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NUMERICAL TAXONOMY OF *ENTEROBACTER* SPP. ISOLATED FROM DIFFERENT AREAS IN SOUTH IRAQ

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ABSTRACT

A numerical classification study was carried out on 153 strains of *Enterobacter* using 64 unit characters including: cell characteristic, growth features, tolerance, biochemical and antibiotic susceptibility. The data were examined using the simple matching (S_{SM}), and clustering was achieved using unweighted pair group average linkage method (UPGMA). The results of the analyses were arranged in the form of dendrogram according to the similarity matrix. At similarity level (83%), all isolates were divided into five groups, the major group was comprised of 80 isolates belong to species *Enterobacter cloacae*. Group 3 was composed of 67 isolates belong to species *E. aerogenes*. Group 5 was composed of 4 isolates representing the species: *E. sakazakii*, and two group (2,4) contain only one isolate representing the species *Enterobacter hormaechei* and *E. asburiae* respectively.

KEYWARDS: Numerical taxonomy, *Enterobacter,* UPGMA.

INTRODUCTION

Numerical taxonomy (also termed adansonian analysis, taxonometrics, taxometrics, phonetics, and computer taxonomy) method has been employed for bacterial classification and identification for many decades.Numerical taxonomy analysis was first suggested by Michel Adanson in 19th century (Loman, 2012). Sneath in 1957 published a first account of the use of computer methods for the classification of bacteria and principles of numerical taxonomy were published in 1963(Sneath, 1995).

This is essentially statistical method that use groups of traits that taken together, point to specific taxa (Staley and colwell, 1973; Sneath, 1984). Application of statistical approaches provides a mechanism for using a wide range of morphological, physiological, biochemical, serological features (frequently 100 or more) each given equal weight (Sneath,1984).This analysis is referred to the "unweighted-pair group method with arithmetic mean" (UWPGA) technique. From this perspective, the computer clusters different strains at selected levels over all similarity (usually that isolate must have at least 80-85% similarity to belong to given species based on unweighted-pair group method analysis (Janda and Abbott, 2002).

Numerical classification provides percentage frequencies of positive character states for all strains within each cluster. such data provide a basis for the construction of a frequency matrix for identification of unknown strains against the defined taxa (Krieg and Holt, 1984). Cluster analysis is the name given to various procedures whereby a set of individuals or units (termed as OTUs "Operational taxonomic units" a useful expression proposed by Sokal and Sneath, 1963, to designate the entities whose classification is in question-individuals, strains, or low-rank taxa already recognized is divided into two or more subgroups (clusters) on the basis of a set of attributes which they share (Meerman, 1993).

Computerized databases have been used to develop diagnostic tests that identify clinically relevant isolates through numerical codes (Krieg and Holt, 1984). This method became the dominant one for classification of bacteria in the latter third of the twentieth century. In this method, researchers chose characteristics that strongly differentiated among taxa when strains were directly compared (Janda and Abbott, 2002). Much of research in numerical taxonomy of bacteria has consisted of detailed analysis of individual taxa. Numerical taxonomy was used to analyze phenotypic data obtained from 126 isolates of *Aeromonas* strains isolated from different sources and the strains clustered into 10 aggregate groups by using the S_{SM} coefficients and UPGMA clustering algorithm (Erdem *etal*., 2011). Greipsson and Priest (1983) found that all Hafnia alvei strains formed a single phenon with a simple matching coefficient by performing a numerical analysis of 101 features because the similarity among all of the strains was very high. Thirteen phena were found at 78% similarity level when tested 89 phenotypic were numerically analyzed against 17 reference strains,using the simple matching coefficient (S_{SM}) where five of these phena were assigned to the family Enterobacteriaceae (Prado *etal*.,2001)

The aim of this study was to classification of *Enterobacter* by numerical taxonomy.

MATERIALS AND METHODS

Samples collection

The samples were collected from different areas of Basrah hospitals (Al-Fayhaa General hospital, Al-Mawanee General hospital, Al-Sadder teaching hospital, Al-Basrah hospital for gynecology and obstetrics, Al-Basrah children's specialty hospital, Al-Basrah General hospital).

Identification of Enterobacter spp.

Enterobacter spp. were identified by conventional methods, they were examined according to the appearance, color and morphology of the colonies and Positive cultures were subjected to biochemical tests (sugar fermentation, IMVC, TSI, Oxidase, Catalase) for identification of bacteria and confirmatory identification of *Enterobacter* spp by Vitek® 2 compact.

Numerical taxonomy

All isolates (OTU) were submitted to numerical taxonomy by using (64) characters (t) Table(1). Clusters analysis was done according to unweighted between group mathematic average linkage (UPGMA) using Simple matching coefecient(S_{SM}). Numerical taxonomy program used within Spss IBM 20 package.Tests results were input to program as (0,1). The program showed the species groups according to similarity percentages, the results appeared as diagram (dendrogram).

Table 1: Phenotypic characteristics were used in numerical taxonomy of *Enterobacter* spp.

No.	Character(t)
	Colony characters
1	White on nutrient agar
2	Mucoid
3	Motility
	Biochemical tests
4	Oxidase
5	Catalase
6	Indole production
7	Methyl red
8	Voges-Proskauer reaction
9	Ornithine decarboxylase
10	H2S production
11	Alpha-Glucosidase
12	Lysine decarboxylase
13	Fermentation/Glucose
14	Beta-Galactosidase

15	Phosphatase
16	Alpha-Galactosidase
17	Urease
18	Beta-Glucosidase
19	Aesculin hydrolysis
20	Blood haemolysis
21	Gelatin liquefaction
22	DNase test
23	Ala-Phe-Pro-Arylamidase
24	Tyrosine arylamidase
	Utilization of:
25	Adonitol
26	L-Arabitol
27	D-Cellobiose
28	D-Glucose
29	D-Maltose
30	D-Mannitol
31	Raffinose
32	D-Mannose
33	Beta-Xylosidase
34	D-Trehalose
35	Citrate(sodium)
36	Malonate
37	L-Proline arylamidase
38	D-Sorbitol
39	Succinate alkalinisation
40	5-Keto-D-Gluconate
41	Lipase
42	Sucrose
43	D-Tagatose
44	Glycine arylmindase
45	L-Histidine assimilation
46	Glu-Gly-Arg-Arylamidase
47	L-Malate assimilation
	Sensitivity to:
48	Piperacillin(100 µg)
49	Cephalothin(30 µg)
50	Amoxicillin/Clavulanic acid(30 µg)
51	Trimethoprim(5 µg)
52	Ceftazidime(30 µg)
	Growth at
53	4°C
54	15°C
55	45°C
	Growth on
56	NaCl(2% w/v)
57	NaCl(8% w/v)
58	Pink colony color on EMB
59	violet colony color on EMB
60	Brown colony color on EMB

61	Cream white colony color on TSA
62	White colony color on TSA
63	Pink colony color on Chrom agar
64	Blue-green colony color on Chrom agar

RESULTS

One hundred and fifty three isolates of Gram-negative, lactose fermentative bacteria, representing members of the Enterobacteriaceae were subjected to numerical taxonomy. A numerical taxonomic study using 64 characters (morphological, cultural, biochemical and physiological characters). The data were analyzed by computer, using the simple matching coefficient (S_{SM}). Clustering was achieved by the unweighted pair group average linkage method (UPGMA). The results of the analyses were arranged in the form of denderogram according to the similarity matrix, which was conducted by Spss IBM 20 package as represented in Figure(1). This results revealed that the dendrogram composed of five major distinct groups at the 83% of similarity level, two of them (2,4 groups) contain only one isolate, where as group(2) contain isolate (33=*Enterobacter hormaechei*), group(4) contain isolate (16=*Enterobacter asburiae*), while groups (1,3) divided into distinct large subgroups within the large group and each large subgroups divide into small subgroups at certain similarity level.

Group(1) is the largest group included 80 isolates (1,4,6,7,8,10,11,13,14,20,21,23,25,30,31, 32,34,36,37,38,39,41,42,43,44,45,48,49,50,52,55,56,58,59,63,64,65,68,69,71,73,77,79,82,85, 86,87,94,95,96,103,104,108,109,111,112,116,117,120,121,123,124,125,127,128,129,131,132 ,133,135,136,138,139,144,145,146,148,149,152,15,3) and divided into two subgroups at 85% similarity level, large subgroup (1A) composed of 75 isolate (1,4,6,7,8,10,11,13,14,20,21,23, 25,30,31,32,34,36,38,41,42,43,44,45,48,49,50,52,56,58,59,63,64,65,68,69,71,73,77,79,82,85, 86,87,94,95,96,103,104,108,109,111,112,116,117,120,121,123,124,125,127,128,129,131,132 ,135,138,139,144,145,146,148,149,152,153 and small subgroup(1B) composed of 5 isolate (37, 39,55,133,136), this group belong to the species Enterobacter cloacae. Group(3) is composed of 67 isolate (3,5,9,12,15,17,18,19,22,24,26,27,28,29,35,40,46,47,51,53,54,57,60, 61,62,66,67,70,72,74,76,78,80,81,83,84,88,89,90,91,92,93,97,98,99,100,101,102,103,107,11 0,113,114,115,118,119,122,126,130,134,137,140,141,142,143,147,151) and divided into three subgroups at 86% of similarity level, subgroup (3B) is the largest one composed of 41 isolates(3,5,9,12,17,18,24,27,29,35,46,51,53,54,57,76,84,88,89,90,91,92,93,97,98,99,100,101 ,102,103,107,114,122,126,130,137,141,142,143,147,151),while subgroup(3A,3C)were the

small subgroups, subgroup3A consist of 19 isolates (19,28,60,62,66,67,70,74,78,80, 81,83,110,113,115,118,119,134,140) and subgroup 3C consisted of 7 isolates (15,22,26,40,47,61,72). All these isolates belong to species E. aerogenes.

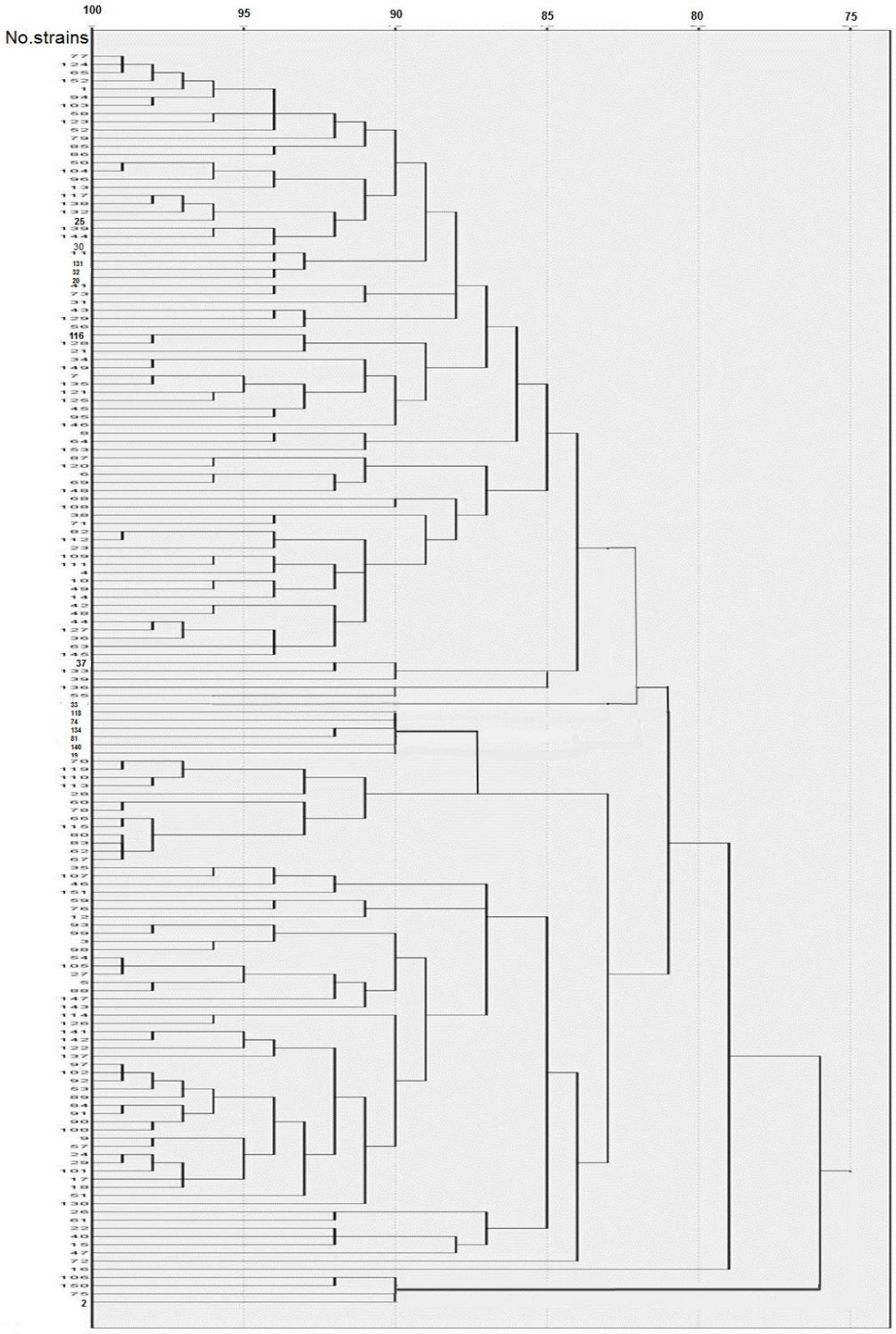

Figure 1: Dendrogram showed the relationship between groups composed of 153 isolates based on the S_{SM} coefficient and unweighted average linkage between groups (UPGMA) at similarity levels(83%,85%86%)

Numerical taxonomy which could evaluate all of the characters simultaneously, is one of the popular methods used in the taxonomy of many species since it is more objective than the traditional ones (Feng and Xie,2013). Some of the papers proposing bacterial classifications

based on computer analysis have included full data on the attributes studied. It has seemed of interest to re-analyse these by the new methods proposed, and compare the results with those obtained by using simple similarity indices and arbitrary levels for distinguishing clusters(Goodall,1966). The taxonomy of *Enterobacter* had been modified for several times, there were still divergences in the classification on the sections level.

In the present study, 153 *Enterobacter* spp. were selected and analyzed by numerical taxonomy method, where conventional identification of all isolates were made in the computer based on the results of the 64 tests. The data were examined using the simple matching coefficient(S_{SM}),which includes both positive and negative matches, and similarity percentage were obtained using unweighted average linkage clustering(UPGMA) (Sokal and Michener,1958;Sneath and Sokal,1973). Defined phena not containing reference strains were identified using keys in Bergeys Manual of Determinative Bacteriology (1974,1994) and Bergeys Manual of Systematic Bacteriology(2004) and the diagnostic tables of Cowan and Steel(2003)(Buchanan *etal*.,1974; Holt *etal*.,1994; Barrow and Feltham,2003; Brenner *etal*.,2004). The relationship between the isolates as revealed by the single linkage cluster analysis method is shown in Figure(1).The results from this study have demonstrated that numerical taxonomy method can record variation in populations of isolates it is appreciated that test error may distort the result (Sneath and Johnson,1972), and that even the commonly used tests are not always reliable (Sneath and Collins,1974). However, in environmental studies, there are special problems which are due to changes occurring after subculture in the laboratory (Austin *etal*.,1979;Austin,1982).The dendrogram obtained by the average linkage method has been divided into four major groups(1,2,3,4) at similarity level 80% and the first one represented the largest one where it is divided into two subgroups and intra subgroup found supgroups contain different species closely related to each other like isolates(117,138,132,2) represented *Enterobacter cloacae* and *Enterobacter sakazakii* and isolates (139,144,19),(11,140,81,134),(118,133), (74, 135, 121,125) represented *Enterobacter cloacae* and *Enterobacter aerogenes* and isolates(33,128)represented *E.cloacae* and *E.hormaechei* and subgroup (1B)contain only one subgroup composed of *E.cloacae* and *E.aerogenes* like isolates (59,76) at similarity level 94%. Found from this results that some groups included strains belonging to two or more different species and this agreement with the findings of (Bascomb *etal*.,1971),while group (3) contain two subgroups each one composed of the same species *Enterobacter sakazakii*. Most of the family Enterobacteriaceae appears to consist of a spectrum of related organisms, and overall similarities provide no

justification for the present separation of the family into numerous species organized into groups(Krieg and Lockhart,1966).Another study showed that number of Enterobacteria have similar relationships within the family in numerical taxonomy analysis(Focht and Lockhart,1965).

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