Identification and characterization of metabolic potential of different strains from genus *Rhizobium*

Karolina Gawryjołek [1], Karolina Furtak [1,*], Jarosław Grządziel [1], Anna Gałązka [1]

Department of Agricultural Microbiology
Institute of Soil Science and Plant Cultivation - State Research Institute
Czartoryskich 8, Puławy, Poland;
kgaw@iung.pulawy.pl; kfurtak@iung.pulawy.pl;
jgrzadziel@iung.pulawy.pl; agalazka@iung.pulawy.pl



Introduction

Bacteria of the *Rhizobium* genus:

- They are common in all types of soils.
- They differ from other soil microorganisms in that they exist in two forms:

1) free-living, saprophytic in the soil,
2) 2) symbiotic - in the root nodules of *Fabaceae* sp. plants [1].

- Inside of root this bacteria differentiation into nitrogen-fixing bacteroids [2].
- The basic function of Rhizobia in the symbiotic system is the reduction of molecular nitrogen to ammonia, directly assimilated by the plant. The plant supplies its symbiotes with carbon compounds produced during the photosynthesis process.
- Nitrogen reduction occurs with the participation of enzymatic nitrogenase complex [3].

$$N_2 + 16\,ATP + 8e^- + 8H^+ \xrightarrow{\text{Nitrogenase; } Mg^{2+}} 2NH_3 + H_2 + 16\,ADP + 16\,P_i$$

The aim of research was genetic identification and characterization of metabolic activity of different strains from *Rhizobium* sp.

Materials

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.

Table 1. Origin of bacteria 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

Methods

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 study of metabolic activity was performed using the GEN III BIOLOG system method (Biolog Inc., Hayward, CA, USA).

The cell suspensions were inoculated into the 134 GEN III™ (100 µl per well) and incubated at 25 °C for 7 d*ays*.

The intensity of colour development was recorded at λ=590 nm at 24 h intervals for a period of 168 h.

Results

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) [5]. All sequences are available at the NCBI database under accession number: SUB7603645.

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 %

Results

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 1).

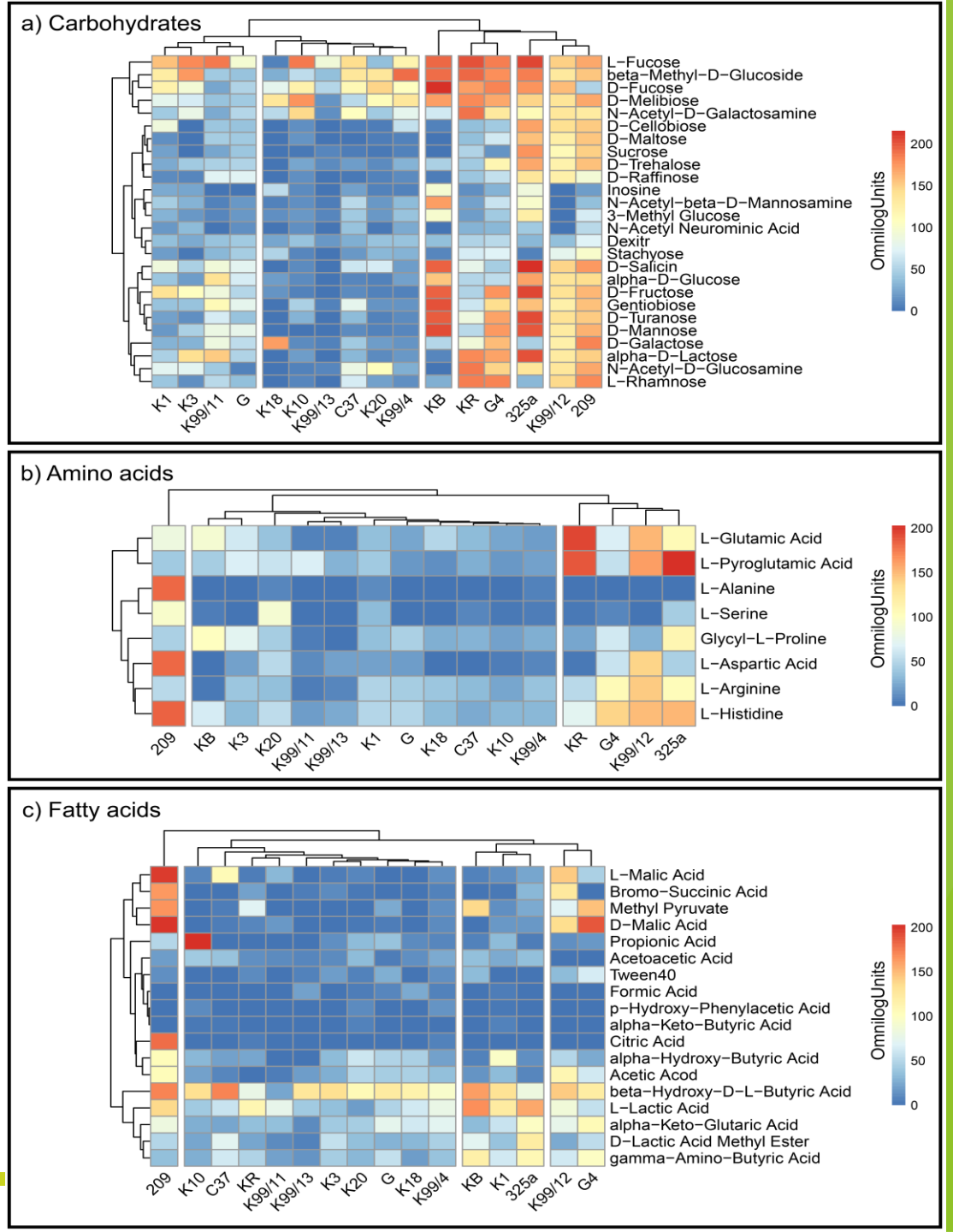

Figure 1. Heatmaps for the carbon utilization patterns of the substrates GEN III grouped into three biochemical groups: (a) carbohydrates, (b) amino acids, (c) fatty acids, by each strain of Rhizobium leguminosarum. Data are shown after 168 hours of incubation. The gradient from light blue to red represents positive utilization.

Results

Based on the cluster analysis, 3 groups of microorganisms were isolated in terms of the intensity of decomposition of the tested compounds (Figure 2).

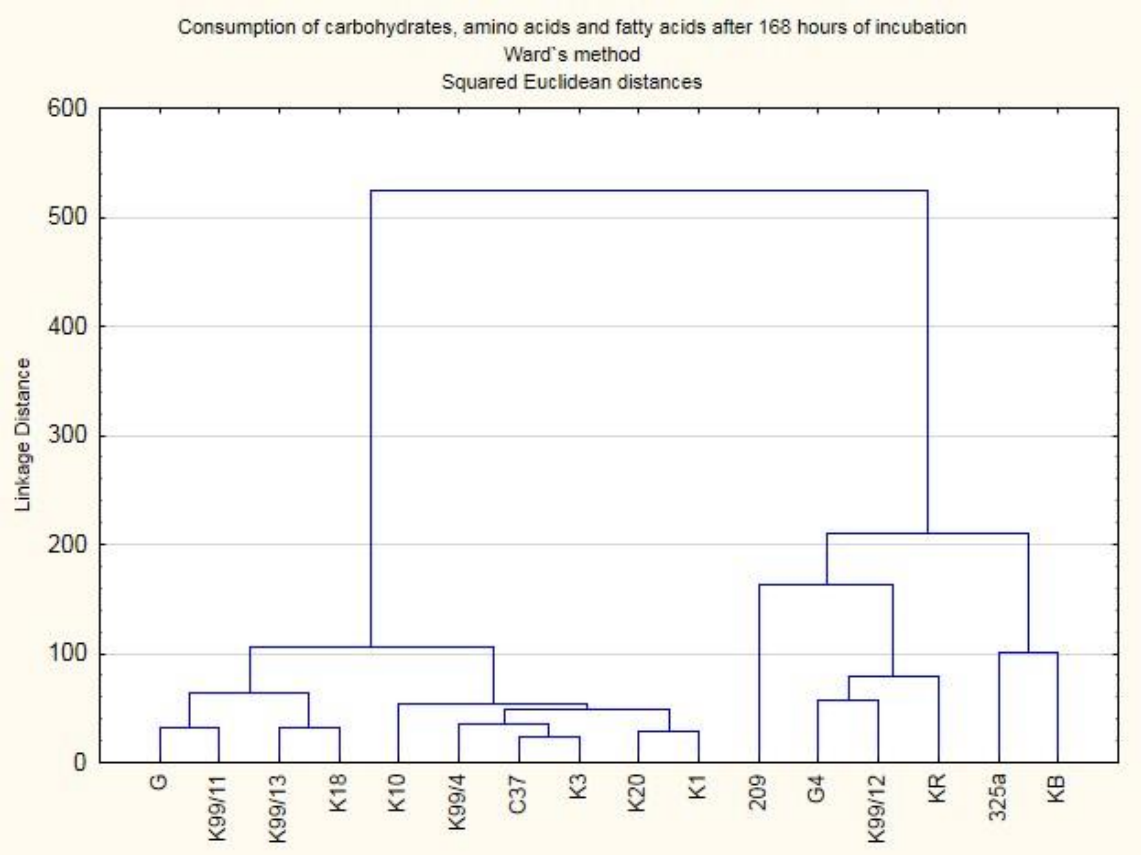

Figure 2. Dendrogram showing division of Rhizobium leguminosarum strains due to the use of carbohydrates, amino acids and fatty acids as a carbon sources after 168 h incubation.

Conclusions

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.

Between the *Rhizobium leguminosarum* strains examined, 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.

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. Blast NCBI Available online: https://blast.ncbi.nlm.nih.gov/Blast.cgi.