

資 料

# Construction of a Database for Yeast Electrophoretic Karyotype

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## <Abstract>

Electrophoretic karyotype analysis is a convincing tool for identification of the genus and the species of a yeast. Chromosomal karyotype analysis is conducted by Pulse-Field Gel Electrophoresis (PFGE). However, researcher previously had to search many karyotype data of articles for comparing a yeast karyotype with them, because electrophoretic patterns were not compiled into a database until now. There are two main reasons why a database had not been built. One is that various electrophoresis were developed as the technique of PFGE progressed, and then the properties of the migration pattern of the chromosome which each showed were different, resulting in not being able to compare it easily visually. The other is that a comparison of the DNA sequence of the specific part became easier, since the genome data of the yeast were compiled into a database and were available on the Internet. However, the genome database is still incomplete and not something which can easily compare a karyotype that is the chromosome pattern (placement of the number and the size) visually. The identification of the yeast genus and species class should be accomplished by means of not only the DNA sequence of the specific part, but also the karyotype. Thus, we decided to compile the karyotypes of yeast listed in research articles into one book and to build an original database. In the construction of the database, we decided to show the size of each chromosome using Miller's bar code type indication form which can be used by any of the electrophoretic methods that had been developed until now. The yeasts are classified as ascomycetous yeasts (18 genus) and imperfect yeasts (9 genus) by their phenotype. The database built in this report was able to publish 217 kinds of electrophoretic migration patterns in total of the kind classified into 5 ascomycetous yeast genus and 2 imperfect yeast genus. Originally, the literature data which was quoted compiled those yeasts which were distributed all over the natural world into a partial database. As a result, it followed that the databas built here covered many yeast which were distributed over the natural world although the yeasts of all genus and species classes are not published. The significance of the compiled electrophoretic karyotype of the yeast being inputted into a database by a constant but versatile method is deeply important.

Keywords: yeast karyotype, PFGE, database

## INTRODUCTION

The karyotype analysis of the yeast is conducted mainly by separation by the gel electrophoresis of the chromosome that is Pulse-Field Gel Electrophoresis (PFGE)<sup>1)</sup>. However, researcher had to search many karyotype data

of articles for comparing a yeast karyotype with them, because electrophoretic patterns were not compiled into a database until now. Besides, the electrophoretic migration pattern of the chromosome considerably distorts it because many documents data which placed karyotype of the yeast are shown using OFAGE

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(Orthogonal-Field-Alteration Gel Electrophoresis)<sup>2)</sup> developed at an initial stage. Various electrophoresis of PFGE had been developed, but the clear nature of the chromosome migration pattern was insufficient afterwards. The main electrophoresis that has been developed includes the following methods before we arrive at the CHEF (Contour-clamped Homogenous Electric Field Gel Electrophoresis)<sup>3)</sup> used most widely at present: TAFE (Transverse Alternating Field Gel Electrophoresis)<sup>4)</sup> and FIGE (Field Inversion Gel Electrophoresis)<sup>5)</sup>. The reason why a database is not built is two mainly. One is that various electrophoresis was developed as a technique of PFGE progresses and then the properties of the migration pattern of the chromosome which each showed were different, resulting that it have not being able to compare it easily visually. Other is that a comparison of the DNA sequence of the specific part became easy, since genome data of the yeast are compiled into a database<sup>6-8)</sup> and were shown on the Internet. Only the specific genus of yeast is compiled into a database, researchers have to utilize the INSDC (International Nucleotide Sequence Database Collaboration/DDBJ, GenBank, NCBI, ENA and EMBL-EBI)<sup>9)</sup> about the search of the yeast of the kind except them. However, the chromosome karyotype is an important element indicating the categorical position of the yeast class although a homology search of the DNA sequence became easy. In addition, it should be used together with DNA sequence in performing accurate identification.

Thus, we decided to compile karyotype of yeast listed in research articles in which karyotype of the yeast partially arranged and compiled, into one book and to build an original database in the unified way with the versatility. In the construction of the database, we decided to show the size of each chromosome using a bar code type indication form which was shown by Miller *et al.*<sup>10)</sup> to be able to use it by any electrophoretic methods that had been developed until now.

## MATERIALS AND METHODS

### ***Method of the illustration***

The karyotype was indicated according to the Miller's method.<sup>10)</sup> A standard line to express chromosome size by the relations of the mobility with a marker strain and calculated the size of each chromosome. One chromosome is expressed in one bar and listed it to reflect difference in size in a frame. Numbers shown over the bar code is based on kbps.

### ***Cited literatures***

All the karyotypes listed in following articles were compiled into the database listed in this study. The circled number is in the database is the same as shown below and shows the article in which the karyotype was quoted. ① indicates the article written by Miller *et al.*<sup>10)</sup> ② indicates the article written by Viljoen *et al.* (1989)<sup>11)</sup> ③ indicates the article written by Coetzee *et al.*<sup>12)</sup> ④ indicates the article written by Viljoen *et al.* (1988)<sup>13)</sup> ⑤ indicates the article written by Kaneko and Sakano.<sup>14)</sup>

### ***Abbreviation***

The genus name was abbreviated as follows. P: *Pichia*, S: *Saccharomyces*, K: *Kluyveromyces*, C: *Candida*, H: *Hansenula*, Y: *Yarrowia*, L: *Lodderomyces*.

## RESULTS

Yeast Karyotype Database (ver.1) was shown as follows.

No	Specific Name	Literature
35	<i>Pamylophila</i> CSIR-Y362	①
36	<i>Pamericana</i> CBS 5644	①
37	<i>Pmississippiensis</i> CSIR-Y306	①
38	<i>Pmississippiensis</i> CBS 7023T	①
39	<i>Peuphorbiae</i> CSIR-Y248	①
40	<i>Peuphorbiae</i> CSIR-Y250	①
41	<i>Peuphorbiae</i> CSIR-Y254	①
42	<i>Phimundalis</i> CBS-5642T	①
43	<i>Phovis</i> CBS 2616T	①
44	<i>Prhodanensis</i> CBS 5518T	①
45	<i>Prhodanensis</i> CSIR-Y315	①
46	<i>Pvernae</i> CSIR-Y357	①
47	<i>Peuphorbiaphila</i> CBS 8083T	①
48	<i>Palni</i> CBS 6986	①
49	<i>Pcanadensis</i> CSIR-Y262	①
50	<i>Pwingei</i> CSIR-Y462	①
51	<i>Pwickerbamii</i> CSIR-Y222	①

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No	Specific Name	Literature
1	<i>Pamethiona</i> var. <i>amethionina</i> NRRL-Y10978	①
2	<i>Pdeserticola</i> CBS 7119T	①
3	<i>Ppseudocactophila</i> CBS 6929	①
4	<i>Phecti</i> CBS 6930T	①
5	<i>Pnakasei</i> NRRL-Y7686T	①
6	<i>Pchambardii</i> NRRL-Y2378T	①
7	<i>Pkyurveri</i> CBS 188T	①
8	<i>Pcactophila</i> CSIR-Y1000	①
9	<i>Pfermentans</i> CSIR-Y55	①
10	<i>Pfermentans</i> CSIR-Y66	①
11	<i>Pfermentans</i> CSIR-Y178	①
12	<i>Pnorvegensis</i> NRRL-Y768T	①
13	<i>Pmembranaefaciens</i> CSIR-Y35	①
14	<i>Pmembranaefaciens</i> CSIR-Y965	①
15	<i>Pmembranaefaciens</i> CSIR-Y83	①
16	<i>Pmembranaefaciens</i> IGC 4275	①
17	<i>Pmembranaefaciens</i> CSIR-Y297	①

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No	Specific Name	Literature
52	<i>Pwickerbamii</i> CSIR-Y283	①
53	<i>Pwickerbamii</i> CSIR-Y284	①
54	<i>Pwickerbamii</i> CSIR-Y282	①
55	<i>Phispora</i> CSIR-Y372	①
56	<i>Phispora</i> CSIR-Y373	①
57	<i>Psparinae</i> CBS 6059T	①
58	<i>Pjadinii</i> CSIR-Y227	①
59	<i>Ponychis</i> CSIR-Y494	①
60	<i>Ppetersonii</i> CSIR-Y622	①
61	<i>Pfabiani</i> CSIR-Y810	①
62	<i>Pmositovora</i> CBS 8006T	①
63	<i>Psegobiensis</i> CBS 6857T	①
64	<i>Ptrehalophila</i> CSIR-Y868	①
65	<i>Pmethanoliza</i> CSIR-Y657	①
66	<i>Pmethanoliza</i> CSIR-Y656	①
67	<i>Pmethanoliza</i> CSIR-Y658	①
68	<i>Pmethanoliza</i> CSIR-Y553	①

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No	Specific Name	Literature
18	<i>Pmembranaefaciens</i> CSIR-Y296	①
19	<i>Pmembranaefaciens</i> CSIR-Y76	①
20	<i>Pmembranaefaciens</i> CSIR-Y179	①
21	<i>Phesseyi</i> NRRL-YB 4711T	①
22	<i>Pdelftensis</i> CBS 2614T	①
23	<i>Popuntiae</i> CBS 7010T	①
24	<i>Pthermotolerans</i> CBS 7012T	①
25	<i>Psalictaria</i> NRRL-Y6780T	①
26	<i>Ppastoris</i> NRRL-Y1603T	①
27	<i>Pquercuum</i> CBS 2283T	①
28	<i>Ppiperi</i> CBS 2887T	①
29	<i>Pdryadoides</i> CSIR-Y650	①
30	<i>Ppopuli</i> CBS 8094T	①
31	<i>Ptoletana</i> CBS 2504T	①
32	<i>Pmeyeriae</i> ( <i>copophila</i> ) CSIR-Y253	①
33	<i>Pmeyeriae</i> CSIR-Y245	①
34	<i>Pmeyeriae</i> CSIR-Y246	①

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1 0 3	<i>Pohmeri</i> CBS 5387	①
1 0 4	<i>Pohmeri</i> CSIR-Y568	①
1 0 5	<i>Pohmeri</i> CBS 568	①
1 0 6	<i>Prabaulensis</i> CBS 6797T	①
1 0 7	<i>Prasburgensis</i> CBS 2939	①
1 0 8	<i>Pguilhermondii</i> CSIR-Y440	①
1 0 9	<i>Pguilhermondii</i> CSIR-Y443	①
1 1 0	<i>Pmexicana</i> CBS 7066T	①
1 1 1	<i>Pscolyti</i> CBS 4802T	①
1 1 2	<i>Plyufordii</i> CSIR-Y802	①
1 1 3	<i>Pmakazae</i> var. <i>akienensis</i> CSIR-Y292	①
1 1 4	<i>Pburtonii</i> CSIR-Y608	①
1 1 5	<i>Pheimii</i> CBS 6139T	①
1 1 6	<i>Panomala</i> var. <i>schnegrii</i> CSIR-Y214	①
1 1 7	<i>Panomala</i> var. <i>anomala</i> CSIR-Y680	①
1 1 8	<i>Panomala</i> var. <i>anomala</i> CSIR-Y112	①
1 1 9	<i>Psubpolliculosa</i> CSIR-Y82	①

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6 9	<i>Pladamae</i> CSIR-Y247	①
7 0	<i>Poellobiosa</i> CSIR-Y554	①
7 1	<i>Phaplophila</i> CSIR-Y870	①
7 2	<i>Phaplophila</i> CSIR-Y233	①
7 3	<i>Phaplophila</i> CSIR-Y225	①
7 4	<i>Pfinlandica</i> CSIR-Y224	①
7 5	<i>Pfinlandica</i> CSIR-Y223	①
7 6	<i>Pphilodendra</i> CSIR-Y458	①
7 7	<i>Pmedia</i> CSIR-Y869	①
7 8	<i>Prostilla</i> CBS 6053T	①
7 9	<i>Pminuta</i> var. <i>minuta</i> NRRL-Y411T	①
8 0	<i>Pminuta</i> var. <i>nongermentans</i> CBS 5764T	①
8 1	<i>Pheiricii</i> CBS 5765T	①
8 2	<i>Pglucosoma</i> CSIR-Y964	①
8 3	<i>Pcapsulata</i> CSIR-Y133	①
8 4	<i>Ppinus</i> CBS 744T	①
8 5	<i>Pharinos</i> CSIR-Y836	①

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1 2 0	<i>Pciferrii</i> CSIR-Y804	①
1 2 1	<i>Paydoviorum</i> CSIR-Y805	①
1 2 2	<i>Scerevisiae</i> UOFS-Y2	②
1 2 3	<i>Scerevisiae</i> CBS 1171	②
1 2 4	<i>Kphaffii</i> CBS 4417	②
1 2 5	<i>Scerevisiae</i> UOFS-Y290	②
1 2 6	<i>Klodderae</i> CBS 2757	②
1 2 7	<i>Sunisporus</i> UOFS-Y386	②
1 2 8	<i>Kafricanus</i> CBS 2654	②
1 2 9	<i>Kpolysporus</i> CBS 6899	②
1 3 0	<i>Cglabrata</i> UOFS-Y164	②
1 3 1	<i>Sexigius</i> UOFS-Y348	②
1 3 2	<i>Kdelphensis</i> UOFS-Y100	②
1 3 3	<i>Chumilis</i> CBS 5658	②
1 3 4	<i>Pguilhermondii</i> UOFS-Y440	②
1 3 5	<i>Cguilhermondii</i> UOFS-Y47	②
1 3 6	<i>Pohmeri</i> UOFS-Y444	②

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8 6	<i>Pharinos</i> CSIR-Y226	①
8 7	<i>Psorbiotophila</i> CSIR-Y170	①
8 8	<i>Psorbiotophila</i> CSIR-Y169	①
8 9	<i>Pmusciola</i> CBS 5800T	①
9 0	<i>Psilvicola</i> CSIR-Y521	①
9 1	<i>Panguishii</i> CSIR-Y286	①
9 2	<i>Ptriangularis</i> CBS 4094	①
9 3	<i>Pacaciae</i> NRRL-Y7117T	①
9 4	<i>Pphillogaea</i> CSIR-Y801	①
9 5	<i>Pphillogaea</i> CSIR-Y800	①
9 6	<i>Pstipitis</i> CSIR-Y567	①
9 7	<i>Pstipitis</i> CSIR-Y633	①
9 8	<i>Pholstii</i> SBS 4140T	①
9 9	<i>Pangusta</i> CSIR-Y264	①
1 0 0	<i>Pangusta</i> CSIR-Y266	①
1 0 1	<i>Pohmeri</i> CSIR-Y559	①
1 0 2	<i>Pohmeri</i> CSIR-Y444	①

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171	<i>C.pararugosa</i> CBS 1010	②
172	<i>C.blankii</i> CBS 1898	②
173	<i>S.kluyveri</i> CSIR-Y412	③
174	<i>S.kluyveri</i> CSIR-Y414	③
175	<i>S.unisporus</i> CSIR-Y386	③
176	<i>S.unisporus</i> CSIR-Y550	③
177	<i>S.exiguus</i> CSIR-Y348	③
178	<i>S.exiguus</i> CSIR-Y572	③
179	<i>S.cerevisiae</i> CSIR-Y290	③
180	<i>S.cerevisiae</i> CSIR-Y2	③
181	<i>H.jadinii</i> UOFS-Y227	④
182	<i>Cutulis</i> CBS 890	④
183	<i>C.shehatae</i> UOFA-Y492	④
184	<i>Patipitis</i> UOFS-Y633	④
185	<i>C.pseudotropicalis</i> UOFS-Y320	④
186	<i>K.marianus</i> CBS 1556	④
187	<i>C.kefir</i> UOFS-Y879	④

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137	<i>C.glabrosa</i> UOFS-Y581	②
138	<i>K.thermotolerans</i> UOFS-Y163	②
139	<i>C.kefir</i> UOFS-Y879	②
140	<i>K.fragilis</i> CBS 1556	②
141	<i>C.pseudotropicalis</i> UOFS-Y320	②
142	<i>C.castellii</i> CBS 4332	②
143	<i>C.shehatae</i> UOFS-Y492	②
144	<i>P.stipiti</i> UOFS-Y663	②
145	<i>C.carissilignicola</i> CBS 8001	②
146	<i>S.kluyveri</i> UOFS-Y412	②
147	<i>C.poludigena</i> CBS 8005	②
148	<i>Phaplophila</i> UOFS-Y225	②
149	<i>P.sorbitophila</i> UOFS-Y169	②
150	<i>C.schatavii</i> CBS 6452	②
151	<i>C.wickerhamii</i> CBS 2745	②
152	<i>C.silvicultrix</i> UOFS-Y481	②
153	<i>P.mexicana</i> CBS 7066	②

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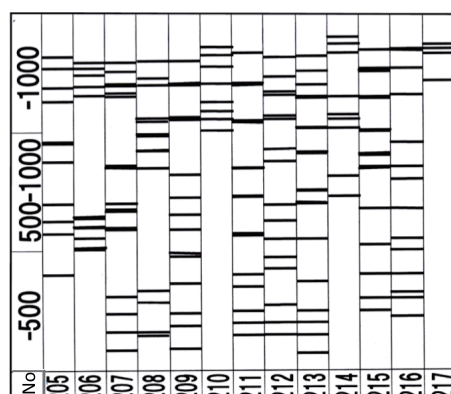
No	Specific Name	Literature <sup>a</sup>
188	<i>H.anomala</i> CBS 605	④
189	<i>C.pelliculosa</i> UOFS-Y5	④
190	<i>C.lipolytica</i> CBS 599	④
191	<i>Y.lipolytica</i> UOFS-Y28	④
192	<i>L.elongisporus</i> UOFS-Y162	④
193	<i>C.parapsilosis</i> UOFS-Y685	④
194	<i>S.dairensis</i> IFO 0211	⑤
195	<i>S.telluris</i> IFO 1331	⑤
196	<i>S.unisporus</i> IFO 0316	⑤
197	<i>S.cerevisiae</i> SH964	⑤
198	<i>S.uvarum</i> IFO 0615	⑤
199	<i>S.steineri</i> IFO 0253	⑤
200	<i>S.oleaginosus</i> IFO 1998	⑤
201	<i>S.oleaceus</i> IFO1997	⑤
202	<i>S.norbornensis</i> IFO 1836	⑤
203	<i>S.inusitatus</i> IFO 1343	⑤
204	<i>S.hiempiensis</i> IFO 1994	⑤

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154	<i>K.blattae</i> UOFS-Y837	②
155	<i>C.tenus</i> UOFS-Y602	②
156	<i>C.cacaoi</i> CBS 2020	②
157	<i>P.farinosa</i> UOFS-Y226	②
158	<i>P.scholyti</i> CBS 4802	②
159	<i>P.parsonii</i> NRRL-Y4275	②
160	<i>C.norvegica</i> UOFS-Y384	②
161	<i>C.atmosphaerica</i> CBS 4547	②
162	<i>C.holmii</i> UOFS-Y441	②
163	<i>C.steatolytica</i> UOFS-Y663	②
164	<i>C.inconspicua</i> UOFS-Y389	②
165	<i>C.maltosa</i> CBS 5611	②
166	<i>P.etchellii</i> UOFS-Y868	②
167	<i>C.berthetii</i> UOFS-Y464	②
168	<i>K.marianus</i> UOFS-Y808	②
169	<i>C.cervautii</i> UOFS-Y127	②
170	<i>C.fermenticicans</i> UOFS-Y854	②

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205	<i>S.heterogenicus</i> IFO 1620	⑤
206	<i>S.globosus</i> IFO 0254	⑤
207	<i>S.diastaticus</i> IFO 1015	⑤
208	<i>S.oreanus</i> IFO 1833	⑤
209	<i>S.chevalieri</i> IFO 0210	⑤
210	<i>S.servazzii</i> IFO 1838	⑤
211	<i>S.sapensis</i> IFO 1991	⑤
212	<i>S.hayanus</i> IFO 1127	⑤
213	<i>S.aceti</i> IFO 10055	⑤
214	<i>S.exiguus</i> IFO 1128	⑤
215	<i>S.prostosedonii</i> IFO 1837	⑤
216	<i>S.carisbergensis</i> IFO 1167	⑤
217	<i>S.kluveri</i> IFO 1685	⑤



## DISCUSSION

The yeast is classified in ascomycetous yeast (18 genus) and imperfect yeast (9 genus) by a phenotype. The names of 18 genus of ascomycetous yeast is as follows: *Saccharomyces*, *Saccharomycopsis*, *Lipomyces*, *Schwanniomyces*, *Debaryomyces*, *Citeromyces*, *Saccharomycopsis*, *Hansenula*, *Pichia*, *Hyphopichia*, *Stephanoascus*, *Pachysolen*, *Kluyveromyces*, *Lodderomyces*, *Wickerhamiella*, *Wingea*, *Metschnikowia* and *Nematospora*. The names of 9 genus of imperfect yeast is as follows: *Candida*, *Oosporidium*, *Trichosporon*, *Phaffia*, *Sympodiomyces*, *Torulopsis*, *Cryptococcus*, *Rhodotorula* and *Selenozyma*. The database built in this report was able to publish 217 kinds of electrophoretic migration patterns in total about a kind classified in 5 ascomycetous yeast genus (*Saccharomyces*, *Hansenula*, *Pichia*, *Kluyveromyces* and *Lodderomyces*) and 2 imperfect yeast genus (*Candida* and *Torulopsis*). Originally the literature data which was quoted compiled the yeast which was distributed a lot

over the natural world into a database partially. As a result, it followed that the database which built here covered much yeast which were distributed over the natural world although the yeast of all genus and species class are not published. The significance that compiled electrophoretic karyotype of the yeast into a database by a constant method with the versatility is deeply imprtant.

There are 41 species are classified for *Sacchamycetes* genus by Lodder.<sup>15)</sup> 24 strains were recorded in this database (as indicated in No.194-217). The phenotypic classification suggests that 4 groups exist in *Saccharomyces cerevisiae*<sup>16)</sup>, several strains show in their karyotypes the slight polymorphism in the length of chromosomes as indicated in No. 179, 180 and 197 in spite of that they are the same strain. There is such example that in spite of the different genus No.156 (*C.cacaoi*) shows very similar karyotype with No.157 (*P.farinose*). On literature investigation<sup>16)</sup>, it is understood that No. 157 (*P.farinose*) is the sexual generation state of No.156 (*C.cacaoi*). It is observed a similar thing between No. 134 (*P.guilliermondii*) and No.135 (*C.guilliermondii*). It is not a same genus class, but it may be thought that the probability that the strain showing a similar band pattern shows the difference of sexual or asexual generation is high. No.41 (*P.euphorbiae*) shows the pattern that is totally different from No.39 (*P.euphorbiae*) and No.40 (*P.euphorbiae*) in domains more than 1000kbps that is a same genus class. *P.euphorbiae* may be a typical case to show a different classification result in karyotype and phenotype. 132 strains of *Pichia* genus were recorded in this data base. They have very small numbers of chromosome different, indicating evolutionarily very far from *Saccharomyces* genus or *Kluyveromyces* genus.

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## 酵母電気泳動核型のデータベースの構築

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### ＜要 旨＞

酵母の電気泳動核型分析は、酵母の属種の同定を行うための有力なツールである。酵母においては、染色体の核型分析はパルスフィールドゲル電気泳動法（PFGE, Pulse-Field Gel Electrophoresis）によってなされる。しかしこれまで電気泳動パターンがデータベース化されていないので、研究者が酵母の核型を照らし合わせるためには、いくつかの文献データを調べて照合しなければならなかった。データベースが構築されていない理由は主に二つある。ひとつは、PFGEの技術が進歩するにつれてさまざまな電気泳動法が開発され、それぞれが示す染色体の泳動パターンの性質が異なるために、視覚的に容易に比較できなかったことである。もうひとつは、酵母のゲノムデータがデータベース化されてインターネット上に公開されたため、特定の部位のDNA配列の比較が容易になったことである。しかしながら、ゲノムデータベースは、核型、つまり染色体パターン（本数と大きさの配置）を容易に視覚的に比較できるものではない。酵母属種の同定は、特定の部位のDNA配列だけでなく、核型によっても併行して遂行されるべきものである。そこでわれわれは既出の学術文献に記載された酵母の核型を集大成して独自のデータベースを構築することにした。データベースの構築にあたっては、これまで開発されてきたどのような泳動法によっても利用し得るものとするために、各々の染色体の大きさをMillerのバーコード型表示形式を用いて示すことにした。酵母は表現型によって子囊酵母（18属）および不完全酵母（9属）に分類されるが、今回構築されたデータベースには、子囊酵母5属と、不完全酵母2属に分類される種について、合計217種の泳動パターンを収載することができた。引用した文献データが、もともと自然界に多く分布する酵母を部分的にデータベース化したものであったため、結果的に今回構築したデータベースは、すべての属種の酵母を収載しているわけではないものの、自然界に分布する多くの酵母をカバーする結果となった。酵母の電気泳動核型を、汎用性のある一定の方法でデータベース化した意義は大きい。

**キーワード：**酵母核型、PFGE、データベース

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