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Identification and Characterization of Metabolic Potential of Different Strains from Genus Rhizobium†

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Abstract: Bacteria of the *Rhizobium* genus form a group of microorganisms existing in the environment in two forms: symbiotic - in the root nodules of *Fabaceae* sp. plants and free-living, saprophytic in the soil environment. The subject of study was genetic identification and characterization of metabolic activity of different strains from *Rhizobium* genus bacteria. The study was conducted on the 16 bacteria strains from the collection of Department of Agricultural Microbiology, Institute of Soil Science and Plant Cultivation in Puławy, Poland. Based on the sequencing of PCR products, we found that all strains belong to one species - *Rhizobium leguminosarum*. The study of metabolic activity was performed using the GEN III BIOLOG system method (Biolog Inc., Hayward, CA, USA). Metabolism analysis of all *R. leguminosarum* strains with the use of GEN III™ plates showed that carbohydrates (CH) were the most intensively utilised group of substrates. Between the *Rhizobium* leguminosarum strains, there are metabolic differences in terms of the studied features.

Keywords: bacterial strain; Rhizobium; Biolog GEN III

1. Introduction

Bacteria of the *Rhizobium* genus form a group of microorganisms existing in the environment in two forms: symbiotic - in the root nodules of *Fabaceae* sp. plants and free-living, saprophytic in the soil environment [1]. Inside of root this bacteria differentiation into nitrogen-fixing bacteroids [2]. The basic function of *Rhizobium* sp. in a symbiosis is to reduce nitrogen to ammonia directly assimilated by the plant. Nitrogen reduction occurs with the participation of enzymatic nitrogenase complex [3]. The subject of study was genetic identification and characterization of metabolic activity of different strains from *Rhizobium* genus.

2. Material and methods

2.1. Bacterial strains

The study was conducted on the 16 bacteria strains from the collection of Department of Agricultural Microbiology, Institute of Soil Science and Plant Cultivation in Puławy, Poland. Bacteria strains were isolated from root nodules derived from plans of the genus *Trifolium*. The collection of bacterial strains was obtained from the roots of plants growing in many locations, mainly in Poland (Table 1).

Table 1. Source of bacterial strains.

Strain symbol	Location	Plant	Year
C37	Poland, Lublin	*Trifolium* sp.	1960
209	USA, Madison	*Trifolium* sp.	1960
325a	USSR, Leningrad (now Russia, Petersburg)	*Trifolium* sp.	1957
G	Poland, Gnojno	*Trifolium* sp.	1994
G4	Poland, Grabów	White clover (*T. repens L.)*	1995
KB	Poland, Grabów	White clover (*T. repens L.)*	1995
KR	Poland, Puławy	Red clover (*T. pratense* L.), var. „Raba"	1996
K1	Poland, Stare Pole	*Trifolium* sp.	2000
K3	Poland, Łabunie	*Trifolium* sp.	2000
K10	Poland, Opatów	*Trifolium* sp.	2000
K18	Poland, Puławy	White clover (*T. repens L.)*	2004
K20	Poland, Puławy	White clover (*T. repens L.)*	2004
K 99/4	Poland, Puławy	*Trifolium* sp. *	1998
K 99/11	Poland, Wielichowo	*Trifolium* sp.	1999
K 99/12	Poland, Wielichowo	*Trifolium* sp.	1999
K 99/13	Poland, Wielichowo	*Trifolium* sp.	1999

* Strain was isolated from plants growing in soil contaminated with heavy metals.

2.2. PCR and sequencing

For PCR, a small amount of material was taken from a single bacterial colony and transferred to a sterile eppendorf with 20 µl of MiliQ water. The samples were thoroughly mixed and 1 µl was taken from the mixture for the PCR reaction. The 16S rDNA region was amplified using primers: 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) [4]. The PCR products were sequenced in Genomed S.A (Warsaw, Poland) using the same primers as at the PCR step. The sequences from both primers were assembled in Unipro UGENE 1.25 software [5] and the Blast search has been taken [6].

2.3. Biolog GEN III

The study of metabolic activity was performed using the GEN III BIOLOG system method (Biolog Inc., Hayward, CA, USA). The GEN III microplate contains 94 phenotypic tests: 71 carbon source utilization assays and 23 chemical sensitivity assays. Tetrazolium dyes from the wells of the microplate are used to indicate the use of carbon sources or resistance to inhibitory chemicals by microorganisms. The cell suspensions were inoculated into the 134 GEN III™ (100 µl per well) and incubated at 25 °C for 7 days. The intensity of colour development was recorded at λ=590 nm at 24 h intervals for a period of 168 h. The results obtained at 168 h are presented because the most intensive substrate decomposition was observed after this incubation time.

2.4. Data analysis and visualisation

Heatmaps were generated using GenIII Omnilog values (data after 168 h of incubation) with R software (version 3.5.1, Northern Ave, Boston, USA) and pheatmap package. Similarity trees were constructed using Bray-Curtis cluster analysis with the UPGMA method [7].

Statistical analyses were performed using the packet Statistica.PL ver. 10.0 (StatSoft. Inc., Tulsa, OK, USA). The dendrogram was applying Ward's method clustering and the squared Euclidean distance matrix calculation method [8].

3. Results

3.1. Bacterial species

PCR products from all the isolates were compared with a size marker and a product with an expected size of about 1500 bp was found (Figure 1). Based on the sequencing of PCR products, we found that all strains belong to one species - *Rhizobium leguminosarum* with a sequence identity of 97-100% (NCBI GenBank, Table 2) [6].

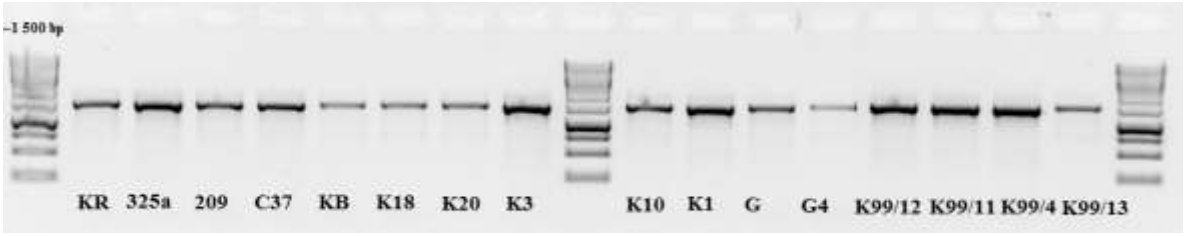

Figure 1. Picture of polyacrylamide gel electrophoresis.

Table 2. The identification of bacteria strains (NCBI GenBank).

Strain symbol	Closest species	Identity
209	*Rhizobium legiuminosarum*	100 %
G	*Rhizobium legiuminosarum*	100 %
K10	*Rhizobium legiuminosarum*	100 %
K99/12	*Rhizobium legiuminosarum*	100 %
K99/4	*Rhizobium legiuminosarum*	100 %
KR	*Rhizobium legiuminosarum*	100 %
C37	*Rhizobium legiuminosarum*	99 %
325a	*Rhizobium legiuminosarum*	99 %
G4	*Rhizobium legiuminosarum*	99 %
K3	*Rhizobium legiuminosarum*	99 %
K99/11	*Rhizobium legiuminosarum*	99 %
K99/13	*Rhizobium legiuminosarum*	99 %
KB	*Rhizobium legiuminosarum*	99 %
K1	*Rhizobium legiuminosarum*	98 %
K20	*Rhizobium legiuminosarum*	97 %
K18	*Rhizobium legiuminosarum*	97 %

All sequences are available at the NCBI database under accession number: SUB7603645.

3.2. Metabolic activity of Rhizobium leguminosarum bacteria

Based on results the heat maps were made (Figure 2) and the cluster analysis according to Ward's method conducted thus illustrating the diversity of strains in terms of the intensity and pace of the individual compounds consumption (Figure 3).

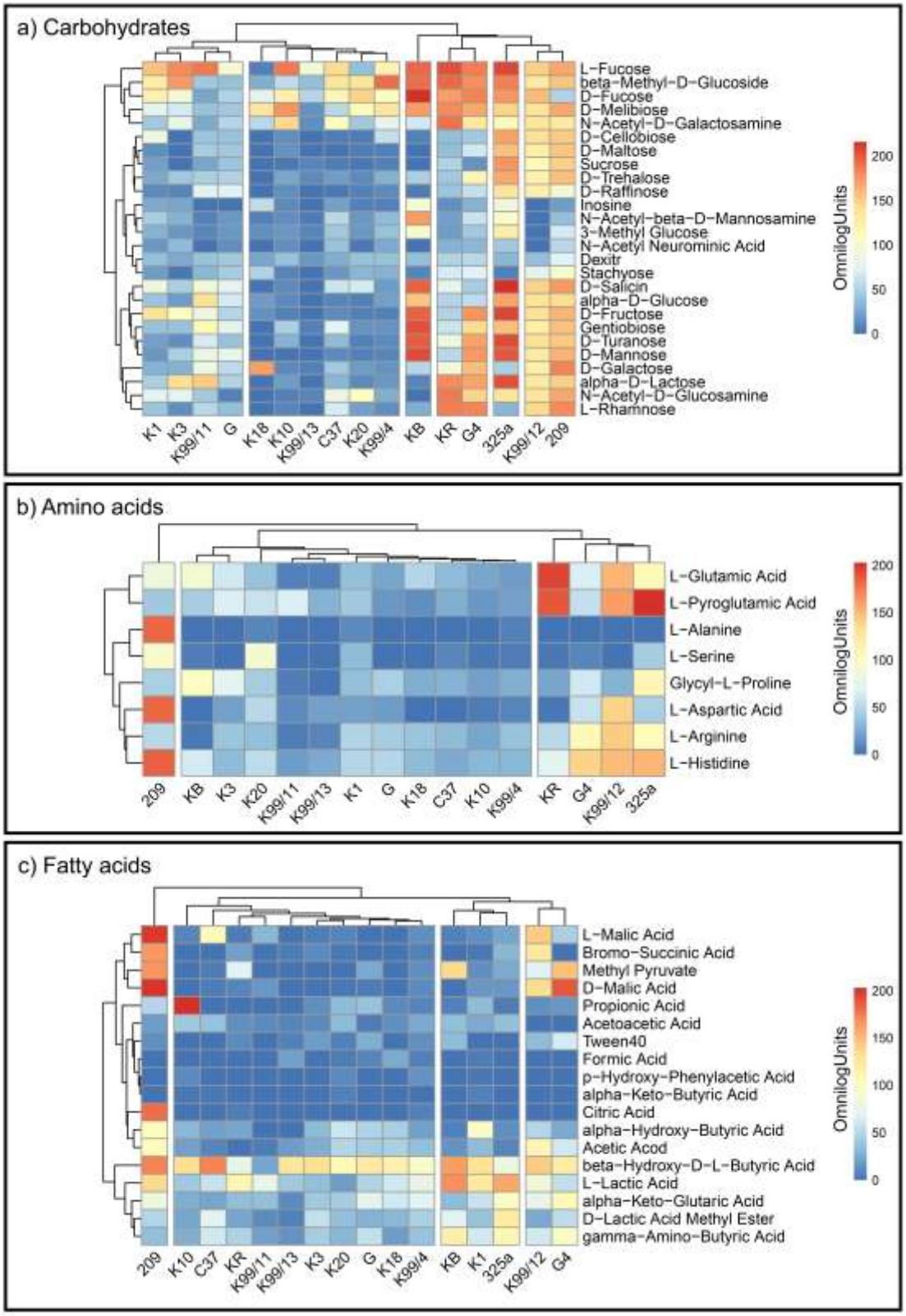

Figure 2. Heatmaps for the carbon utilization patterns of the substrates GEN III grouped into three biochemical groups: (**a**) carbohydrates, (**b**) amino acids, (**c**) fatty acids, by each strain of *Rhizobium leguminosarum*. Data are shown after 168 hours of incubation. The gradient from light blue to red represents positive utilization.

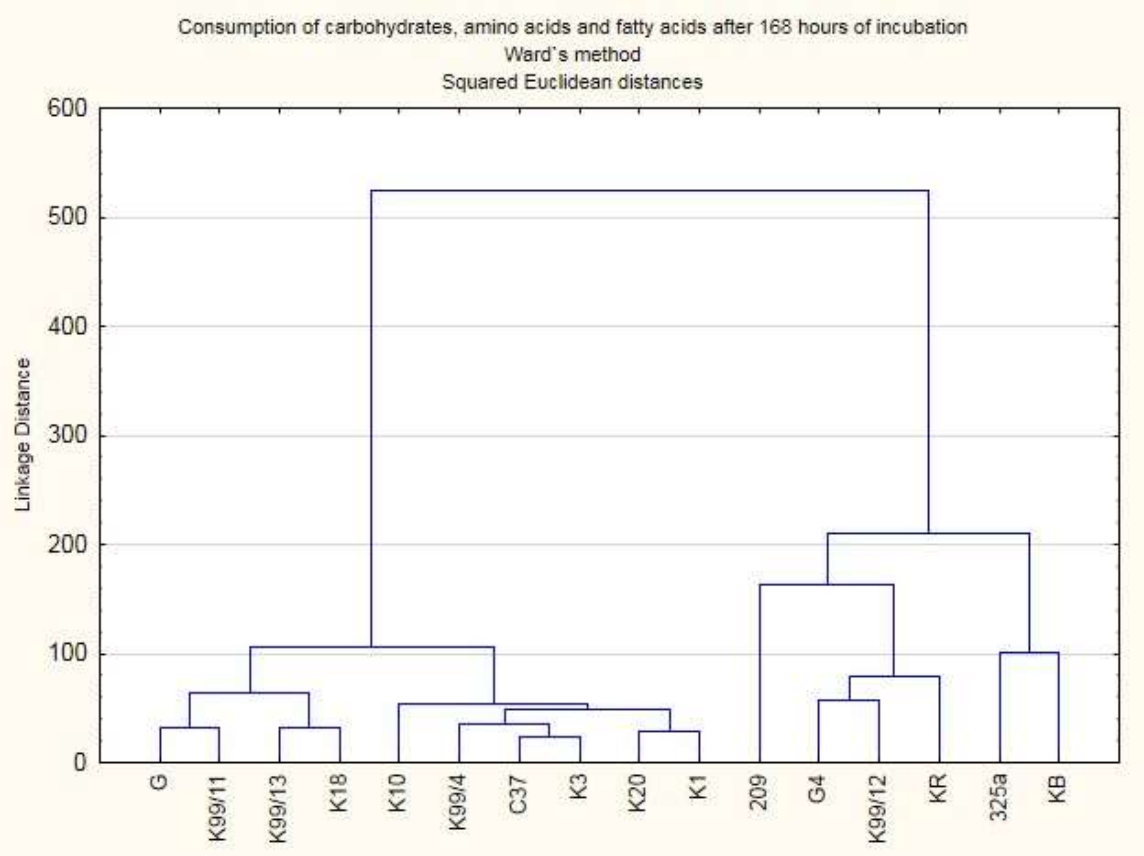

Figure 3. Dendrogram showing division of *Rhizobium leguminosarum* strains due to the use of carbohydrates, amino acids and fatty acids as a carbon sources after 168 h incubation.

Metabolism analysis of all *R. leguminosarum* strains with the use of GEN III™ plates showed that carbohydrates (CH) were the most intensively utilised group of substrates. Between the *Rhizobium leguminosarum* strains, there are metabolic differences in terms of the studied features (Figure 2). That may indicate the adaptive capacity of microorganisms to the environmental conditions in which they currently live. Based on the cluster analysis, 3 groups of microorganisms were isolated in terms of the intensity of decomposition of the tested compounds (Figure 3). The most active strains in terms of using as a carbon source all three types of compounds are strains 209, K99/12, 325a and G4.

4. Conclusions

In conclusion, it can be stated that, between the *Rhizobium leguminosarum* strains, there are metabolic differences in terms of the studied features. That may indicate the adaptive capacity of microorganisms to the environmental conditions in which they currently live.

Author Contributions: K.G. and A.G. conceived and designed the experiments; K.G., K.F. and J.G. performed the experiments and analysed the data; K.G. and K.F. wrote the paper

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Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Stasiak, G.; Mazur, A.; Koper, P.; Żebracki, K.; Skorupska, A. Symbiosis of rhizobia with legume plants (Fabaceae). *Postep. Mikrobiol.* **2016**, *55*, 289–299.
2. Kereszt, A.; Mergaert, P.; Kondorosi, E. Bacteroid development in legume nodules: Evolution of mutual benefit or of sacrificial victims? *Mol. Plant-Microbe Interact.* 2011, *24*, 1300–1309.
3. Oke, V.; Long, S.R. Bacteroid formation in the Rhizobium-legume symbiosis. *Curr. Opin. Microbiol.* 1999, *2*, 641–646.
4. Weisburg, W.G.; Barns, S.M.; Pelletier, D.A.; Lane, D.J. 16S Ribosomal DNA Amplification for Phylogenetic Study. *J. Bacteriol.* **1991**, *173*, 697–703.
5. Okonechnikov, K.; Golosova, O.; Fursov, M.; UGENE team Unipro UGENE: a unified bioinformatics toolkit. *Bioinformatics* **2012**, *28*, 1166–1167, doi:10.1093/bioinformatics/bts091.
6. Blast NCBI Available online: https://blast.ncbi.nlm.nih.gov/Blast.cgi.
7. McMurdie, P.J.; Holmes, S. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS One* **2013**, *8*, e61217, doi:10.1371/journal.pone.0061217.
8. Ward, J.H. Hierarchical Grouping to Optimize an Objective Function. *J. Am. Stat. Assoc.* **1963**, *58*, 236–244, doi:10.1080/01621459.1963.10500845.