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Classification of Strains of *Bacillus sphaericus* by Different Statistical Methods

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Abstract: In this study, the sensitivies of 21 strains of *Bacillus sphaericus* to 20 different antibiotics were determined with the antibiotic disc susceptibility test. By measuring the diameters of the inhibition zones in terms of millimetres, the obtained values were analysed using the SPSS for Windows package and classifications were made. With these analyses, 21 strains were clustered into 5 groups. In our study, strains IAB 59 and 2297 were placed into a separate class. In addition, those antibiotics which caused a differentation in strains and a discrimination of strains according to the level of antibiotic effect were determined.

Key Words: *Bacillus sphaericus*, classification.

Farklı İstatistiksel Metotlar Kullanılarak *Bacillus sphaericus* Suşlarının Sınıflandırılması

Özet: Bu çalışmada *Bacillus sphaericus*'a ait toplam 21 suşun 20 değişik antibiyotiğe olan duyarlılıkları, antibiyotik disk duyarlılık yöntemi ile ölçüldü. İnhibisyon zonu çapları milimetre cinsinden ölçülerek elde edilen değerler, SPSS for Windows paket programı ile analiz edildi ve sınıflandırmalar yapıldı. Analizler sonunda 21 suşun 5 küme oluşturduğu belirlendi. Çalışmamızda IAB 59 ve 2297 nolu suşların diğer suşlardan ayrı bir sınıfta yer aldığı saptanmıştır. Ayrıca suşlarda ortaya çıkan farklılaşmanın hangi antibiyotiklerden kaynaklandığı belirlenmiş ve antibiyotiklerin etkinlik derecelerine göre suşların ayrımının sağlanabileceği sonucuna varılmıştır.

Anahtar Sözcükler: *Bacillus sphaericus*, sınıflandırma.

Introduction

Chemical pesticides are largely used to fight against harmful organisms. However, because of the mutagenic and cancerogenic effects of such chemicals and the ability of organisms to develop resistance to pesticides, biological methods have been developed as an alternative. Bacteria (especially *Bacillus*, viruses, fungi and protozoa) are the natural pathogenic agents of insects (1). While a large number of strains have been isolated by researchers since the 1900s, the most successful results have been obtained using *Bacillus thuringiensis* and *B. sphaericus* strains (2). *B. sphaericus*, first described by Neide, is a saprophytic micro-organism and its spores are found in soil and aquatic environments (3). There are at present a great number of known strains. However, the development of new techniques to discriminate between strains and test the reliability of strain identification are on the agenda now. The first classification studies were generally based on morphological and biochemical characteristics. As phenotypic properties cannot be enough alone to separate different strains, other methods have been developed to show strains' differences. The H-flagellar agglutination method was first successfully used for the separation of insect pathogenic agent *B. thuringiensis*. This method has also been used to distinguish different strains of *B. sphaericus*, and the strains are clustered in different serotypes accordingly (4,5). Pathogenic strains were clustered in one group according to DNA homology (6). Strains of the species have also been determined through their fatty acid

content and reaction against bacteriophage (7,8). Research using polyacrylamide gel electrophoresis showed the separation possibilities of the strains in accordance with bacteriocine activities (9), while numerical methods are widely used for the classification of bacteria (10-13). Bağcı et al. have reported the separation of the same serotypical varieties into distinct groups linking *B. thuringiensis* using the numerical taxonomic method. Furthermore, a *B. sphaericus* strain used as a control has shown the same characteristics in all methods, and so researchers report that the use of the antibiotic disc method is sufficient for its distinction (14). Ismail and Tohamy reported that *Bacillus, Micrococcus* and *Staphylococcus* were isolated and selected for further study using numerical taxonomy based on 33 morphological and biochemical character units. Distance matrices were analysed by weighted pair group and complete linkage clustering methods. The researchers proposed that *B. pumilus* be reclassified as a new genus (15).

In this study, the classification of *B. sphaericus* strains is investigated via antibiotic disc sensitivity and statistical methods.

Materials and Methods

The bacteria used in this research were obtained from the bacteriology culture stocks of the bacteriology laboratory, Department of Biology, Faculty of Science, Ankara University. *B. sphaericus* strains' serogroups, DNA homologies, phage groups and the antibiotics used in this study are given in Table 1 (4,6,16).

Bacteria were activated 3 times at 30 °C in nutrient broth medium. The activated culture was cultivated by a sterile ecuvion, and then the antibiotic discs were placed on the surface of the nutrient medium. The inhibition zones were measured in millimetres using a pair of compasses after 24 h incubation at 30 °C. Statistical analysis was applied with the SPSS for Windows package for a 21 x 20 data matrix. The obtained data were used to discover the strains' similarities and differences. Strain classifications by similarity and difference were performed through clustering analysis. Clustering analysis sought to classify the strains into homogeneous groups within themselves and heterogeneous groups among themselves. Hierarchic clustering methods, single-linkage, complete linkage, between groups average linkage (BGAL), within groups average linkage (WGAL), centroid

Table 1. *B. sphaericus* strains serogroups, DNA homologies, phage groups and antibiotics.

No	Antibiotics	Strains	Serogroups	DNA Homologies	Phage Groups
1	Cephalothin	2362	H5a5b	-	3
2	Cefoxitin	34-2	H9a9c	-	8
3	Cefadroxil	IAB 460	H6	-	3
4	Trimethoprim-Sulfhamethoxazole	NRS 592	-	III	-
5	Oxacillin	IAB 467	H6	-	3
6	Cefamandole	2297	H25	-	4
7	Cefonicid	1883	H2a2b	-	2
8	Doxycycline	31-2	H9a9c	-	8
9	Polymyxin B	IAB 59	H6	-	3
10	Cefazolin	ATCC 14577	-	I	-
11	Carbenicillin	Kellen Q	H1a	IIA	1
12	Mezlocillin	1881	H5a5b	IIA	3
13	Amoxicillin	NRS 400	-	IV	-
14	Azithromycin	NRS 1198	-	V	-
15	Tetracycline	SSII-1	H2a2b	IIA	2
16	Gentamycin	1-2	-	-	-
17	Sulbact.-Amp.	ATCC 12300	-	IIB	-
18	Erythromycin	NCTC 9602	-	I	-
19	Vancomycin	1404	H2a2b	IIA	2
20	Chloramphenicol	1593	H5a5b	IIA	3
21	-	ATCC 7055	-	IIB	-

linkage, median linkage and the Ward method were applied to the data (17,18).

Together with the hierarchic clustering method and nonhierarchic clustering method, k-average linkage was also applied to the data. Nonhierarchic clustering methods are used when there is no previous information in relation to cluster numbers or if the researcher finds the cluster numbers satisfactory. Another reason for the preference of this method is the powerful theoretical support available for it (18). Finally, factor analysis was applied to the correlated data to obtain a small number of unrelated hypothetical variables (factors) which explained large amounts of the variance of the original variables. The loads of antibiotics were revealed using factors obtained from factor analysis. After this analysis varimax rotation was performed to receive the simple structure for factors and the causal analysis between antibiotics and strains. In the results of the rotation, strains different from those of factor analysis were determined, and so information about antibiotics causing strain differences was obtained. Finally, the strains' differences were studied by cluster analysis.

Results and Discussion

B. sphaericus strain serogroups, DNA homologies and phage groups are given in Table 1. In our study of the effect of 20 distinct antibiotics on 21 strains, hierarchic cluster analysis was first applied. Tree graphics obtained by complete linkage, BGAL and WGAL were observed to be the same, while tree graphics obtained through 7 different methods were studied. Relative similarities between the tree graphics of the Ward, median, centroid and single linkage methods were also determined. Twenty-one strains of *B. sphaericus* strain tree graphics procured through WGAL techniques are shown in Figure 1.

The most successful cluster number was shown to be '3' after studying the tree graphics of the 7 methods used in hierarchic clustering. The results obtained from hierarchic clustering methods are illustrated in Table 2.

As seen in Table 2, 2297 and IAB 59 strains made 2 different clusters. Though different from other linkage techniques in the Ward method, there were no units forming a cluster alone and the 2 strains were also observed to have been placed in the same cluster.

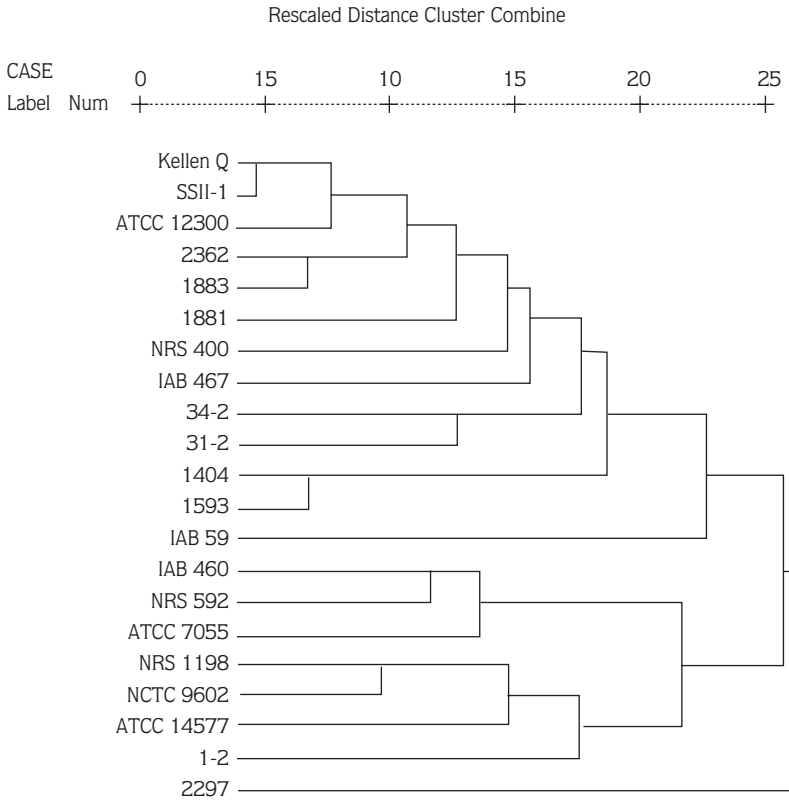

Figure 1. Phenogram obtained by within groups average linkage.

Table 2. The results obtained with the separation of the strains into three clusters by hierarchic clustering methods.

Methods	Strains			
Complete Linkage	2297	IAB 59		others
Single Linkage	2297	IAB 59		others
Median	2297	IAB 59		others
Centroid	2297	IAB 59		others
BGAL	2297	IAB 59		others
Ward	2297	IAB 460	1-2	
	IAB 59	NRS 592	ATCC 7055	others
		NRS 1198	ATCC 14577	
		NCTC 9602		
WGAL	2297	IAB 460	1-2	others
		NRS 592	ATCC 7055	
		NRS 1198	ATCC 14577	
		NCTC 9602		

Moreover, different from other linkage techniques strain 2297 formed a cluster alone in the WGAL method, and in this method strain IAB 59 was placed with other strains in the same cluster. Pathogenic and nonpathogenic strains dissimilar from the viewpoint of DNA homologies (6), phage groups and serogroups (4) were observed to have been placed in the same cluster in the Ward and WGAL methods. Another chosen cluster number after examination of the 7 hierarchic clustering techniques tree diagram was 5. The results obtained from the separation of the strains into 5 clusters are given in Table 3. The strains were separated into 4 clusters in the Ward, centroid and single linkage methods.

In Table 3, strains 2297 and IAB 59 each formed a cluster, very different from other clustering methods, while these strains collected in the same cluster in the Ward technique; the formation of the same clusters in BGAL, WGAL and Ward techniques was interesting.

Table 3. The results obtained with the separation of the strains into five clusters by hierarchic clustering methods.

Methods	Strains				
Complete Linkage	2297	IAB 59	1-2	1881	31-2
			1404	IAB 460	34-2
			1593	IAB 467	1883
			NRS 1198	NRS 400	2362
			NCTC 9602	NRS 592	SSII-1
			ATCC 14577	ATCC 7055	Kellen Q
					ATCC 12300
BGAL	2297	IAB 59	1-2	IAB 460	
			NRS 1198	NRS 592	others
			NCTC 9602	ATCC 7055	
			ATCC 14577		
WGAL	2297	IAB 59	1-2	IAB 460	
			NRS 1198	NRS 592	others
			NCTC 9602	ATCC 7055	
			ATCC 14577		
Median	2297	IAB 59	1-2	1404	others
Ward*	2297		1-2	IAB 460	
	IAB 59		NRS 1198	NRS 592	others
			NCTC 9602	ATCC 7055	
			ATCC 14577		
Single Linkage*	2297	IAB 59	1-2		others
Centroid*	2297	IAB 59	1-2		others

*Cluster number is determined 4.

Strains IAB 460, NRS 592 and ATCC 7055 formed just a cluster in the Ward, BGAL and WGAL techniques, but the addition of strains IAB 467, 1881 and NRS 400 strains were seen in the complete linkage method. The formation of two different clusters by strains 1-2 and 1404 has been determined in median linkage. Furthermore, the cluster number has been determined to be 4 in the Ward method, and in this technique strains 2297 and IAB 59 formed only a cluster just similar to strains divided into 3 clusters in Table 2. The settlement of mosquito pathogen and nonpathogen strains in the same cluster was very interesting. Strains 2362, 1593 and 1881, which are in the same serogroup and phage group and have a high virulence (19), formed quite different clusters in the complete linkage method.

In analysis by the k-average technique, which is a nonhierarchic clustering method, the cluster number of which is already known, the cluster number was considered to be 3 and 5 for comparison with the hierarchic clustering method results and the results obtained were examined. Among all the studied clusters, the strains 2297 and IAB 59 were noted for forming 2 different clusters. The results are shown in Table 4.

For cluster number 3, strains 2297 and IAB 59 formed 2 distinct clusters, but the third cluster was developed by all the other strains used in the study. In cluster number 5, strains 2297 and IAB 59 were observed to form 2 different clusters. A collection of nonpathogenic strains was observed in the third cluster obtained as a result of separation. In addition, the results obtained through the k-average method were the same as those taken from hierarchic clustering analysis. Especially if the cluster number changes, strains 2297 and IAB 59, except for the Ward method, are interesting as they compose different clusters on their own. For a cluster of 5, strains 1-2, NCTC 9602, ATCC 14577 and NRS 1198 in the Ward, complete linkage, BGAL and WGAL methods were clearly placed in the same cluster. In the Ward and BGAL techniques, whereas other strains except for strain 2297 were discriminated, 2 different clusters, in the case of cluster number 3, strains 2297, IAB 59 and others were determined to be in the same cluster in the k-average method.

Total variance description proportions obtained by applying factor analysis to the 20 antibiotics data used in 21 strains in a sensitivity study are given in Table 5. The first factor describes 25.925% of the total variance. The 7 factors also extract 81.224% of the total variance.

In the original value study of the factors, it was found that different factors such as Cephalothin, Cefoxitin, Cefadroxil, Cefamandole, Cefonicid, Cefazolin and Tetracycline antibiotics weighted in the first factor, Mezlocillin, Amoxicillin and Chloramphenicol antibiotics weighted in the second factor, Trimethoprim-

Table 4. Three and five clusters obtained by k-Average technique.

Number of Cluster	Strains				
	I. Cluster	II. Cluster		III. Cluster	
For 3 cluster	2297	IAB 59	1-2 31-2 34-2 1404 1593 1881 1883	2362 SSII-1 IAB 460 IAB 467 NRS 400 NRS 592 NRS 1198	Kellen Q NCTC 9602 ATCC 7055 ATCC 12300 ATCC 14577
	I. Cluster	II. Cluster	III. Cluster	IV. Cluster	V. Cluster
For 5 cluster	2297	IAB 59	1-2 NRS 1198 NCTC 9602 ATCC 14577	31-2 34-2 2362 IAB 460 IAB 467 NRS 592 ATCC 7055	1404 1593 1881 1883 SSII-1 NRS 400 Kellen Q ATCC 12300

Table 5. Results of factor analysis.

	Initial Eigenvalues			Rotation Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	5.185	25.925	25.925	4.783	23.914	23.914
2	2.941	14.707	40.632	2.189	10.946	34.859
3	2.424	12.12	52.752	2.158	10.788	45.647
4	1.921	9.604	62.356	2.069	10.347	55.994
5	1.636	8.179	70.534	1.759	8.794	64.788
6	1.11	5.548	76.082	1.716	8.582	73.37
7	1.028	5.142	81.224	1.571	7.854	81.224
8	0.757	3.787	85.01			
9	0.661	3.307	88.318			
10	0.582	2.909	91.227			
11	0.519	2.593	93.82			
12	0.388	1.94	95.76			
13	0.249	1.246	97.006			
14	0.203	1.015	98.021			
15	0.149	0.746	98.767			
16	0.118	0.592	99.359			
17	6.21E-02	0.311	99.67			
18	4.92E-02	0.246	99.916			
19	1.17E-02	5.87E-02	99.974			
20	5.10E-03	2.55E-02	100			

Extraction Method: Principal Component Analysis

Sulphamethoxazole, Oxacillin, Vancomycin and Azithromycin antibiotics weighted in the third factor, Sulbact.-Amp. antibiotic weighted in the fourth factor, and Gentamicin antibiotic weighted in the fifth factor. However, it is difficult to determine the factors Doxycycline, Polymyxin B, Carbenicillin and Erythromycin antibiotics weighted on.

Thus, the varimax method was applied to determine the loads of the antibiotics in the factors, and the results are explained in Table 6.

After varimax rotation, we observed that Cephalothin, Cefoxitin, Cefamandole, Cefonicid, Cefazoline and Tetracycline were in the first factor, Polymyxin B and Mezlocillin were in the second factor, Erythromycin and Chloramphenicol were in the third factor, Carbenicillin, Amoxicillin and Vancomycin were in the fourth factor, Oxacillin and Doxycyline were in the fifth factor, Trimethoprim-Sulphamethoxazole, Azithromycin and Sulbact.-Amp. were in the sixth factor and finally Gentamicin was in the seventh factor.

After examining the antibiotic loads in factors, the factor scores showing the factors causing the distinction of the strains were determined (Table 7).

How and on which factors the antibiotics weighted are seen in Table 6 and commented upon according to the scores to be compared with the factors seen in Table 7. In accordance with the interpretation as seen in the table strains 1881, 1404 and 1593 and strains IAB 467 and 1883 are different from the others according to the first factor in which positive loads of Cephalothin, Cefoxitin, Cefadroxil, Cefamandole, Cefonicid, Cefazoline and Tetracycline were weighted. According to the second factor in which Polymyxin B's positive and Mezlocillin's negative loads are weighted, strains 1883, Kellen Q, SSII-1 and ATCC 12300 and strains 2362, IAB 59 and NRS 1198 are different from the other strains. According to the third factor in which the positive loads of Erythromycin and Chloramphenicol are weighted, strains 1883, ATCC 14577, 1-2 and NCTC 9602 and strains 34-2, 31-2, IAB 467 and 1881 are different from the other

Table 6. Results of varimax rotation of factor analysis.

Antibiotics	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Cephalothin	.876	.282	.122	-.223	9.39E+01	6.74E+00
Cefoxitin	.847	-.172	9.30E+01	6.49E+00	-1.09E-02	-3.37E-02
Cefonicid	.792	.230	-.221	-1.22E-02	.239	.167
Cefamandole	.765	.312	8.91E+00	9.31E+01	-.366	.161
Cefadroxil	.741	-.362	1.24E+01	.237	-.181	-9.69E-02
Tetracycline	.738	.382	4.19E+01	2.47E+01	-.165	-.163
Cefazolin	.710	2.99E+01	.232	.228	2.42E+01	-5.63E-02
Mezlocillin	-4.50E-02	-.854	4.21E+01	.286	.145	1.41E+01
Polymyxin B	.335	.781	-.239	.119	.111	2.09E+01
Chloramphenicol	.131	-.233	.851	.168	-.148	-9.74E-02
Erythromycin	-1.80E-02	-2.41E-03	.808	.137	.216	.281
Carbenicillin	.172	.107	.108	.929	-6.86E-02	1.02E+01
Amoxicillin	7.77E+00	-.244	.112	.788	6.07E+00	-5.78E-02
Vancomycin	.390	.247	.396	-.418	.295	8.11E+01
Oxacillin	-.122	2.98E+01	-3.06E-02	-5.82E-02	.853	.125
Doxycycline	.338	-5.66E-02	.336	-.151	.494	-.258
Trimethoprim-Sulfamethoxazole	9.90E+01	-2.48E-02	.237	-.173	-3.22E-03	.822
Sulbact.-Amp.	7.02E+01	-.217	.484	-.164	-8.81E-02	-.658
Azithromycin	-.176	-.270	5.76E+01	6.08E+01	.552	.572
Gentamycin	-9.78E-03	5.05E+01	6.28E+01	.122	-.144	6.31E+01

Table 7. Factor scores.

Strains	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7
2362	-0.82861	**-1.06367**	-0.05377	0.27325	0.59930	0.45676	-0.82920
34-2	-0.90404	-0.52915	**-1.88353**	0.41437	0.57999	**1.09216**	0.06056
IAB 460	-0.92663	0.30174	0.21100	0.55607	0.26660	**-1.26457**	0.59684
NRS 592	-0.31171	0.36967	-0.12830	-0.25465	-0.10742	**-2.26325**	**1.26593**
IAB 467	**-1.20054**	0.32682	**-1.05379**	0.78621	0.47575	**-1.17875**	**-1.35073**
2297	-0.50368	-0.19507	-0.29682	**-3.28592**	-0.04983	0.11567	-0.36919
1883	**-1.31814**	**1.33468**	**1.75952**	-0.01086	0.52522	0.56716	**-1.40432**
31-2	-0.64456	-0.26173	**-1.13716**	0.94386	**1.74980**	0.97496	**1.62916**
IAB 59	-0.35499	**-1.77045**	0.58596	**-1.64261**	0.38004	-0.19896	0.06940
ATCC 14577	-0.67010	-0.0682	**1.93649**	0.22106	0.65669	0.70949	**1.76326**
Kellen Q	-0.01999	**1.11002**	0.19490	0.17014	-0.81116	0.84605	-0.23027
1881	**1.19640**	-0.07800	**-1.07946**	0.33030	-0.61905	0.11862	**-1.04539**
NRS 400	-0.68674	0.29857	-0.35702	-0.06942	**-3.29117**	0.20419	**1.49392**
NRS 1198	0.37093	**-2.67486**	-0.01897	0.94830	**-1.02324**	0.16464	-0.76168
SSII-1	0.17514	**1.25954**	-0.84689	-0.53007	-0.00262	0.15483	0.50547
1-2	0.97954	-0.05838	**1.23679**	**1.51512**	-0.38057	0.36631	0.38491
ATCC 12300	0.17944	**1.52625**	0.00491	0.30499	-0.04242	0.09284	**-1.43392**
NCTC 9602	0.51532	-0.56783	**1.57653**	-0.07530	-0.28538	0.28993	-0.79472
1404	**2.67110**	0.28956	0.09316	-0.60403	**1.34232**	0.10421	0.73422
1593	**1.29933**	0.62383	-0.79543	-0.34461	-0.23547	**1.15202**	-0.04132
ATCC 7055	0.98254	-0.17336	0.05188	0.35378	0.27262	**-2.50431**	-0.24296

strains. In the fourth factor when positive loads of Carbenicillin and Amoxicillin and negative loads of Vancomycin are weighted, strains 1-2 strain, 2297 and IAB 59 differ from the other strains. In the fifth factor on which positive loads of Oxacillin and Doxycycline are weighted, strains 31-2 and 1404 and strains NRS 400 and NRS 1198 are different from the other strains. In the sixth factor on which positive loads of Trimethoprim.-Sulphamethoxale and Azitromycin and the negative loads Sulbact.-Amp. are weighted, strains 34-2 and 1593 and strains IAB 460, NRS 592, IAB 467 and ATCC 7055 differ from the other strains. According to the seventh factor on which Gentamicin positive load is weighted, strains NRS 592, 31-2, ATCC 14577 and NRS 400, and strains IAB 467, 1883, 1881 and ATCC 12300 differ from the other strains.

In this research strains IAB 59, IAB 460 and IAB 467 in the same phage group have been illustrated to be classified in different groups through taxonomic analysis. In addition, strains IAB 59 and 2297 in distinct serogroups, phage groups and DNA homologies have been found to gather in 2 clusters different from other strains. In analysis of clustering, these 2 strains completely differed from the others, according to a fourth factor obtained by factor analysis, they were discriminated from the other strains. It was determined that the differences of factor were caused by Carbenicillin, Amoxicillin and Vancomycin. Strains 1198 and ATCC 14577, which have different DNA homologies, have been observed to be placed in the same group by statistical methods. Strains 31-2 and 34-2 placed in different serogroups and phage groups have been found in the same group with high virulence strain 2362 (19).

In this research, the separation of strains placed in different serological, phage and DNA homologies were determined by statistical approaches. Strains 2297 from various serogroups and phage groups was observed to differ from the other strains statistically. In addition, strains IAB 59 formed a cluster alone that was different from other IAB strains belonging to the same serogroup and phage group. Furthermore, the antibiotics which caused the exchange in the strains were studied and the separation of the strains was concluded to be possible according to antibiotic effect values.

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References

1. Burges HD. Microbial Control of Pests and Plant Diseases 1970-1980. Academic Press, London. 1981. p.949.
2. Bursalıoğlu M. Böceklerin Biyokontrolu. Ege Üniversitesi Fen Fakültesi. İzmir. Ph.D. p.172. 1985.
3. Neide E. Botanische Beschreibung einiger sporenhildenden Bacterien. Zentralbl Bacteriol Parasitenk Infektionskr Hyg Abt 12: 1-32. 1904.
4. de Barjac H, Veron M, Cosmao-Dumanoir V. Characterization biochimique et serologique de souches de *Bacillus sphaericus* pathogenes ou non four les moustiques. Ann Microbiol (Inst. Pasteur). 131B: 191-201. 1980.
5. de Barjac H, Larget-Thiery I, Cosmao-Dumanoir V, et al. Serological classification of *Bacillus sphaericus* strains on the basis of toxicity to mosquito larvae. Appl Microbiol Biotechnol 21: 85-90. 1985.
6. Krych VK, Johnson JL, Yousten AA. Deoxyribonucleic acid homologies among strains of *Bacillus sphaericus*. Int J Syst Bacteriol 30: 476-484. 1980.
7. Frachon E, Sylviane H, Nicolas L, et al. Cellular fatty acid analysis as a potential tool for predicting mosquitocidal activity of *Bacillus sphaericus* strains. Appl Environ Microbiol 57: 3394-3398. 1991.
8. Yousten AA. Bacteriophage typing of mosquito pathogenic strains of *Bacillus sphaericus*. J Invertebr Pathol 43: 124-125. 1984.
9. Çökmüş C, Yousten AA. Bacteriocin production by *Bacillus sphaericus* strains. J Invertebr Pathol 61: 323-325. 1993.
10. Kildsgaard J, Engesgaard P. Numerical analysis of biological cloggind in two-dimensional sand box experiments. J of Contaminant Hydrology. 50: 261-285. 2001.
11. Dabboussi F, Hamze M, Elomari M, et al. Taxonomic study of bacteria isolated from Lebanese spring waters: Proposal for *Pseudomonas cedrella* sp. nov. and *P. orientalis* sp. nov. Research in Microbiology. 150: 303-316. 1999.
12. Borsodi AK, Farkas I, Kurdi P. Numerical analysis of planktonic and reed biofilm bacterial communities of Lake Fertö (Neusiedlersee, Hungary/Austria). Wat. Res. 32: 1831-1840. 1998.

13. Toth E. The species composition of oligotrophic bacterial communities of Lake Balaton - a numerical analysis. Acta Microbiol Immunol Hung 43: 333-338. 1996.

14. Bağcı H, Shareef SR, Özdamar K. *Bacillus thuringiensis* varyetelerinin sınıflandırılmasında sayısal taksonominin uygulanması. Doğa - Tr J of Biology 5: 70-81. 1991.

15. Ismail A, Tohamy E. Phenetic and biochemical numerical taxonomy of bacteria isolated from some medical products. J Union Arab Biol Cairo 6(B): 209-222. 1998.

16. Alexander B, Priest FG. Numerical classification and identification of *Bacillus sphaericus* including some strains pathogenic for mosquito larvae. Journal of General Microbiology 136: 367-376. 1990.

17. Özdamar K. Paket Programlar İle İstatistiksel Analiz-2. Kaan Kitabevi. 502 Eskişehir; 1999.

18. Tatlıdil H. Uygulamalı Çok Değişkenli İstatistiksel Analiz. Cem Web Ofset Ltd Şti. 424 Ankara; 1996.

19. Russell BL, Jelley SA, Yousten AA. Carbohydrate metabolism in the mosquito-pathogen *Bacillus sphaericus* 2362. Appl Environ Microbiol 55: 294-297. 1989.