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# IDENTIFICATION OF SPECIES OF THE GENUS *LISTERIA* BY FERMENTATION OF CARBOHYDRATES AND ENZYMATIC PATTERNS*

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Patterns of carbohydrates and determination of API ZYM and API oxidases can be considered a useful way to differentiate the strains of *Listeria*. With all this information it is possible to work out a schematic table that allows the identification of *Listeria* strains with a remarkable certainty. By numerical analysis four differentiated clusters have been demonstrated.

In recent years, the use of new taxonomic techniques has permitted the reclassification of the species within the genus *Listeria*. Rocourt et al. [1] divide the genus into five genomic groups composed of *Listeria monocytogenes*, *Listeria bulgarica* (serovar 5 of *L. monocytogenes)*, described later as *Listeria ivanovii* [2], *Listeria innocua*, and groups 4 and 5 which correspond to the species *Listeria welshimeri* and *Listeria seeligeri*, respectively [3]. Finally, *Listeria denitrificans* whose inclusion in the genus was never completely admitted, has been reclassified in a new genus as *Jonesia denitrificans*.

In this work previous studies of a biochemical character have been completed [4], using enzymatic microtests and fermentation of carbohydrates with a collection of strains which included all the species of the genus.

## Materials and methods

Thirty strains corresponding to the following species have been studied: *L. monocytogenes* (20), *L. welshimeri* (1), *L. seeligeri* (1), *L. innocua* (1), *L. murrayi* (2), *L. grayi* (2), *L. ivanovii* (2) and *J. denitrificans* (1). Among these are included the strain-types of each of the species studied.

The micromethod API 50CH has been used to observe the fermentation of carbohydrates, employing phenol red broth base and incubating for three days, whilst evaluating the reaction every 24 h. The enzymatic profiles were obtained by the methods API ZYM and LRA

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**Table I**

*Carbohydrate fermen*

| | *L. m.* 1/2ba | *L. m.* 1/2a | *L. m.* sv 1/2a 7973 | *L. m.* 1/2b | *L m..* 1/2c | *L. m.* c | *L. m.* c 19 116 | *L. m.* 1e | *L. m.* 2 | *L. m.* 3a | *L. m.* 4ab | *L. m.* 4a | *L. m.* 4b |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Glycerol | + | + | − | − | − | − | − | − | − | − | − | − | − |
| Erythritol | − | − | − | − | − | − | − | − | − | − | − | − | − |
| D-Arabinose | − | − | − | − | − | − | − | − | − | − | − | − | − |
| L-Arabinose | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Ribose | − | + | − | + | − | − | − | − | − | + | + | ± | − |
| D-Xylose | − | − | − | − | − | − | − | − | − | − | − | − | − |
| L-Xylose | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Adonitol | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Beta-methyl-xyloside | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Galactose | − | + | − | − | − | − | + | + | + | + | + | − | − |
| D-Glucose | + | + | + | + | + | + | + | + | + | + | + | + | + |
| D-Fructose | + | + | + | + | + | + | + | + | + | + | + | + | + |
| D-Mannose | + | + | + | + | + | + | + | + | + | + | + | + | + |
| L-Sorbose | − | ± | − | − | − | − | − | − | − | + | − | − | − |
| Rhamnose | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Dulcitol | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Inositol | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Manitol | − | + | − | ± | − | − | − | + | − | + | − | − | − |
| Sorbitol | − | + | − | + | − | − | − | − | − | + | − | − | − |
| Alpha-methyl-D--mannoside | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Alpha-methyl-D--glucoside | + | + | + | + | + | − | + | + | + | + | + | + | + |
| N-Acetyl-glucosamine | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Amygdaline | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Arbutin | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Esculin | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Salicin | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Cellobiose | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Maltose | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lactose | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Melibiose | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Sucrose | − | ± | + | ± | − | − | − | − | + | + | − | − | − |
| Trehalose | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Inulin | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Melezitose | − | ± | − | − | − | − | − | + | + | − | + | − | − |
| D-Raffinose | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Starch | + | + | ± | ± | + | + | + | ± | + | + | + | + | + |
| Glycogen | − | − | − | − | − | − | − | − | − | + | − | − | − |
| Xylitol | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Beta-gentibiose | + | + | + | + | + | + | + | + | + | + | + | + | + |
| D-Turanose | + | + | ± | ± | − | − | − | − | − | + | − | − | − |
| D-Lyxose | − | − | − | − | − | − | ± | − | − | − | − | − | − |
| D-Tagatose | − | − | − | − | − | − | − | − | − | − | − | − | − |
| D-Fucose | − | − | − | − | − | − | − | − | − | ± | − | − | − |
| L-Fucose | − | − | − | − | − | − | − | − | − | − | − | − | − |
| D-Arabitol | + | + | + | + | + | + | + | + | + | + | + | + | + |
| L-Arabitol | − | − | − | − | − | − | − | − | − | − | − | − | − |
| Gluconate | − | − | − | − | − | − | − | − | − | ± | − | − | − |
| 2-Keto-gluconate | − | − | − | − | − | − | − | − | − | − | − | − | − |
| 5-Keto-gluconate | − | − | − | − | − | − | − | − | − | − | − | − | − |

*tation patterns*

| *L. m.* 4d | *L. m.* 4d 19 117 | *L. m.* 4f | *L. m.* 4g | *L. m.* 5 19 119 | *L. bulgarica* | *L. m.* 6a | *L. innocua* 11 288 | *L. m.* 6b | *L. m.* 7 | *L. grayi* | *L. grayi* 19 120 | *L. murrayi* | *L. murrayi* 25 501 | *L. denitrificans* | *L. seeligeri* sv 1/2b | *L. welshimeri* sv 6a |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + |
| − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| − | − | − | − | − | − | − | − | − | − | − | − | − | − | + | − | − |
| + | + | − | − | + | + | − | − | ± | − | + | + | + | + | ± | − | − |
| − | − | − | − | + | + | − | − | − | − | + | − | − | − | + | + | + |
| − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| − | + | − | − | + | + | − | − | − | + | + | + | + | + | + | − | − |
| + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| + | + | + | + | + | + | + | + | + | + | + | − | + | − | − | − | − |
| − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| − | − | − | − | + | − | − | − | − | + | + | + | + | + | − | − | − |
| − | − | − | − | − | − | − | − | − | − | − | − | ± | − | − | − | − |
| + | + | + | + | + | − | + | + | + | + | + | + | + | + | − | − | + |
| + | + | + | + | + | + | + | + | + | + | + | − | + | − | − | + | + |
| + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | + | + |
| + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| − | − | − | − | − | − | − | − | − | − | − | − | − | − | + | − | − |
| − | − | − | − | − | + | − | − | + | − | − | − | − | − | + | − | ± |
| + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| + | + | − | − | − | − | − | − | + | ± | − | − | − | − | ± | ± | ± |
| − | − | − | − | − | − | − | − | − | − | − | − | + | − | − | − | − |
| + | − | + | + | − | − | + | + | + | + | + | + | + | − | + | ± | ± |
| − | − | − | − | − | − | − | − | − | − | − | + | − | − | + | − | − |
| + | + | + | + | + | + | + | − | + | + | + | + | + | + | − | + | + |
| + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| ± | − | − | ± | − | − | + | ± | + | − | − | − | − | − | + | ± | − |
| − | − | − | − | − | − | − | − | − | − | + | + | + | − | + | − | − |
| − | − | − | + | − | − | − | − | − | − | − | − | − | − | − | − | + |
| − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| + | + | + | + | + | + | + | + | − | + | + | + | + | + | − | + | + |
| − | − | − | − | − | − | − | − | − | − | ± | + | ± | + | − | − | − |
| − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |
| − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − |

ZYM oxidases, incubating for 4 h, and interpreting the reaction immediately after the reagents were added.

The results obtained were submitted to a numerical analysis, using the similarity coefficients of Sokal and Michener ($S_{sm}$) and of Jaccard ($S_j$), and employing the UPGMA as technique of clustering.

## Results

The pattern of fermentation of carbohydrates permits the clear recognition of the species of the genus *Listeria*. Thus, whereas a series of substrates were fermented usually by all strains (glucose, fructose, mannose, amigdalin, esculin, salicin, celobiose, maltose, lactose, gentibiose, and others, Table I), some others were fermented by specific strains; differential patterns of special interest have been found. *L. murrayi* is distinguished by the fermentation

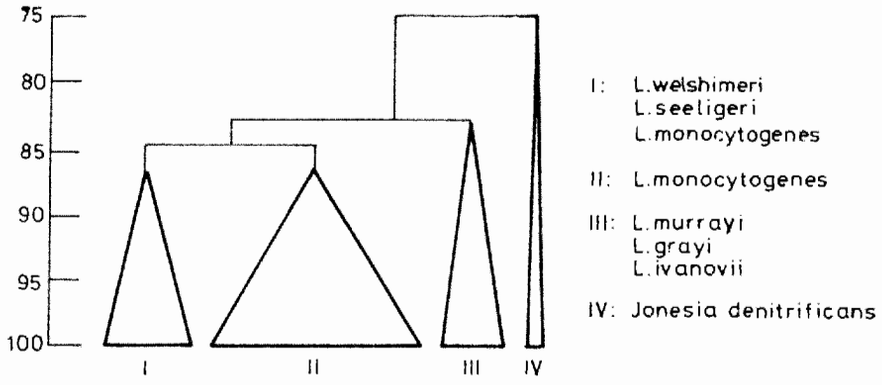


*Fig. 1.* Dendogram of clustering in phenons of *Listeria* spp

of raffinose and gluconate, sharing this characteristic with *L. grayi*. *J. denitrificans* maintains important differences from the rest of the strains studied, such as the fermentation of L-arabinose and melibiose. Some substrates, such as alpha-methyl-mannoside allow the recognition of very closely related species such as *L. seeligeri* and *L. welshimeri*. The serovars of *L. monocytogenes* present very variable results which do not allow a determined biochemical pattern to be assigned to each one of them.

The API ZYM (Table II) show the presence of phosphohydrolase and acid but not alkaline phosphatase. There are esterases from fatty acids of short chain, leucine-aryl-amidase and alpha- and beta-glucosidases (using substrates with a beta-naphthyl group conjugated). With the LRA ZYM osidases (Table III) the sensitivity of the substrates conjugated with p-nitrophenol, is superior and a greater activity can be detected, outstanding in the case of the hydrolysis of alpha- and beta-glucosidase, N-acetyl-beta-D-glucosaminidase, alpha-maltosidase, and alpha- and beta-mannosidase.

**Table II**

*Enzymatic profiles (API ZYM)*

| | *L. m.* 1/2ba | *L. m.* 1/2a | *L. m.* sv 1/2a 7973 | *L. m.* 1/2b | *L. m.* 1/2c | *L. m.* e | *L. m.* c 19 116 | *L. m.* 1e | *L. m.* 2 | *L. m.* 3a | *L. m.* 4ab | *L. m.* 4a | *L. m.* 4b | *L. m.* 4d | *L. m.* 4d 19 117 | *L. m.* 4f | *L. m.* 4g | *L. m.* 5 19 119 | *L. bulgarica* | *L. m.* 6a | *L. innocua* 11 288 | *L. m.* 6b | *L. m.* 7 | *L. grayi* | *L. grayi* 19 120 | *L. murrayi* | *L. murrayi* 25 401 | *L. denitrificans* | *L. seeligeri* sv 1/2b | *L. welshimeri* sv 6a |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alkaline phosphatase | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| Esterase (c4) | 3 | 3 | 1 | 5 | 3 | 5 | 3 | 5 | 5 | 5 | 5 | 5 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 5 | 3 | 5 | 3 | 5 | 1 | 5 | 0 | 0 | 3 | 0 |
| Lipase esterase (c8) | 1 | 1 | 1 | 3 | 1 | 3 | 1 | 3 | 3 | 3 | 1 | 3 | 1 | 5 | 1 | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 1 | 3 | 1 | 1 | 0 | 1 | 3 | 1 |
| Lipase (c14) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Leucine aryl-amidase | 1 | 1 | 0 | 3 | 3 | 3 | 1 | 5 | 5 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 3 | 3 | 1 | 1 | 1 | 1 | 0 | 1 | 5 | 5 | 3 | 1 |
| Valine aryl-amidase | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| Cystine aryl-amidase | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Trypsin | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Alpha-chemotrypsin | 1 | 0 | 0 | 3 | 3 | 1 | 0 | 5 | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 1 | 0 | 1 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Acid-phosphatase | 3 | 1 | 1 | 5 | 3 | 3 | 5 | 5 | 3 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 3 | 5 | 3 | 3 | 3 | 1 | 3 | 0 | 0 | 0 | 0 | 0 | 3 | 1 |
| Phosphohydrolase | 3 | 3 | 1 | 5 | 5 | 1 | 3 | 5 | 0 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 1 | 1 | 1 | 1 | 1 | 3 | 3 |
| Alpha-galactosidase | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Beta-galactosidase | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Beta-glucuronidase | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Alpha-glucosidase | 0 | 5 | 5 | 3 | 0 | 0 | 3 | 0 | 5 | 0 | 1 | 3 | 5 | 5 | 3 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 5 | 1 | 0 | 3 | 0 | 5 | 5 | 0 |
| Beta-glucosidase | 3 | 3 | 1 | 1 | 3 | 5 | 5 | 1 | 0 | 5 | 5 | 5 | 0 | 3 | 5 | 0 | 3 | 5 | 0 | 3 | 1 | 3 | 3 | 5 | 1 | 5 | 0 | 1 | 3 | 3 |
| N-Acetyl-beta-glucosaminidase | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 1 | 0 | 1 | 0 | 0 |
| Alpha-mannosidase | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Alpha-fucosidase | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

0 = 0 nanomol; 1 = 5 nanomoles; 3 = 20 nanomoles; 5 = 40 nanomoles

**Table III**

*Enzymatic profiles (LRA ZYM oxidases)*

| | *L. m.* 1/2na | *L. m.* 1/2a | *L. m.* sv 1/2a 7973 | *L. m.* 1/2b | *L. m.* 1/2c | *L. m.* c | *L. m.* c 19 116 | *L. m.* 1e | *L. m.* 2 | *L. m.* 3a | *L. m.* 4ab | *L. m.* 4a | *L. m.* 4b | *L. m.* 4d | *L. m.* 4d 19 117 | *L. m.* 4f | *L. m.* 4g | *L. m.* 5 19 119 | *L. bulgarica* | *L. m.* 6a | *L. innocua* 11 288 | *L. m.* 6b | *L. m.* 7 | *L. grayi* | *L. grayi* 19 120 | *L. murrayi* | *L. murrayi* 25 401 | *L. denitrificans* | *L. seeligeri* sv 1/2b | *L. welshimeri* sv 6a |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Alpha-D-galactosidase | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Beta-D-galactosidase | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Phospho-beta-D-galactosidase | 3 | 3 | 1 | 1 | 1 | 1 | 5 | 3 | 5 | 3 | 0 | 0 | 5 | 3 | 1 | 3 | 3 | 1 | 3 | 0 | 1 | 3 | 5 | 1 | 0 | 0 | 1 | 0 | 3 | 1 |
| Alpha-L-arabinosidase | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 5 | 0 | 0 |
| Alpha-D-glucosidase | 3 | 5 | 3 | 5 | 0 | 0 | 5 | 1 | 5 | 0 | 5 | 1 | 5 | 5 | 3 | 5 | 3 | 1 | 0 | 5 | 0 | 3 | 5 | 3 | 1 | 1 | 3 | 5 | 5 | 0 |
| Beta-D-glucosidase | 5 | 5 | 3 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 3 | 5 | 5 | 5 | 3 | 5 | 5 | 5 | 5 | 3 |
| Beta-D-galacturonohydrolase | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Beta-D-glucuronidase | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Alpha-maltosidase | 3 | 5 | 0 | 5 | 0 | 1 | 3 | 3 | 3 | 3 | 5 | 1 | 3 | 5 | 3 | 5 | 5 | 1 | 0 | 5 | 0 | 0 | 5 | 5 | 3 | 5 | 3 | 3 | 0 | 0 |
| Beta-maltosidase | 1 | 5 | 0 | 3 | 0 | 0 | 0 | 0 | 1 | 0 | 3 | 0 | 1 | 3 | 0 | 1 | 1 | 0 | 0 | 3 | 0 | 1 | 3 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| N-Acetyl-alpha-D-glucosaminidase | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N-Acetyl-beta-D-glucosaminidase | 5 | 5 | 1 | 5 | 3 | 3 | 5 | 1 | 5 | 5 | 3 | 1 | 5 | 5 | 5 | 5 | 5 | 3 | 3 | 5 | 1 | 5 | 5 | 5 | 1 | 5 | 3 | 0 | 3 | 1 |
| Alpha-L-fucosidase | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Beta-D-fucosidase | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| Beta-L-fucosidase | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Beta-D-lactosidase | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Alpha-D-mannosidase | 5 | 5 | 0 | 5 | 0 | 0 | 1 | 1 | 0 | 5 | 5 | 0 | 0 | 5 | 1 | 0 | 0 | 0 | 0 | 5 | 0 | 5 | 5 | 3 | 0 | 0 | 1 | 0 | 0 | 0 |
| Beta-D-mannosidase | 5 | 5 | 0 | 5 | 1 | 1 | 3 | 3 | 3 | 5 | 3 | 0 | 5 | 5 | 3 | 5 | 5 | 1 | 1 | 5 | 0 | 5 | 5 | 5 | 0 | 1 | 3 | 0 | 1 | 0 |
| Alpha-D-xylosidase | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| Beta-D-xylosidase | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 |

For explanation see Table II

The processing of the data in order to obtain the coefficients of similarity and the clustering by means of UPGMA demonstrates the formation of four differentiated clusters (Fig. 1). Three, with a similarity of 85% ($S_{sm}$) groups the phenon I *(L. welshimeri, L. seeligeri, L. innocua* and three strains accepted as *L. monocytogenes)*; the phenon II, which includes the rest of the strains of *L. monocytogenes* together with the strain type; and the phenon III composed of *L. murrayi, L. grayi* and *L. ivanovii.* Further away, with 75% similarity, one finds the phenon IV which includes as only strain *Jonesia denitrificans.*

## Discussion

The patterns of fermentation of carbohydrates can be considered as a useful method in the differentiation of strains of *Listeria*, and has thus been recognized in previous works [4, 5]. API ZYM and API oxidases bring important data. With all this information it is possible to elaborate reduced tables which permit the identification of *Listeria* with a notable level of reliability.

In the same way, the numerical analysis of these data shows us the homogeneity of the genus, once *J. denitrificans* has been separated, also the convenience of maintaining *L. grayi* and *L. murrayi* in the genus, in agreement with data of other authors [3, 6].

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