一致した[10]．

ITS1では，上述の28S rDNA D1/D2領域で同定可能であった分離菌株のほかに，菌株Ba-4，Gr-1，Jp-1 Ma-1およびOa-2の同定が可能であった(Table 3)．しかし一方で，Co-1およびOa-1については，28S rDNA D1/D2領域で同定が可能であったのに対し，ITS1では同定できなかった．その他の4分離株については，100.0％のホモロジーを示したリファレンス菌株が複数の菌種にわたり，候補を1菌種に絞ることが不可能であった．同定が可能であった菌種の内訳を見ると，28S rDNA D1/D2領域の場合と比較して，さらに*F. proliferatum*および*F. semitectum*であれば必ず同定可能となったが，逆に*F. verticillioides*である菌株は1株も同定できないという違いが認められた．*F. equiseti*，*F. tricinctum*および*F. sporotrichioides*は，28S rDNA D1/D2領域の場合と同様に1菌株も同定できていなかった．以前，筆者ら[10]は，*Fusarium*属菌系統樹のすべての枝長を足し合わせることによって*Fusarium*属菌に平均的な塩基置換速度を算出し，ITS1は28S rDNA D1 D2領域よりも塩基置換速度が速く同定に適していることを示唆した．*F. proliferatum*および*F. semitectum*については，28S rDNA D1/D2領域では不可能であった菌種でも同定が可能となったことから，以前の筆者らの研究と本研究の結果は一致した．しかし，*F. verticillioides*については，それと反する結果となった．これは，*F. verticillioides*の系統においては，ITS1の塩基置換速度は*Fusarium*属菌全体の傾向と逆転し，28S rDNA D1/D2領域よりも遅かったためと考えられる．また，ITS1で菌株Ma-2の同定を試みた際には，*F. semitectum* MAFF236521と最もホモロジーが高いことから*F. semitectum*と同定された．しかし，形態学的手法による同定結果からは，菌株Ma-2は小型分生子を多量に産出すること，分生子形成様式はmonophialideのみであるなどの特徴を有し，*F. semitectum* は明確に否定され，*F. equiseti*と同定された．さらに，*β-tub*および*lys2*の示すホモロジーによっても菌株Ma-2は*F. equiseti*と同定された．これらの同定結果を総合的に判断すると，本菌株は*F. equiseti*であり，ITS1はこの場合誤同定を起こしたものと考えられた．ITS1を*F. equiseti*および*F. semitectum*分離株の同定に用いる際には注意が必要である．

*lys2*では，PCRで遺伝子増幅が見られシークエンスの決定が可能であれば，すべての菌株について候補を1菌種に絞ることが可能であった(Table 3)．さらに，この場合，Ch-1およびTo-1を除きPCRで遺伝子の増幅が認められたすべての菌株において6遺伝子中でホモロジー1位と2位との差が最も大きく，塩基置換速度が最も速い遺伝子であるとするWatanabeらの研究結果[10]と一致した．シークエンスを決定することが可能でさえあれば，塩基配列の特異性を認識しやすい同定に適したマー

Table 3. Identification of candidate species of *Fusaium* isolates based on mophological methods and nucleotide sequence homologies

Isolate	Identification based on morphological methods[a]	Identification based on nucleotide sequence homology	Nucleotide sequence homology (%)						
			Reference strain	18S rDNA	ITS1	5.8S rDNA	28S rDNA	*β-tub*	*lys2*
Ap-1	*F. oxysporum*	ITS1, 28S rDNA, *β-tub*, *lys2* → *F. oxysporum*	*F. oxysporum* MAFF240304	100.00	100.00	100.00	99.78	98.64	96.72
			F. oxysporum MAFF240321	100.00	100.00	100.00	99.78	98.64	96.72
			F. proliferatum (*F. phylophilum*) CBS216.76	100.00	99.17	100.00	99.35	98.46	96.23
Ba-1	*F. solani*	*28S rDNA*, *β-tub*, *lys2* → *F. solani*	*F. solani* MAFF238538	100.00	97.54	100.00	100.00	98.46	—
			F. solani NBRC8505	100.00	94.22	100.00	99.14	97.95	—
			F. solani MAFF239038	99.39	90.08	98.11	98.27	93.69	—
			F. decemcellulare MAFF238421	99.80	71.90	98.74	97.19	92.83	
Ba-2	*F. subglutinans*	*28S rDNA*, *β-tub*, *lys2* → *F. subglutinans* (*F. sacchari*)	*F. subglutinans* (*F. sacchari*) MAFF235376	100.00	100.00	100.00	100.00	100.00	99.82
			F. subglutinans (*F. subglutinans*[c]) ATCC38016	100.00	99.17	100.00	100.00	95.90	94.35
			F. oxysporum MAFF240304	100.00	98.33	100.00	99.35	97.27	94.35
			F. oxysporum MAFF240321	100.00	98.33	100.00	99.35	97.27	94.35
Ba-3	*F. semitectum*	ITS1, *β-tub*, *lys2* → *semitectum*	*F. semitectum* MAFF236521	100.00	100.00	100.00	100.00	99.83	90.37
			F. equiseti MAFF236434	100.00	99.17	100.00	100.00	98.98	**92.01**
			F. equiseti MAFF236723	100.00	99.17	98.73	100.00	98.46	90.98
Ba-4	*F. proliferatum*[c]	*28S rDNA*, → *F. proliferatum*[b]; *b-tub*, *lys2* → *F. proliferatum*[c]	*F. proliferatum* (*F. proliferatum*[c]) MAFF238035	100.00	100.00	100.00	100.00	100.00	100.00
			F. proliferatum (*F. fujikuroi*) MAFF237651	100.00	100.00	100.00	100.00	99.49	98.93
			F. proliferatum[b] (*F. phyllophilum*) CBS216.76	100.00	100.00	100.00	99.57	97.10	97.10
			F. subglutinans (*F. sacchari*) MAFF235376	100.00	99.17	100.00	100.00	96.76	97.32
			F. oxysporum MAFF240304	100.00	99.17	100.00	99.35	96.93	94.72

Table 3. Continued

Isolate	Identification based on morphological methods[a]	Identification based on nucleotide sequence homology	Nucleotide sequence homology (%)						
			Reference strain	18S rDNA	ITS1	5.8S rDNA	28S rDNA	*β-tub*	*lys2*
Co-1	*F. verticillioides*[b]	*28S rDNA*, → *F. verticillioides*[b]; *b-tub, lys2* → *F. verticillioides*[c]	*F. verticillioides*[b] (*F. verticillioides*[c]) CBS576.78	100.00	100.00	100.00	100.00	100.00	100.00
			F. proliferatum[b] (*F. phyllophilum*) CBS216.76	100.00	100.00	100.00	99.57	98.64	97.32
			F. verticillioides[b] (*F. thapsinum*) CBS100312	99.80	100.00	100.00	100.00	97.95	97.17
Co-2	*F. subglutinans*[b]	*β-tub, lys2* → *F. subglutinans*[c]	*F. subglutinans*[b] (*F. subglutinans*[c]) ATCC38016	100.00	100.00	100.00	100.00	100.00	100.00
			F. oxysporum MAFF240304	100.00	99.17	100.00	99.35	97.44	94.72
			F. oxysporum MAFF240321	100.00	99.17	100.00	99.35	97.44	94.72
			F. subglutinans[b] (*F. sacchari*) MAFF235376	100.00	99.17	100.00	100.00	95.90	95.17
Ch-1	*F. spotrichiodes*	*28S rDNA*, *β-tub, lys2* → *F. sporotrichioides*	*F. sporotrichioides* ATCC34914	100.00	100.00	100.00	100.00	100.00	99.80
			F. sporotrichioides CBS119839	100.00	100.00	100.00	100.00	100.00	99.80
			F. sporotrichioides MAFF236639	100.00	100.00	100.00	100.00	100.00	99.80
			F. langsethiae CBS113234	100.00	100.00	100.00	100.00	99.15	99.39
Eg-1	*F. tricinctum*	*28S rDNA*, *β-tub, lys2* → *F. tricinctum*	*F. tricinctum* MAFF235551	100.00	100.00	100.00	100.00	100.00	—
			F. tricinctum ATCC38183	100.00	100.00	100.00	100.00	100.00	—
			F. avenaceum MAFF239206	100.00	100.00	100.00	100.00	97.95	—
Gmi-1	*F. nivale*	*28S rDNA*, *β-tub* → *F. nivale*	*F. nivale* MAFF236681	99.80	83.76	100.00	98.75	91.98	—
			F. nivale CBS116205	99.80	84.62	100.00	98.70	91.81	—
			F. dimerum CBS632.76	96.10	62.60	96.23	93.31	91.30	—
Gr-1	*F. proliferatum*	ITS1, *β-tub, lys2* → *F. proliferatum*	*F. proliferatum* (*F. proliferatum*[c]) MAFF238035	100.00	100.00	100.00	100.00	99.90	100.00
			F. proliferatum (*F. fujikuroi*) MAFF237651	100.00	100.00	100.00	100.00	99.49	98.93
			F. subglutinans (*F. sacchari*) MAFF235376	100.00	99.17	100.00	100.00	96.76	97.32
			F. proliferatum[b] (*F. phyllophilum*) CBS216.76	100.00	100.00	100.00	99.57	97.10	96.24

Table 3. Continued

Isolate	Identification based on morphological methods[a]	Identification based on nucleotide sequence homology	Nucleotide sequence homology (%)						
			Reference strain	18S rDNA	ITS1	5.8S rDNA	28S rDNA	*β-tub*	*lys2*
Jp-1	*F. semitectum*	ITS1, *β-tub*, *lys2* → *F. semitectum*	*F. semitectum* MAFF236521	100.00	100.00	100.00	100.00	100.00	100.00
			F. equiseti MAFF236434	100.00	99.17	100.00	100.00	98.81	92.01
			F. equiseti MAFF236723	100.00	99.18	98.73	100.00	98.29	93.44
Ma-1	*F. semitectum*	ITS1, *β-tub*, *lys2* → *F. semitectum*	*F. semitectum* MAFF236521	100.00	100.00	100.00	100.00	100.00	100.00
			F. equiseti MAFF236434	100.00	99.17	100.00	100.00	98.81	92.01
			F. equiseti MAFF236723	100.00	99.18	98.73	100.00	98.29	93.44
Ma-2	*F. equiseti*	ITS1 → *F. semitectum* *β-tub*, *lys2* → *F. equiseti*	*F. equiseti* MAFF236434	100.00	99.17	100.00	100.00	98.98	93.65
			F. semitectum MAFF236521	100.00	100.00	100.00	100.00	98.81	91.80
			F. equiseti MAFF236723	100.00	99.18	98.73	100.00	98.81	92.62
Oa-1	*F. verticillioides*[c]	28S rDNA, → *F. verticillioides*[b] *b-tub*, *lys2* → *F. verticillioides*[c]	*F. verticillioides*[b] (*F. verticillioides*[c]) CBS576.78	100.00	100.00	100.00	100.00	99.83	100.00
			F. proliferatum[b] (*F. phyllophilum*) CBS216.76	100.00	100.00	100.00	99.57	98.81	97.32
			F. verticillioides[b] (*F. thapsinum*) CBS100312	99.80	100.00	100.00	99.57	98.12	97.10
Oa-2	*F. semitectum*	ITS1, *β-tub*, *lys2* → *F. semitectum*	*F. semitectum* MAFF236521	100.00	100.00	100.00	100.00	99.66	98.77
			F. equiseti MAFF236434	100.00	99.17	100.00	100.00	98.64	91.80
			F. equiseti MAFF236723	100.00	99.17	98.73	100.00	99.15	92.62
Po-1	*F. oxysporum*	ITS1, 28S rDNA, *β-tub*, *lys2* → *F. oxysporum*	*F. oxysporum* MAFF240304	100.00	100.00	100.00	99.60	97.78	97.00
			F. oxysporum MAFF240321	100.00	100.00	100.00	99.60	97.78	97.00
			F. proliferatum CBS216.76	100.00	99.17	100.00	99.30	97.35	96.00
So-1	*F. solani*	28S rDNA, *β-tub*, *lys2* → *F. solani*	*F. solani* NBRC8505	100.00	97.52	100.00	99.57	98.12	—
			F. solani MAFF238538	100.00	93.33	100.00	99.57	97.10	—
			F. solani MAFF239038	99.40	90.91	98.11	98.27	94.37	—
			F. dimerum MAFF237465	99.60	71.05	98.74	97.41	91.98	—

Isolate	Identification based on morphological methods[a]	Identification based on nucleotide sequence homology	Nucleotide sequence homology (%)						
			Reference strain	18S rDNA	ITS1	5.8S rDNA	28S rDNA	*β-tub*	*lys2*
To-1	*F. proliferatum*[b]	ITS1, *β-tub*, *lys2* → *F. proliferatum*[b]	*F. proliferatum* (*F. fujikuroi*) MAFF237651	100.00	100.00	100.00	100.00	100.00	99.82
			F. proliferatum (*F. proliferatum*[c]) MAFF238035	100.00	100.00	100.00	100.00	99.13	99.10
			F. proliferatum[b] (*F. phyllophilum*) CBS216.76	100.00	100.00	100.00	99.57	96.93	96.10
			F. oxysporum MAFF240304	100.00	99.17	100.00	99.35	96.76	91.70

[a] Nelson, et al. *Fusarium* species: An illustrated manual for identification, 1983.
[b] sensu lato.
[c] sensu strict.
The grey box indicates that the nucleotide sequence homology leaded to misidentification.
The open box indicates that the nucleotide sequence homology leaded to accurate identification.
Species in parenthesis is re-identified by nucleotide sequence homology in the new taxonomic systems.

カーであると言える．しかし，今回供試した19菌株中4菌株でPCRでの遺伝子増幅が見られず，同定が不可能であった．*lys2*ではPCRでの遺伝子増幅が見られなかった菌種は以前の筆者らの研究においても報告されており[10]，系統特異的に*lys2*の塩基置換速度がさらに加速しているためと考えられる．また，*lys2*で菌株Ba-3の同定を試みた際には，*F. equiseti* MAFF236434と最もホモロジーが高いことから*F. equiseti*と同定された．しかし，形態学的手法による同定結果からは，菌株Ba-3は小型分生子を産出しないこと，分生子形成様式はpolyphialideが観察されるなどの特徴を有し，*F. equiseti*は明確に否定され，*F. semitectum*と同定された．ITS1におけるMa-2の場合と同様に同定結果を総合的に判断すると，本菌株は*F. semitectum*であり，*lys2*はこの場合誤同定を起こしたものと考えられた．*lys2*を*F. equiseti*および*F. semitectum*分離株の同定に用いる際には注意が必要である．

*β-tub*では，すべての分離株で同定が可能であった．本遺伝子座は*Fusarium*属菌分離株の同定に適したマーカーであることがすでに示されているが[10]，species complexを形成する菌種の分離株であっても十分に識別が可能であること，および他の遺伝子座と異なり，誤同定が起こらなかったことが確認された．

本研究の結果から，*Fusarium*属のspecies complexを形成するような互いに極近縁な菌種を，最も高い相同性を示した菌種を参照し正確に同定するための遺伝子指標としては，従来広く用いられてきたリボゾーム関連遺伝子群や，以前の筆者らの研究において最も速い塩基置換速度をもち優れたマーカーであることが示唆された*lys2*よりも，*β-tub*が適するということが明らかとなった．ただし，*β-tub*を用いた場合でも，*F. equiseti*および*F. oxysporum*など塩基配列相同率の差が1.0%以下程度と小さい場合もあり高いシークエンス精度が求められ，実験的な操作による誤同定が起こる場合もあると考えられる．そのため，形態学的指標による同定も合わせて行う必要がある．

なお，遺伝子塩基配列ホモロジーを指標とした真菌分離株の同定を行う際には，対象群についての分類学的な知識に基づいて検索結果の解釈を行い，適正な判断を行うことが必要不可欠である．1990年代以降，*Gibberella fujikuroi* species complexに含まれる分類群に新たな菌種名が提唱された[3]．これらの菌種は形態学的指標に基づいた場合非常に区別し難く[3]，本研究で参照したNelsonら[1]の分類体系においては別種として扱われてはいなかったが，その後交配集団および分子生物学的な解析結果を根拠として，この新たな菌種名が認知されつつある．これに基づくと，*F. subglutinans* MAFF235376は*F. sacchari*，*F. proliferatum* MAFF237651は*F. fujikuroi*，*F. proliferatum* CBS216.76は*F. phyllophilum*，*F. verticillioides* CBS100312は*F. thapsinum*とそれぞれ再同定され(Table 3)，*β-tub*によれば，分離株Ba-2，Ba-4，Co-2，Gr-1およびTo-1で示された(Table 3)ように，分離株の同定もこのレベルまでの識別が可能であることが明らかとなった．本研究では，現在広く用いられる形態学的指標を用いた伝統的な分類体系による菌種名を用いたが，同定目的に応じて適した菌種名を選択する必要がある．

遺伝子塩基配列ホモロジー検索による同定を行う際には，複数の既知の菌種と非常に高いホモロジーを示し1菌種に同定できない，または，既知のいずれの菌種にも適合せず同定が不可能など，確からしい同定が不可能である場合がしばしば生じる．その原因として，データ

ベース登録配列の多様性が低く近縁種を識別するための情報を有していない，また，登録されている菌種や種内集団にかぎりがあることなどが挙げられる．前者の問題は，本研究によって*Fusarium*属菌の多くの菌種に属する分離株について適したマーカーとなることが示された*β-tub*を用いれば，おおよその解決が可能であると考えられる．しかし，後者の問題については，本研究で作製したデータベースにも収録されていない菌種および種内集団が存在するため，可能なかぎりこれらのデータをデータベースに加えることが必要不可欠であると考えられる．以上の点について改善を行い，すべての*Fusarium*属菌分離株の同定にも適したデータベースの作製を行うことが今後の検討課題であろう．

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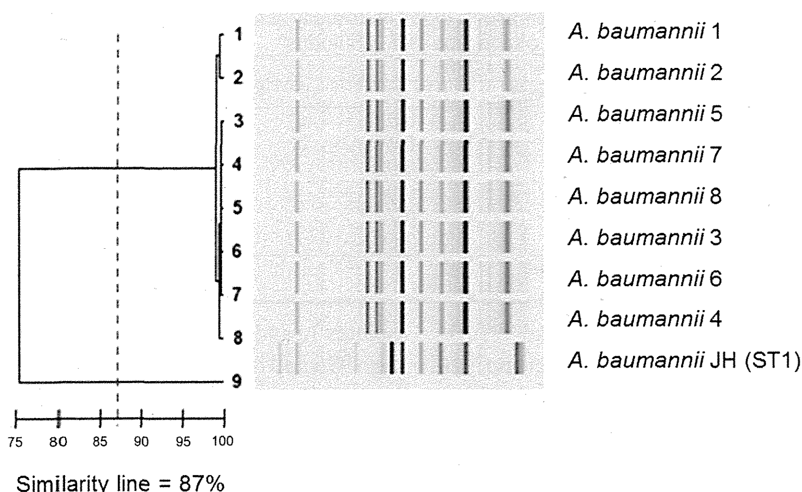

Figure. Results of Diversilab system (bioMérieux, La Balme-les-Grottes, France) analysis of *Acinetobacter baumannii* isolates. Similarity line shows the cutoff that separates the different clones.

identified mainly carbapenem-hydrolyzing carbapenemase OXA-58 (*9*).

Recently, we showed that the bla_{NDM-1} gene was carried by a composite transposon bracketed by 2 copies of IS*Aba125* in *A. baumannii* (*10*). Cloning and sequencing of the genetic context of the bla_{NDM-1} in the first isolate showed that transposon Tn*125* was truncated at its 3′-end extremity by insertion sequence IS*Aba14*, giving rise to a truncated Tn*125* (ΔTn*125*). PCR mapping of all isolates showed that they possessed this truncated isoform of Tn*125*, which was therefore probably no longer functional.

The identification of several clinical *A. baumannii* isolates that possessed the bla_{NDM-1} gene and originated from North Africa, with no obvious link to the Indian subcontinent, strongly suggests that 1 NDM-producing *A. baumannii* clone is probably widespread in North Africa and that it might now act as a reservoir for NDM-1. This finding might indicate that control of spread of multidrug-resistant *A. baumannii* would have a primary role in controlling spread of NDM-1.

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Genomic Analysis of *Salmonella enterica* Serovar Typhimurium Definitive Phage Type 104

To the Editor: *Salmonella enterica* is among the leading causes of foodborne diseases worldwide. Multidrug-resistant *S. enterica* serovar Typhimurium definitive phage type (DT) 104 emerged during the early 1990s in the United Kingdom and spread worldwide thereafter (*1*). This phage-type strain harbors a chromosomally encoded genomic island, Salmonella Genomic Island 1, which is typically responsible for resistance to ampicillin, chloramphenicol,

streptomycin, sulfonamide, and tetracycline (*2*). Multilocus variable-number tandem-repeat analysis (MLVA) is an established molecular epidemiologic tool; its high-resolution power has been applied to the subtyping of a variety of bacterial species (*3*). An MLVA system has been developed for analyzing *S. enterica* serovar Typhimurium (*4,5*).

The design of an MLVA system relies on the analyzed genome sequences. In this study, we found and evaluated a variable-number tandem-repeat region, or locus, designated DT104o. The locus is specific to *S. enterica* ser. Typhimurium DT104, according to the sequence of NCTC 13348 (available from the Sanger Institute, http://www.sanger.ac.uk/resources/downloads/bacteria/salmonella.html). The repeat unit sequence was CTCAGAA/TTCTGAG, spanning 1952121–1952274 on the reference genome or 22 repeats of 7 nt.

We used 266 apparently independent isolates of *S. enterica* serovar Typhimurium collected during 1981–2012; 103 were from human samples and 163 from non-human sources. Bacteriophage typing was performed according to Anderson's method and scheme (*6*). Types of 100 isolates were in the DT104 group, comprising DT104, DT104B, and U302, the latter being related to DT104 (*2*); MLVA was performed by using the 5 loci (STTR3, STTR5, STTR6, STTR9, and STTR10) with slight modifications (*4,5*). The DT104o locus was tested by using primers o-for (5′-GTCAACATGAACTGCCCCTCA-3′), labeled with NED, and o-rev (5′-TTTGCTCTTCGCTCTTAGCAATC-3′); this spanned 1952367–1952043 on the reference sequence, resulting in a 325-bp product with 171-bp offset.

For all 266 isolates tested, the number of alleles and the Simpson's index of diversity score (*D*) identified in each locus are summarized in the Table. The 5 common and DT104o loci displayed high discriminatory power:

Table. Number of alleles and Simpson's index of diversity score in *Salmonella* enterica serovar Typhimurium definitive phage type 104 in humans*

Locus	All, n = 266		DT104-group, n = 100	
	No. alleles†	*D*	No. alleles†	*D*
STTR9	5	0.62	1	0.00
STTR5	17	0.87	10	0.76
STTR6	20	0.93	16	0.90
STTR10	25	0.90	20	0.93
STTR3	12	0.73	2	0.20
DT104o	24	0.60	23	0.92

**D*, Simpson's index of diversity score.
†Including the null allele.

DT104o was specific for the DT104 group, and all 100 DT104 group isolates displayed amplified products with 13–40 repeat copy numbers; the others showed the null allele. Focusing only on the 100 DT104 group isolates, the discriminatory power of STTR9 and STTR3 were poor, whereas STTR5, STTR6, STTR10, and DT104o displayed high discriminatory powers (Table). In addition, using the 5 common loci (MLVA5) in analysis, we identified 66 types with a *D* value of 0.974; use of MLVA5 plus the DT104o locus (MLVA6) identified 83 types with a *D* value of 0.984. These results indicate that the DT104o locus is highly specific and therefore useful as an additional molecular epidemiologic marker for analyzing *S. enterica* ser. Typhimurium DT104.

Because DT104o was highly variable, 5 DT104 strains were tested for the frequency of variants at each locus after 5 serial passages by using liquid culture: cultures were diluted 1:1,000 at each passage. Sixteen colonies of each strain were tested by using MLVA6 (online Technical Appendix Table 1, wwwnc.cdc.gov/EID/article/19/5/12-1395-Techapp1.pdf). No variants were observed in STTR3, STTR9, or STTR10. STTR5, STTR6, and DT104o each showed 1 variant of 80 colonies. The results suggest that DT104o would not be less stable than other loci.

We also found that DT104o could provide more discriminatory power to MLVA5 in some settings (online Technical Appendix Table 2). We compared 2 settings using isolates from non-human samples. Setting 1 comprised isolates 1a and 1b from an outbreak during 1996 and isolate 1c in 2007. Isolates 1a and 1b were identical by MLVA6. Isolate 1c was identical by MLVA5 but not by MLVA6. In Setting 2, three isolates obtained in different years also were identical by MLVA5, but differed from each other by MLVA6. This suggests that MLVA6 could be useful in some epidemiologic settings such as in an outbreak investigation, though more extensive study would be required to confirm this suggestion.

The DT104o locus is located at the proximal region of fragment 180 comprised of a prophage structure, which was proven to be DT104-specific in a previous study (*7*). This finding is consistent with the results of our study.

In conclusion, development of an MLVA system is dependent upon the genome sequences available, and the system is usually used for molecular subtyping of a certain serotype in a particular organism. However, a specific group of strains could cause a pandemic and become a target of public health concern, as was *S. enterica* ser. Typhimurium DT104. The MLVA system could be improved by adding loci based on the genome sequence of such pandemic strains. In this study, we showed that the newly identified DT104o locus could be useful in identification and subtyping of *S. enterica* ser. Typhimurium DT104.

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Single Genotype of *Anaplasma phagocytophilum* identified from Ticks, Camargue, France

To the Editor: Granulocytic anaplasmosis is a tickborne zoonosis caused by *Anaplasma phagocytophilum* bacteria, which are emerging in Europe. Besides infecting humans, *A. phagocytophilum* infect a wide range of wild and domestic mammals (*1*). In Europe, the *Ixodes ricinus* tick is the main vector for the bacteria, but *A. phagocytophilum* has also been detected in association with *Rhipicephalus* and *Dermacentor* spp. ticks (*2*). The climate and biotopes of the Mediterranean region are particularly favorable for several species of ticks and, therefore, for tickborne diseases.

Although *I. ricinus* ticks are rare or absent in the Mediterranean Basin, serosurveys performed on equine populations in Camargue, southern France, indicated an *A. phagocytophilum* seroprevalence of ≈10% (*3*). To investigate the prevalence and diversity of *A. phagocytophilum* bacteria in ticks in Camargue, we collected questing ticks from horse pastures and feeding ticks from horses.

Ticks feeding on horses were collected in randomly selected stables during 2007 (84 stables), 2008 (72 stable), and 2010 (19 stables). The stables were chosen among those where evidence of *A. phagocytophilum* seroconversion in horses had been previously found (*3*). In 2008 and 2010, questing ticks were collected by the dragging method in 19 pastures, around bushes, and in areas where horses spent the most time. Surveys were conducted in the spring, which represents the peak activity time of *Ixodes* ticks.

A total of 406 adult ticks were collected, representing 6 species: *Rhipicephalus bursa, R. sanguineus, R. turanicus, R. pusillus, Dermacentor marginatus*, and *Hyalomma marginatum*. Tick species were identified by morphologic criteria and molecular analyses based on mitochondrial 12S rDNA sequences (*4*). Total DNA was extracted from the ticks by using the NucleoSpin Tissue Kit (Macherey-Nagel, Düren, Germany) (*5*). *A. phagocytophilum* was detected by nested PCR targeting the 16S rDNA (online Technical Appendix 1, wwwnc.cdc.gov/EID/article/19/5/12-1003-Techapp1.pdf).

Of the 406 ticks, 40 were infected with *A. phagocytophilum*. The infected group included ticks from all 6 collected species except *R. pusillus*. Infection rates among the species ranged from 0 to 22% (online Technical Appendix 2, wwwnc.cdc.gov/EID/article/19/5/12-1003-Techapp2.pdf). The prevalence of *A. phagocytophilum* infection did not differ significantly between species (logistic regression model, p = 0.76) but was higher among questing ticks than feeding ticks (p<0.001; odds ratio 1.15).