Extremophiles (2000) 4:267–274

ORIGINAL PAPER

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Genetic diversity analysis of *Rhodothermus* reflects geographical origin of the isolates

Received: January 21, 2000 / Accepted: April 27, 2000

Abstract The genetic diversity and relationships of 81 *Rhodothermus* isolates from different geothermal environments in Iceland were examined by analysis of electrophoretically demonstrable allelic variation of 13 genes encoding enzymes. All the enzymes were polymorphic. A total of 71 distinctive multilocus genotypes (electrophoretic types, ETs) were identified. The mean genetic diversity per locus (H_t) was 0.586. The relatively high genetic variance observed within *Rhodothermus* isolates from different locations is most likely the result of genetic changes occurring independently in the locations studied. A high G_{st} value (0.284) indicates that a considerable part of the variance observed is due to differences between locations. Cluster analysis revealed two major groups of ET clusters diverging at a genetic distance of 0.75, reflecting strongly the geographic origin of isolates. Estimation of the association index (I_A) indicates that *Rhodothermus marinus* is a clonal species in which recombination events occur rarely. Partial or whole sequencing of the 16*S* rRNA genes of *Rhodothermus* isolates grouping at genetic distance of 0.40 confirmed that all the isolates belonged to the species *Rhodothermus marinus*. The results of this study confirm that, despite phylogenetic and phenotypic similarity, genetic diversity within *Rhodothermus marinus* is quite high.

Key words Multilocus enzyme electrophoresis (MEE) · Genetic diversity · Geographic population · 16*S* rRNA sequencing · *Rhodothermus marinus*

Communicated by W.D. Grant

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Introduction

Rhodothermus marinus is a gram-negative eubacterium originally isolated from a submarine alkaline hot spring in Isafjarðardjúp in northwest Iceland (Alfredsson et al. 1988). It is obligately aerobic, moderately halophilic, with optimum growth at 65°C and 2% NaCl. It does not grow without salt added to the medium. By 16*S* rRNA gene sequencing of *R. marinus* (type strain DSM 4252), it has been placed in the *Cytophaga–Flexibacter–Bacterioides* group (Andrésson and Fridjonsson 1994; GenBank 95014058). *R. marinus* has also been isolated from shallow marine hot springs in St. Miguel in the Azores (Nunes et al. 1992) and from hot springs in Naples in Italy (Moreira et al. 1996). Whole genomic DNA–DNA hybridization on *Rhodothermus* strains from the Azores, Naples, and the type strain from Iceland has revealed 77%–84% homology, indicating that they all belong to the same species (Moreira et al. 1996). Another species of the genus, *Rhodothermus obamensis* (EMBL X95071), was isolated from Tachibana Bay in Japan (Sako et al. 1996).

Several methods have been introduced to estimate genetic variation in bacterial populations, most of them based on DNA analysis such as restriction fragment-length polymorphism (RFLP) of 16*S* rDNA and random amplification of polymorphic DNA (RAPD), the former using different restriction enzymes in examining the variability at one locus but the latter aimed at the total genome. For estimating the genetic diversity of *R. marinus* we used multilocus enzyme electrophoresis (MEE), which has increasingly been accepted as a tool to explore genetic variation within bacterial species (Selander et al. 1986). A major advantage of MEE for the study of genetic population structure is that it covers more than one locus and the observed variation is usually clear (Young et al. 1987).

Thermophilic habitats are confined to small areas that may be separated by great distances. They can, therefore, be defined ecologically as islands with large dispersal barriers. This habitat structure provides a unique

opportunity to study the mechanisms and species divergence of the microbial world, the importance of selective pressures, and the extent of gene flow within a particular habitat or over greater distances. MEE is particularly suitable for population studies. The method is relatively fast and inexpensive, and many samples can be processed in parallel. Allelic differences are easily detectable, and genetic diversity is estimated on the basis of more than one locus. Statistical methods for analyzing the data are also well developed for estimating the influence of geography on population structure and population parameters such as genetic diversity, genetic distances, clonality, and gene flow (Selander et al. 1986).

It is commonly accepted that most bacterial species are clonal, that is, the lineages differing at many chromosomal genetic loci coexist over a wide geographic range with little recombination between them (Young et al. 1987). In recent years, this idea has been disputed (Istock et al. 1992; Souza et al. 1992), and attempts have been made to answer the questions as to how clonal bacteria really are (Maynard-Smith et al. 1993) and to what extent genetic exchange can influence genetic variation within a population and prevent adaptive divergence between bacterial populations (Cohan 1996). Population genetics and the extent of clonality of several bacterial species studied by MEE suggest that not all bacteria are equally clonal. Some species are clonal, i.e., showing high linkage disequilibrium, such as *Salmonella* (Maynard-Smith 1995). Other species studied are nonclonal or show some intraspecies recombination, such as *Bacillus subtilis* (Istock et al. 1992), *Neisseria gonorrhoeae* (O'Rourke and Stevens 1993), *Rhizobium leguminosarum* (Souza et al. 1992), and *Burkholderia cepacia* (Wise et al. 1995).

The genus *Rhodothermus* has become increasingly popular as a research subject, and members of the genus appear to be a good source for many thermostable enzymes. However, the ecology and population structure of the species have not been much studied apart from the taxonomic studies of Moreira et al. (1996) previously mentioned. In this study, genetic diversity within 81 *Rhodothermus marinus* strains from four different saline geothermal sites in Iceland was studied by analysis of electrophoretically demonstrable allelic variation of 13 genes encoding enzymes. Two additional strains from the Azores were also analyzed. An attempt was made to answer to what extent *R. marinus* is a clonal species and to estimate which factors influence the genetic structure of the species.

Materials and methods

Isolation and growth media

Samples were collected from four different geothermal sites in Iceland. These locations were coastal springs at Reykjanes in Isafjardardjup in northwest Iceland, from which the type strain of *R. marinus* was isolated; an effluent from the geothermal powerplant at the Blue Lagoon in southwest Iceland; the effluent from the Saltfactory at Reykjanes in southwest Iceland; and from coastal springs and effluent from a borehole in Oxarfjordur in northeast Iceland (Fig. 1).

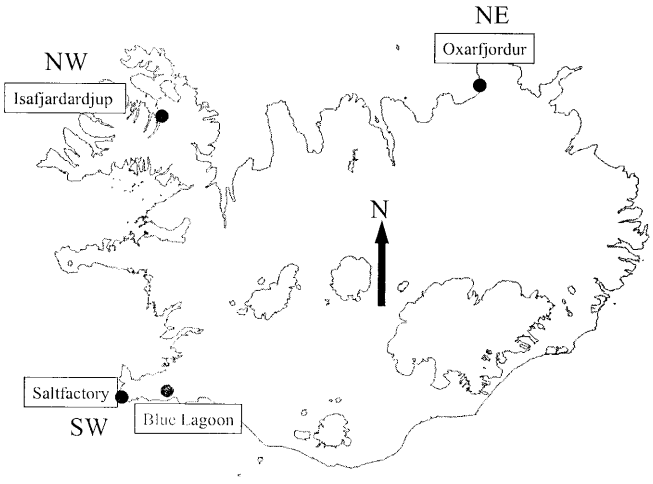

Fig. 1. Map of Iceland showing the origin of *Rhodothermus marinus* isolates in this study

Isolation of strains was done as previously described (Alfredsson et al. 1987). The antibiotic pattern of *R. marinus*, i.e., resistance to the aminoglycosides streptomycin, kanamycin, and gentamycin, was used as initial identification of *R. marinus* strains.

Preparation of samples for MEE

The strains were cultivated at 65°C in 250 ml of medium 162 (Degryse et al. 1978) containing 1% NaCl. The cultures were centrifuged at 11 080 *g* for 10 min, the cells were suspended in TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0) 5 ml g^{-1}, and the cell wall was ruptured in a French press (700 psi). The crude extract was centrifuged twice at 37 123 *g* in a Sorvall SS-34 for 30 min and the supernatant collected. Before use, the samples were centrifuged at 21 700 *g* for 30 min. Supernatant and loading buffer (7.0 ml, 4× stacking gel buffer; 3.0 ml glycerol, 1.2 mg bromophenol blue) were mixed in 1:3 ratio before loading on gels.

Electrophoresis and staining of gels

The samples were run on 7.5% polyacrylamide gels (Sigma A-6050) at 30–40 mA and unlimited voltage. Enzyme staining has been described elsewhere (Selander et al. 1986). The 13 enzymes assayed were adenosine deaminase (ADA), aspartate aminotransferase (AAT), alkaline phosphatase (AP), glucose-6-phosphate isomerases 1 and 2 (GPI-1, GPI-2), isocitrate dehydrogenases 1 and 2 (IDH-1, IDH-2), malate dehydrogenase (MDH), nucleoside phosphorylase (NPH), phosphoglucomutase (PGM), superoxide dismutase (SOD), and starch-degrading enzymes 1 and 2 (ST-1, ST-2). Comparison of the mobilities of enzymes

from different isolates were made visually, as described by Selander et al. (1986).

Electromorphs of each enzyme were given numbers in the order of decreasing anodal mobility. The numbers are equated with alleles at the corresponding structural gene locus. An absence of enzyme activity is attributed to a null allele. Distinctive combination of alleles over the 13 enzyme loci (multilocus genotypes) is obtained for each strain that is designated as its electrophoretic type (ET).

Statistical analysis

Genetic diversity and relationships among 81 *Rhodothermus* strains characterized by multilocus enzyme electrophoresis (MEE) were analyzed as previously described by Selander et al. (1986) using computer software ETDIV and ETCLUS by Dr. T.S. Whittam (Whittam 1986). Genetic diversity at an enzyme locus among either ETs or isolates was calculated from the allele frequencies among ETs or isolates as $h = (1 - \Sigma x_i^2)(n/n - 1)$ where x_i is the frequency of the ith allele and n is the number of ETs or isolates. Mean genetic diversity (H) is the arithmetic average of h values over all loci. H_S, the within-group diversity, was calculated as the mean of the diversity values obtained for the separate sampling sites (subpopulations). Total genetic diversity, H_T, is the diversity value calculated for the population as a whole. For a subdivided population, the total diversity H_T will be greater than the diversity within subpopulations. Nei's coefficient of genetic differentiation (Nei and Chesser 1983), G_{ST}, was then calculated as $(H_T - H_S)/H_T$ (Whittam 1986) using the ETDIV software. This coefficient indicates how large a proportion of the overall variation is the result of differences between subsamples. We prepared a dendrogram based on the average linkage algorithm of all the isolates with the Azores strains included. Distance was measured as the proportion of mismatched loci between pairs of ETs. Genetic distance between pairs of ETs was expressed as the proportion of enzyme loci at which dissimilar alleles occurred (mismatches), and clustering of ETs was performed from a matrix of genetic distances by the average linkage method (UPGMA) (Caugant et al. 1987).

Multilocus linkage disequilibrium is calculated on the basis of the distribution of allelic mismatches between pairs of bacterial isolates over all the loci examined. The ratio of the observed variance in mismatches, V_O, to the expected variance in a corresponding population at linkage equilibrium, V_E, provides a measure of multilocus linkage disequilbrium that can be expressed as the index of association (I_A). Linkage disequilibrium between two geographically distant populations was measured by calculating I_A between loci by $I_A = V_O/V_E - 1$ (Maynard Smith et al. 1993) using the ETLINK computer program (Whittam 1986).

16*S* rDNA PCR/RFLP

A total of 28 representative strains were selected randomly from ET clusters formed at less than 0.40 genetic distance. DNA was isolated directly from cells with Dynal magnetic beads as described by the manufacturer. Amplification of the 16*S* rRNA gene was done in 40-µl volumes using universal Eubacterial primers F9 (5′-CCGAATTCGTCG ACAAGAGTTTGATCCTGGCTCAG-3′) and R1544 (5′-CCCGGGATCCAAGCTTAGAAAGGAGGTGAT CCA-3′) and Dynazyme polymerase (Finnzymes). To confirm the purity and size of the PCR products, they were run on agarose gels (1%) with lambda *Hin*dIII (Pharmacia) as a marker. The PCR products were digested in 23-µl volumes using five different restriction endonucleases: *Rsa*I, *Alu*I, *Eco*RI, *Hae*III, and *Hin*fI (Pharmacia) as described by the manufacturer. The choice of restriction endonucleases was based on the 16*S* rRNA restriction map of *R. marinus* type strain (Andrésson and Fridjonsson 1994). Restriction fragments were run on polyacrylamide gels (12.5%) at 30 mA and unlimited voltage for 18 h. The gels were stained with ethidium bromide, and restriction fragment patterns were visualized by UV.

Sequencing of 16*S* rRNA genes

Sequencing of 16*S* rRNA genes was done on representative strains from the major groups revealed by MEE. The primers used were F9 (5′-CCGAATTCGTCGACAAG AGTTTGATCCTGGCTCAG-3′), F515 (5′-GTGCCAGCAGCCGCGGTAAATAC-3′), R357 (5′-CTGCTGCCXCCCGTAGG-3′) (x = inosine), R805 (5′-GACTACCCGGGTATCTAATCC-3′), R1195 (5′-GACGTCXTCCCCXCCTTCCTC-3′) (x = inosine), R1510 (5′-GGTTACCTTGTTACGACTT-3′), and R1544 (5′-CCCGGGATCCAAGCTTAGAAAGGAGGTGA TCCA-3′). Cycle sequencing was performed on a Perkin-Elmer automatic sequencer using DNA sequencing kit with dRhodamine terminator (Perkin-Elmer, Norwalk, CT, USA).

Results

Origin of isolates

A total of 83 *R. marinus* strains were used in this study, 81 strains from Iceland and 2 strains from the Azores. The sample sites in this study were all coastal, but differed in proportions and in physicochemical conditions (Table 1). The sample site on the northwest (NW) coast was a large geothermal area on the coast and subjected to tidal variations. Furthermore, it was different from the other

Table 1. Sample sites, number of isolates, and environmental factors

Sample site	No. of isolates	T (°C)	pH	Salinity (%)
Isafjordur (NW)	38	75–95	7	0.5–3
Blue Lagoon (SW)	19	36–70	7.3–7.6	2.2–3.8
Saltfactory (SW)	18	55–70	6.7–6.9	5.6–5.8
Oxarfjordur (NE)	6	40–85	6	0.2–0.4

sites in having a typical and abundant coastal algal vegetation. Two of the strains in this study were isolated originally from this site (Alfredsson et al. 1988), i.e., the type strains DSM 4252 and ITI-378. In this study, we isolated 36 additional strains from this same site.

The other two sites were both saline effluents from coastal boreholes. The southern site effluents formed large thermal ponds with high silica concentration and with a profusion of Cyanobacteria as primary producers. Eighteen strains were isolated from the effluent of the Saltfactory in southwest Iceland and 19 strains from the effluent of the powerplant in Svartsengi in southwest Iceland (the Blue Lagoon), jointly discussed as SW strains. Finally, 6 strains came from an effluent of a borehole in northeast Iceland forming a small stream or trickle on a sandy shore (see Fig. 1). Two additional strains isolated from the Azores were used for part of the study. All the strains showed the characteristic aminoglycoside resistance pattern of *R. marinus* (Alfredsson et al. 1988).

Genetic diversity among isolates

All the 13 enzyme loci studied were polymorphic, and the number of alleles ranged from 3 for SOD and GP-2 to 8 for ST-1. The average number of alleles per locus was 5.5 (Table 2). Among the 81 Icelandic isolates, 71 unique ETs were identified (Table 3). Eight ETs had multiple isolates but included only 2 to 3 isolates, which were always of the same origin.

Genetic diversity based on allele frequencies for individual loci is listed in Table 2. The most diverse locus was ST-1 (0.795) and the least diverse was SOD (0.040). The mean genetic diversity (H_t) for the population as a whole was 0.586. The interregional component of diversity, G_{st}, was 0.284 (Table 2), indicating a considerable degree of regional differentiation. G_{st} values for individual loci vary considerably, or from 0 for the SOD to 0.671 for GP-2.

Genetic relationships of ETs

Cluster analysis of all strains revealed two major groups of ETs diverging at a genetic distance of 0.75 (Fig. 2). Almost all the NW isolates are in one major group, reflecting the geographic origin of the isolates. Other isolates form the other major group, which consists more or less of three subgroups (Fig. 2; Table 4): a distinct subgroup of red isolates from the SW sites diverging at 0.5 (F; Fig. 2, Table 4), and two distinct subgroups of colorless isolates that reflect their geographic origin, one from the Saltfactory (SW) diverging at 0.55 (D; Fig. 2, Table 4) and the other including all the *R. marinus* isolates from the NE site diverging at 0.5 (E; Fig. 2, Table 4). The phenotypic trait of colorlessness found among the isolates from the SW and NE sites is not found among NW isolates.

The connection of geographic origin and clustering is visible, as summarized in Table 4. The two Azores isolates diverge at almost 0.6 within the SW/NE isolates, and one of them forms a branch (I; Fig. 2, Table 4) together with three of the SW strains at 0.45.

Geographic variation

Mean genetic diversity within locations was higher among the ETs of NW isolates (0.513) than SW isolates (0.477). It was considerably lower at the NE site, or 0.269 (Table 3).

Table 2. Genetic diversity for different loci

Locus	No. of alleles	H_s	H_t	G_{st}
NPH	6	0.728	0.783	0.070
ST1	8	0.746	0.795	0.061
ST2	6	0.445	0.678	0.344
ALP	6	0.274	0.566	0.516
GP1	5	0.461	0.684	0.326
GP2	3	0.139	0.422	0.671
ADA	7	0.436	0.642	0.320
SOD	3	0.040	0.040	0.000
PGM	4	0.479	0.675	0.290
MDH	6	0.275	0.344	0.203
ID1	5	0.457	0.617	0.259
ID2	5	0.313	0.612	0.489
AAT	7	0.663	0.758	0.124
Mean	5.5	0.420	0.586	0.284

The index of genetic diversity for a locus is given by $h = n(1 - \Sigma x_i^2)/(n - 1)$, where x is the frequency of the i^{th} allele at the locus, n is the number of isolates in the sample, and $n/(n - 1)$ is a correction for samples (Selander et al. 1986). H_S represents the index of diversity across subpopulations; H_T represents the index of diversity for the pooled population, and G_{ST} is Nei's coefficient of gene differentiation, calculated as $(H_T - H_S)/H_T$ (Whittam 1986)

Table 3. Genetic diversity within locations

Location	No. of strains	No. of ETs	Polymorphic loci	Mean no. of alleles	ETs (H)	Isolates (H)
SW (Blue Lagoon & Saltfactory)	37	33	1.00	4.00	0.477	0.460
NW (Isafjardardjup)	38	33	1.00	4.08	0.513	0.484
NE (Oxarfjordur)	6	5	0.46	1.62	0.269	0.241
Total	81	71	–	–	–	–

ET, electrophoretic type

Table 4. Origin of isolates clustering at 0.5 in the dendrogram (Fig. 2)

Group	Ísafjordur (NW)	Blue Lagoon (SW)	Saltfactory (SW)	Öxarfjordur (NE)	Azor	Total
A	1					1
B	1					1
C		1	1			2
D			9[a]			9
E				6[a]		6
F	1	16	5			22
G		1				1
H		1				1
I			3		1	4
J					1	1
K	2					2
L	4					4
M	29					29
Total	38	19	18	6	2	83

[a] Colorless isolates

Table 5. Degree of differentiation (G_{st}) between locations

Location	NE (Oxarfjordur)	NW (Isafjardardjup)
SW (Blue Lagoon and Saltfactory)	$G_{st} = 0.182$	$G_{st} = 0.209$
NE (Oxarfjordur)		$G_{st} = 0.286$

When G_{st} is calculated for two populations at a time, the resulting values are still quite high, but lower for SW strains compared to NE strains than for any other possible pair, indicating a lower degree of differentiation between these two populations (Table 5).

Linkage disequilibrium

Measurements of linkage disequilibrium by calculating the index of association (I_A) of *R. marinus* was done separately for the SW populations and the NW population. I_A is near zero as a population approaches linkage equilibrium and increases in value with increasing multilocus linkage disequilibrium (Whittam 1995). I_A values obtained for the two populations were 0.75 for the SW populations and 1.47 for the NW population, indicating a clonal species (Maynard Smith 1995).

Previous classification studies

Geographic classification of the isolates was observed earlier using the RAPD method in a study on 20 strains using 20 different oligonucleotide primers (data not shown). The consistency of these two methods, i.e., MEE and RAPD, has been mentioned elsewhere in studies on *Burkholderia ceparia* (Cello et al. 1997). Results from tests on utilization of nine different polysaccharides did not reflect the genetic clusters or origins obtained by MEE and RAPD (data not shown), nor did results of a numerical taxonomy study using 43 different phenotypic tests on 24 of the isolates. The only phenotypic trait that could be assigned to specific clusters obtained by MEE studies was colorlessness. All other traits were sporadically distributed (data not shown).

16*S* rDNA/RFLP and 16*S* rRNA sequencing

Restriction fragment-length patterns (RFLP) analysis with five different restriction endonucleases on 16*S* rDNA PCR products from 28 representative strains chosen from 15 ET clusters showed identical fragment size patterns for all the strains (data not shown). Partial or whole sequencing of the 16*S* rRNA gene of representative isolates revealed almost identical sequences.

Discussion

Multilocus enzyme electrophoresis analysis is a reliable tool to quantitatively estimate genetic diversity of a species and to assess genetic differences between different geographic populations. It is also a valuable method for estimating the extent of lateral gene transfer within a species. The study of allelic variation by MEE has yielded significant insights into the genetic diversity and population structure of various bacteria and appears to be particularly suitable for the study of divergence of bacterial populations (Maynard Smith 1995). In this study, we examined the phylogenetic relationships, phenotypic properties, and allelic variation of 13 loci in three geographically distant populations of *Rhodothermus marinus* in Iceland.

Level of recombination

Lateral gene transfer or interspecies recombination may be very important for the transfer of a selectively advantageous trait within a species and between species. Generally, recombination acts to lower the genetic diversity within a species (Cohan 1996). It has been shown to act with a

varying degree within different bacterial species, from being very low among the Enterobacteria such as *E. coli* and *Salmonella* to being very high in *Neisseria* and natural isolates of *Bacillus subtilis* (Maynard Smith 1995). This study indicates that the extent of recombination within *Rhodothermus marinus* is low. The I_A values obtained for the two largest study populations were 0.75 for the SW populations and 1.47 for the NW population. Consequently, lateral gene transfer does not seem to be an important mechanism for shaping the genetic structure of the species. *Rhodothermus marinus* appears to be predominantly a clonal species, which by itself should result in increased genetic diversity in the population as a whole. The genetic diversity for the whole population was H_t 0.586, which is relatively high compared to other species (Souza et al. 1999).

Genetic diversity

A similar degree of genetic diversity was observed within the populations from the NW and SW sites, 0.48 and 0.46, respectively, but was considerably lower, 0.241, for the NE site (Table 3). Genetic diversity (H_t) for the population of *Rhodothermus marinus* as a whole was significantly higher, 0.586 on average (Table 2). One locus, SOD, was however notably less diverse than the others with H_t of 0.04, confirming the well-known structural conservation of superoxide dismutase among Bacteria (May and Dennis 1989; Joshi and Dennis 1993).

Geographic variation

The allelic distribution within the species is clearly discontinous and is apparently influenced by geography. This characteristic is reflected by the significantly higher average H_t value for the population as a whole compared to the intersite diversities and is also quite clearly shown by the high G_{st} value between the populations, ranging from 0.18 to 0.29 (Table 5). These values show that the most similar populations (SW and NE) differ by 18% in allele composition and the least similar populations (NW and NE) by close to 30%. These results clearly reveal that the populations at the different sites are evolving independently of each other and indicate that genetic drift in conjunction with geographic isolation is an important mechanism of species divergence. The very different genotypes, or ETs, obtained at each sample site also indicate that migration events are rare or that colonization is prevented by the locally adapted strains which outcompete incoming strains.

Clustering of the isolates

The geographic distinctiveness of the populations is also revealed by the genetic relationships of *R. marinus* strains calculated on the basis of the MEE data. The isolates fall into two main clusters separated by a genetic distance of 0.75, corresponding to 10 of 13 different loci between the

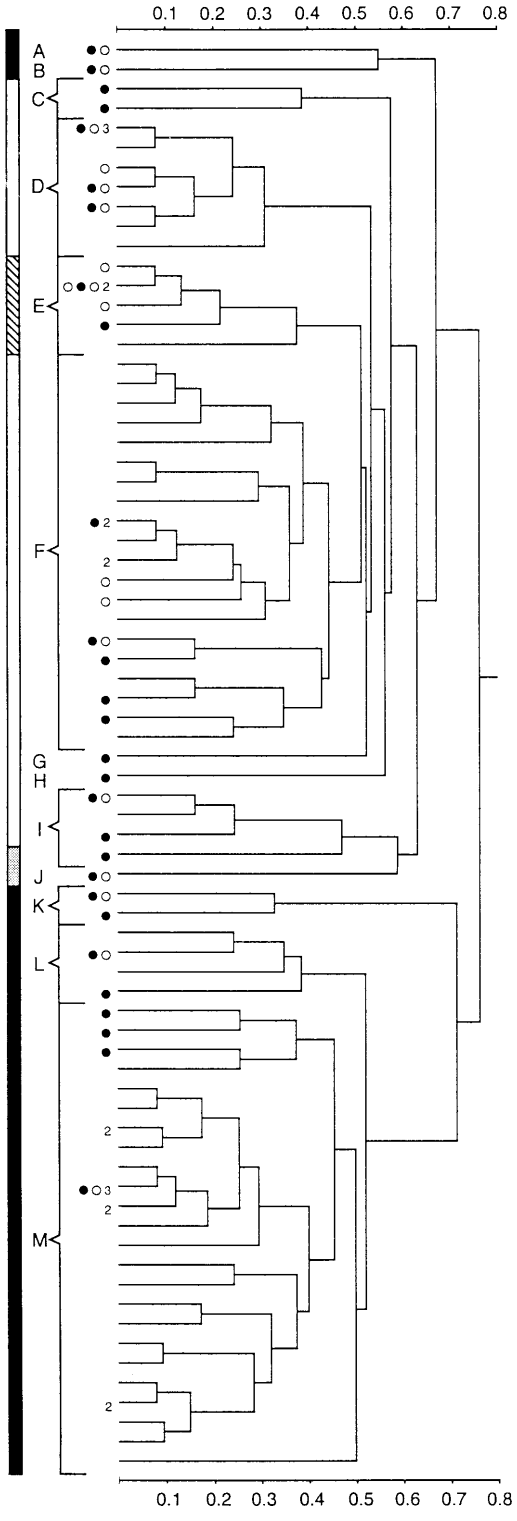

Fig. 2. Clustering of 83 *Rhodothermus* isolates by MEE. Geographic clustering of the isolates is shown: ■, NW isolates; □, SW isolates; ▨, NE isolates; ⊡, Azores isolates. A–M show clusters formed at less than 0.5 genetic distance. ●, isolates tested by RFLP; ○, partial or whole sequencing of the 16*S* rRNA gene. *Numbers* indicate identical ETs

most distant members of these two clusters. One of those two clusters contains only isolates from the NW site and the other cluster contains the populations from the NE and SW sites as well as the two Azores isolates. Within this latter cluster, the isolates from different sites form distinct genetic lineages, separated by a genetic distance of approximately 0.5. Furthermore, distinct clonal lineages within each site were observed at the same genetic distance. Especially noteworthy is the apparent genetic distinction between the red and the colorless isolates, which clearly shows that colorlessness is not the result of sporadic mutations in the populations but is a stable and vertically transmissible trait associated with a distinct genotype. The colorlessness may indicate adaptation to a sheltered existence. In this view, it is interesting to note that the colorless isolates that were found only in the NE and the SW sites were effluents from boreholes and that only colorless isolates were found at the NE site. The colorless lineages from each site were, however, not specifically related, which indicates convergent adaptation to similar niche conditions at these sites. Apart from color, no phenotypic trait examined could be specifically associated with any particular lineage. The traits examined included the ability to utilize different carbon sources and extracellular enzymes.

The greater diversity of *Rhodothermus marinus* in the NW and the SW sites compared to the small NE site is noteworthy. Geothermal regions can be considered as islands in the ecological sense, and it has been established that the number of species of an island is directly related to its area and is a balance between immigration and extinction of species (MacArthur and Wilson 1967). The larger the area, the greater the diversity of niches that is to be expected. Also, greater abundance of any particular species or ecotype makes it less vulnerable to temporal or long-term hostile conditions. It has also been proposed that the diversity of bacterial niches relates to the abundance of heterogeneous substrates available (Wise et al. 1995). The relatively high genetic diversity and uniqueness of the NW population (0.484) may therefore be explained by its relatively large area, by great fluctuations in environmental conditions, and by the availability of an abundance of nutritional factors available from the rich algal populations. Similarly, the SW site, even though it is apparently a more uniform environment, is also comparatively large and the primary production is substantial because of a profusion of Cyanobacteria in the ponds (Petursdóttir and Kristjansson 1996). In contrast, the NE borehole effluent forms a small stream or a trickle on a sandy shore, resulting in a more homogenous habitat but also in a less stable environment and less diversity.

Periodic selection

Palys et al. (1997) pointed at periodic selection as one of the major factors of divergence of populations within species, leading to ecologically distinct populations and ultimately to new species. According to this theory, each adaptive mutation produces a more competent cell, and the descendants of that cell will outcompete other cells in that habitat or niche, resulting in a population of lesser diverstity. If different populations are separated in space, this will lead to genetic divergence of the species. The sample sites of this study are clearly different in physicochemical conditions. The individual *Rhodothermus marinus* populations may therefore be genetically adapted by periodic selection to the local conditions, and furthermore the large areas may harbor different niches and therefore different ecotypes of the same species. In this context, the low genetic diversity (0.241) of the population at the NE site is interesting and may indicate that periodic selection has influenced the genetic structure of this population to a greater degree than the others. Although the sample effort at this site was no less than at the other sites, only a few *R. marinus* strains could be isolated at the NE site, and all of them were colorless.

16*S* rRNA analysis

The geographic divergence observed within the strains by MEE in this study was not reflected in the highly conserved 16*S* rRNA molecule. Partial or whole sequences of the 16*S* rRNA gene from representative strains were almost identical and independent of geographic origin. This result was surprising compared to the large genetic distances obtained between the different populations, especially between the NW and the other populations. However, it also exemplifies the fact that genes and proteins that are part of the informative cell apparatus, such as the protein translation machinery, are not likely to reveal differences between ecologically or geographically different populations of the same species**.** It is also possible that differences in divergence of 16*S* rRNA versus divergence of enzyme coding sequences may vary between species (Palys et al. 1997). The habitat structure of thermophilic bacteria may enhance these differences. Thermophilic habitats are confined to small areas that are separated by great geographic distances. This isolation essentially hinders the mixing of different populations of the same species, thereby restricting interspecies competition, which may therefore lead to accelerated divergence in enzyme coding sequences of the species as a whole.

Conclusion

In conclusion, it can be stated that this study clearly shows the population structure of *Rhodothermus marinus* to be endemic. Despite phenotypic similarity and phylogenetic homogeneity as estimated by 16*S* rRNA analysis, the species forms genetically distinct populations that can be explained by geographic isolation and genetic drift. Whether these populations are also ecologically distinct remains to be demonstrated, but there are indications that this may be so.

On a geological scale, geothermal habitats are confined to small spots that are relatively rare and separated by large

geographic distances. As discrete ecosystems, they are solely occupied by microbes that furthermore are very different from those of the surrounding areas. This study supports our postulate that geothermal areas can be considered as ecological islands and that large geographic distances may act as dispersal barriers for thermophilic microorganisms (Kristjansson et al. 1996). We propose that this may create opportunities for isolated and unique adaptations and lead to accelerated divergence of a bacterial species. It is our view that thermophilic organisms, because of their special habitat structure, are particularly well suited for studying ecological diversity and the mechanisms of speciation and evolution.

Acknowledgments This work was supported by grant no. 952140096 from the National Research Council of Iceland and by grant MAS-3 CT95-003U from the EU-MAST program.

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