GENETIC AND BIOCHEMICAL IDENTIFICATION OF MICROORGANISMS ISOLATED FROM AGRICULTURAL AND FOOD WASTES FOR METHANE FERMENTATION PROCESS

Magdalena Frąc[1], Anna Pawlik[2], Karolina Oszust[1], Anna Siczek[1], Nina Bilińska[1]

[1]Institute of Agrophysics Polish Academy of Sciences,
Doświadczalna 4, 20-290 Lublin 27, POLAND
e-mail: m.frac@ipan.lublin.pl

[2]Maria Curie-Skłodowska University, Department of Biochemistry,
Akademicka 19, 20-033 Lublin, POLAND
e-mail: anna.pawlik@poczta.umcs.lublin.pl

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INTRODUCTION

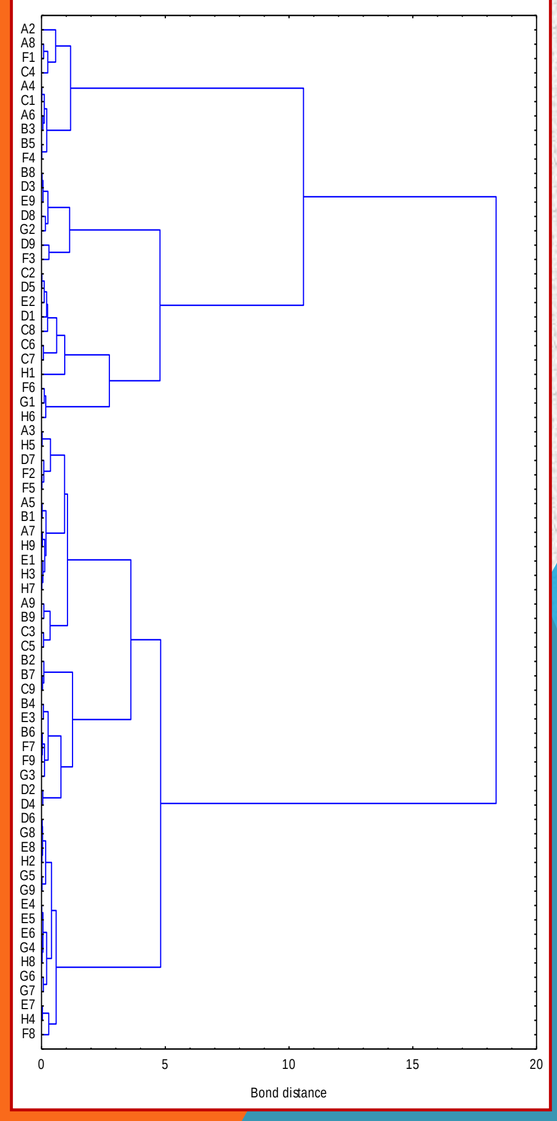
Agricultural and food wastes contain a large amount of organic matter content, which can be important source of microorganisms. The development of tools aimed at the clear-cut and safe identification and assessment of genetic, as well as biochemical variability of the wild strains is a fundamental goal of molecular genetic research. These study are also important in evaluation of strains as potential microorganisms used in biotechnology sector, like bio-preparations for waste utilization.

OBJECTIVE, MATERIALS AND METHODS

The aim of this study was the genetic and biochemical identification of selected bacterial strains isolated from organic waste originating from agricultural and food sector. Microorganisms were identified with molecular technique using comparative sequencing of the bacterial 16S rDNA region. The MicroSEQ ID software were used for genetic identification and clustering with Neighbor-Joining method. The binary information was used to calculate Jaccard's pairwise similarity coefficients. The Biolog Gen III MicroPlate and OmniLog Data System was applied for identification microorganisms from their phenotypic pattern.

Fungal strain	Molecular identification					Metabolic identification
	Specimen Score	Top match	% Match	Consensus Length	Library Entry Length	
B_23_11	36	*Micrococcus luteus* (ATCC=4698)	99.95	457	465	*Micrococcus luteus*
B_40_11	32	*Bacillus megaterium* (ATCC=14581)	99.98	500	497	*Bacillus idriensis*
B_58_11	36	*Paenibacillus favisporus* (DSM=17253)	99.92	502	499	*Paenibacillus glycanilyticus*
B_93_11	35	*Bacillus mycoides* (ATCC=6462)	100.00	499	498	*Bacillus hemicellulosilyticus*
B_95_11	31	*Bacillus simplex* (ATCC=49097)	99.98	434	495	*Brevibacterium frigoritolerans*
B_97_11	35	*Bacillus atrophaeus* (ATCC=49337)	92.64	357	496	*Bacillus licheniformis*

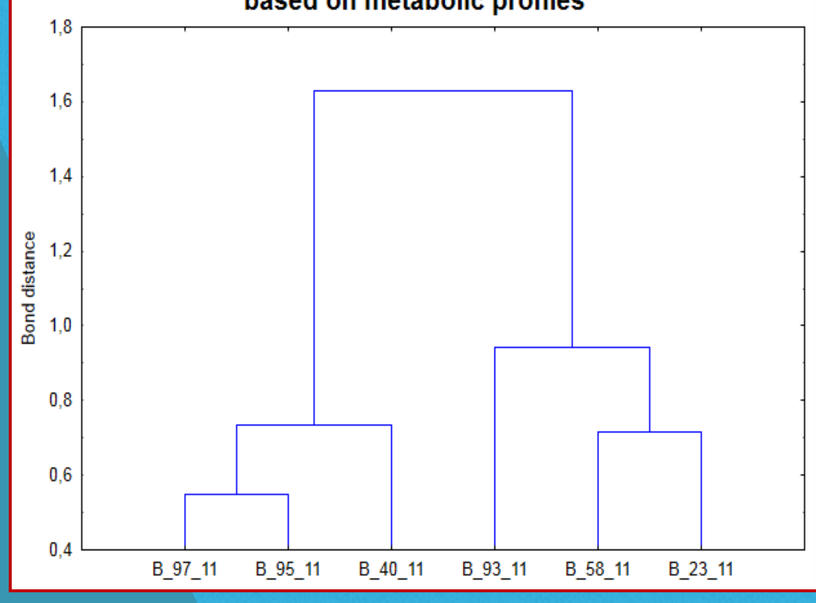
Results of cluster analysis for carbon substrates from GEN III plate based on metabolic profiles of bacterial strains



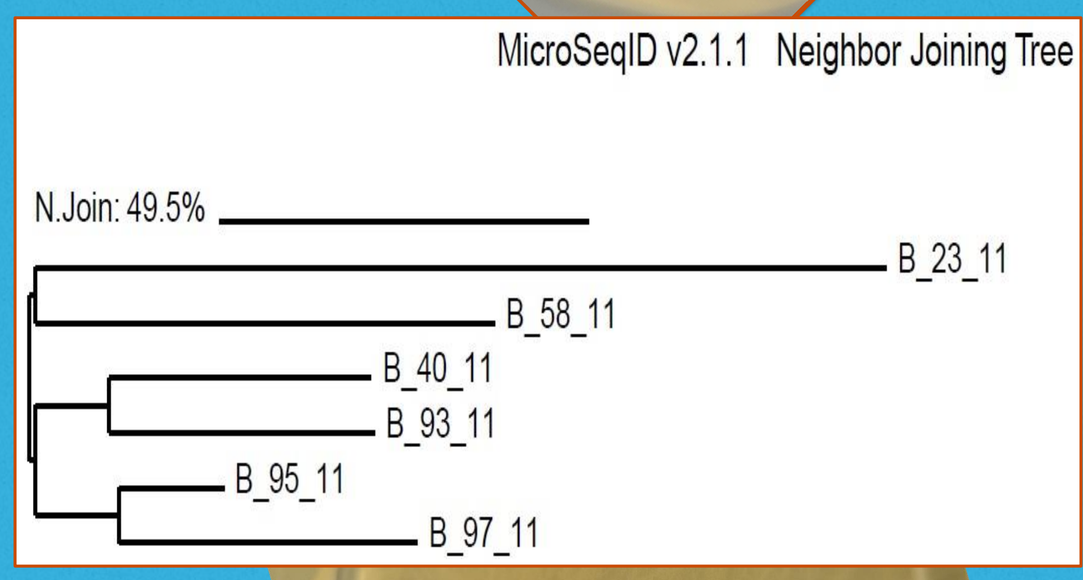
Percent similarity/divergence between analysed strains based on 16 S rDNA sequences

	Fungal strain	Percent identity (similarity)					
		B_97_11	B23_11	B_40_11	B_58_11	B_93_11	B_95_11
Percent divergence	B_97_11		76.5	84.4	81.8	85.3	91.4
	B_23_11	26.0		77.0	77.9	78.6	78.1
	B_40_11	15.5	26.6		84.0	90.2	90.8
	B_58_11	19.3	26.0	17.2		83.5	87.1
	B_93_11	15.0	24.8	10.0	18.2		88.9
	B_95_11	6.3	24.8	8.7	13.1	11.1	

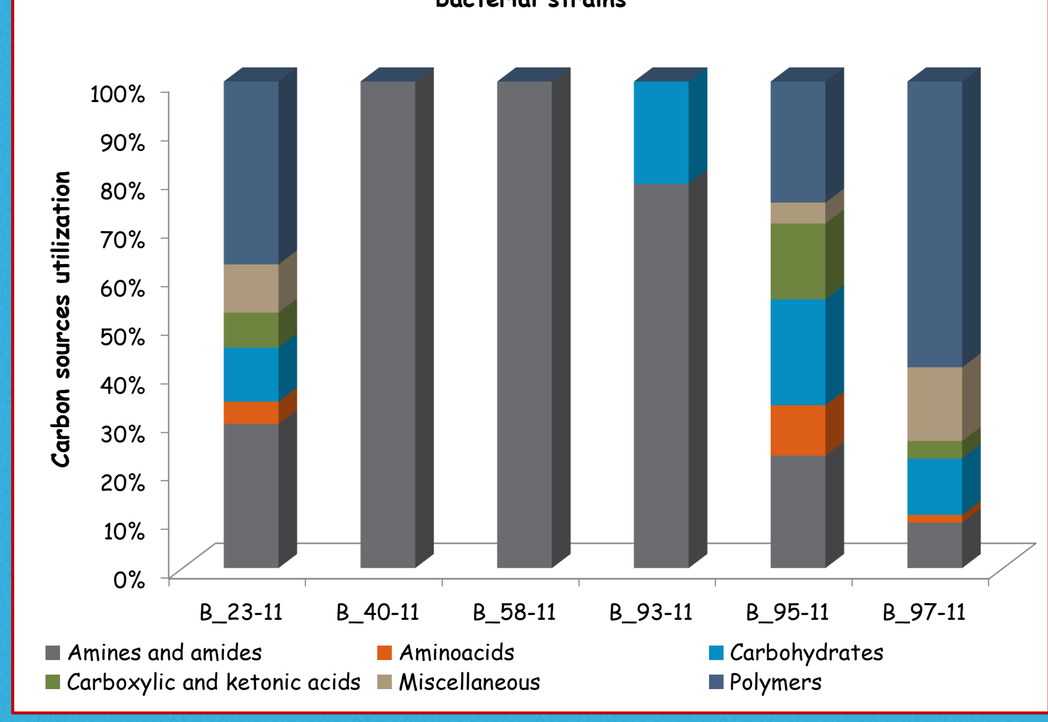
The results of cluster analysis for selected bacterial strains based on metabolic profiles



MicroSeqID v2.1.1 Neighbor Joining Tree



Utilization of different substrates in GEN III Plates be selected bacterial strains



RESULTS

Both the molecular, as well as, biochemical analysis allowed the microorganisms identification at the species level. Correlation between genetic identification (16S rDNA analysis) and substrate utilization fingerprints (Gen III MicroPlate) were depended on the bacteria strains. Sometimes there were found strong correlations and for other strains there were no correlations between molecular and metabolic identification.