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Numerical taxonomy of sporulated bacteria from marine soils : preliminary results.

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Introduction

The data in our previous technical reports of 1973 showed that :

1) the aerobic, heterotrophic bacteria of marine mud samples formed a highly diversified population ;

2) the most important taxon, both with regard to the number of isolates and to the number of types, was that of the Sporulales ;

3) it was difficult to identify most isolates, whether sporulated or not, by the classical procedures.

These findings induced us to try to regroup and identify the sporulated isolates using the methods of numerical taxonomy.

Methods

At the time of writing, 57 different isolates were examined, the origin of which is as follows :

Marine soil	4 isolates from separate locations, taken before September '72
	6 isolates from samples M2552 and M2841, September '72
	10 isolates from sample MO1 of January 25th, '73
	9 isolates from sample M14 of July 12th, '73
	2 isolates from sample M15 of October 4th, '73
Soil of Spuikom[(a)]	16 isolates from a sample of February 19th, '73
	10 isolates from a sample of September 19th, '73

[(a)] Bassin de Chasse, Harbour of Ostend.

In addition, 10 strains representing different species of Bacillus were obtained from the Institut Pasteur of Paris (IPP), and examined along with our isolates. The calculations are at present completed for 43 of our own isolates and 7 of the IPP test strains.

The analysis consisted of the 58 tests listed in Table 1, which can be divided into 18 morphologic, 7 physiologic and 33 biochemical tests. The number of answers to each test was either 2 (encoded as 6 or 0), 3 (6, 3 or 0), 4 (6, 4, 2 or 0) or 7 (6, 5, 4, 3, 2, 1 or 0). Additional tests were developed (3 morphologic, 5 physiologic and 1 biochemical) and partly applied, but the results were not yet incorporated into the calculations.

For all pairs of isolates, the Simple Matching Coefficient (S) was computed at the V.U.B.-U.L.B. Computer Centre. From these figures, clusters were formed by the Unweighted Pair Group Method with Arithmetic Averages, and a Similarity Matrix was built. This was slightly rearranged by hand to yield the best apparent grouping for all pairs for which $S \geqslant 0.75$ (i.e., about 1/5 of all pairs).

Results

The first group of 43 isolates yielded five clusters Ato E encompassing 31, and excluding 12 isolates. Only the largest cluster (E) could be considered a phenon because of its relatively large (9) number of members and homogeneity. Its description is given below (Table 2). The other clusters are still likely to join among themselves or split up when the analysis is extended.

Description of phenon E : Table 2

The incorporation of seven IPP test strains into the similarity matrix gave the following results :

1) Bac. pantothenticus IPP 5124, Bac. coagulans IPP 5263 and Bac. cereus var. mycoides IPP 5259 did not join any cluster ;

2) Bac. anthracis IPP A3, Bac. subtilis IPP 5265 and Bac. licheniformis IPP 5271 formed a high S value with a single member of one of the clusters ;

3) Bac. pumilus IPP 5267 had a very high S value when paired with 8 of the 9 members of phenon E. This phenon is therefor tentatively identified as Bacillus pumilus.

Preliminary conclusions

1) Some regrouping of the otherwise staggering variety of sporulating bacteria was already evident from this limited analysis ;

2) a cluster comprising more than 1/5 of the isolates was identified as Bacillus pumilus with exclusion of the related species Bac. subtilis and Bac. licheniformis ;

3) the analysis will be extended and refined by the incorporation of additional tests.

Table 1

		Number of States
1.	Occurrence of chains, vegetative cells	3
2.	Occurrence of chains, sporangia	3 (a1)
3.	Average length of vegetative cells	4
4.	Average diameter of vegetative cells	4
5.	Ratio of average length to average diamter of vegetative cells	4
6.	Average length of sporangia	3
7.	Ratio of average diameter of sporangia to average diameter of vegetative cells	3
8.	Number of spores per sporangium	2
9.	Position of spores in sporangium	2 (a8)
10.	Abundance of spores	3
11.	Thermoresistance of spores	3
12.	Abundance of ghosts	2
13.	Diameter of colonies on Marine Agar (30°C - 10 days)	4
14.	Margin of colonies on Marine Agar	2 (a13)
15.	Opacity of colonies on Marine Agar	3
16.	Intensity of pigmentation	3
17.	Lustre of colonies	2
18.	Motility by phase contrast examination	2
19.	Motility by stab inoculation	2
20.	Growth under anaerobic conditions (Hugh and Leifson)	2
21.	Salt tolerance	7
22.	Influence of 3.5 per cent NaCl	3 (a21)
23.	Temperature maximum	7
24.	Modification of Litmus Milk	3
25.	Alkalinisation of Simmons Citrate	2
26.	Hydrogen sulfide production	3
27.	Acetylmethylcarbinol production	3

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		Number of States	
28.	Hydrolysis of casein	3	
29.	Liquefaction of gelatin	3	
30.	Hydrolysis of starch	3	
31.	Production of catalase	3	
32.	Production of oxidase	3	
33.	Reduction of nitrate to nitrite	3	
34.	Reduction of nitrite	2	(a33)
35.	Production of lecithinase	3	
36.	Hydrolysis of tween 80	3	
37.	Production of urease	3	
38.	Production of phosphatase	3	
39.	Production of acid from glucose with $NH_4H_2PO_4$ as the source of nitrogen	2	
40.	Production of acid from glucose in peptone	2	
41.	Production of acid from glucose under anaerobic conditions (Hugh and Leifson)	2	(a20) (a39, 40
42.	Methyl red test	3	(a39, 40)
43.	Production of acid from fructose (peptone)	2	" "
44.	" " " " mannitol	2	" "
45.	" " " " lactose	2	" "
46.	" " " " saccharose	2	" "
47.	" " " " arabinose	2	" "
48.	" " " " galactose	2	" "
49.	" " " " glycerol	2	" "
50.	" " " " dulcitol	2	" "
51.	" " " " maltose	2	" "
52.	" " " " mannose	2	" "
53.	" " " " raffinose	2	" "
54.	" " " " sorbitol	2	" "
55.	" " " " xylose	2	" "
56.	" " " " salicine	2	" "
57.	" " " " inositol	2	" "
58.	Hydrolysis of aesculin	3	

a : the results of this test may be disregarded or not, depending on the results of another test, the number of which is given.

Table 2

Description of phenon E.

This cluster includes 9 isolates.
Gram positive rods, mean length 2.18 to 3.86 µm, mean diameter 0.57 to 0.80 µm, not in chains. Ghost-forms are present in 7 cases.
Sporangia not definitely swollen (ratio of mean diameter of sporangia to mean diameter of vegetative cells = 1.00 to 1.38)
Spores central and ellipsoidal, in all cases resistant to 80°C (20 minutes) and in 4 cases also resistant to 100°C.
Marine Agar colonies (30°C - 10 days) : 6 to 11 mm in diameter, opaque, glistening, slightly cream-colored.
6 strains are able to grow under anaerobic conditions.(Hugh and Leifson), but only 2 of them produce acid from glucose.
Temperature relations : optimum 30°C, minimum between 4 and 18°C, maximum between 44 and 70°C (4 strains are able to grow at a temperature of 65°C)
NaCl broth : addition of NaCl to broth is not required.
salt tolerance in all cases at least 7 per cent (3 strains still grow in 20 per cent NaCl)
Litmus milk : coagulated and reduced, by 3 strains also peptonized.
Acetyl methylcarbinol is produced
Aesculin, casein and gelatin are hydrolysed
Catalase and lecithinase are produced
Starch is not hydrolysed
Citrates are not utilized as sole source of carbon
H_2S is not produced
Urease is not produced
The tests oxidase, reduction of nitrate, hydrolysis of tween 80 and phosphatase and the methyl red test yield either positive or negative results.
In the presence of peptone acid is produced from glucose, fructose, mannitol and salicin, but not from arabinose, galactose, maltose, raffinose, sorbitol, xylose and inositol. In some cases acid is also produced from lactose, glycerol and mannose. Only 5 strains yielded acid from glucose with $NH_4H_2PO_4$ as the source of nitrogen.