UDC 577

Mariola Paściak[1], Ewelina Kasprów[1], Ying Huang[2], Andrzej Gamian[1]

[1]Ludwik Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Rudolfa Weigla 12, 53-114 Wrocław, Poland, tel. +48 71 370 99 22, e-mail: pasciak@iitd.pan.wroc.pl
[2]Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, PR China.

POLAR LIPIDS OF *RUANIA ALBIDIFLAVA*, A NOVEL MEMBER OF THE SUBORDER *MICROCOCCINEAE*

Ruania albidiflava represents a novel species and new genus within the suborder Micrococcineae in Actinobacteria classis. The aim of the studies was to establish a polar lipid profile of Ruania and to compare it with other representatives of actinobacteria. Additionally, isolation, purification and chemical characteristics of major lipid compounds were elaborated. Major lipids were isolated using column adsorption chromatography and purified by TLC and high performance liquid chromatography. The polar lipids of Ruania were phosphatidylglycerol, diphosphatidylglycerol and two unknown lipids containing sugar and phosphorus. GLC-MS and methylation analysis of sugar part of major glycolipids revealed the presence of glycerol, inositol; and terminal residues of mannose and galactosamine. Fatty acids were mainly iso and anteiso C15:0 branched. Comparative TLC showed its glycolipid profile of Ruania different from previously reported for Rothia, Arthrobacter, Micrococcus and Propionibacterium, but was similar to Oerskovia which belongs to Micrococcineae suborder.

K e y w o r d s: bacterial lipids, glycolipids, Ruania, chemical markers.

Actinobacteria class was introduced by Stackebrandt and coworkers in 1997 and contains phylogenetically related microorganisms on the basis of 16 S rRNA and rDNA sequence similarity, previously known as actinomycetes [10].

During the past several years numerous novel taxa in the class *Actinobacteria* were cultured and described. One of them is *Ruania albidiflava*, which represents a novel species and new genus within the suborder *Micrococcineae* [3]. Besides that new families in this suborder are created, *Ruania* up to now represents a separate genus [4] and the nearest phylogenetic neighbour was determined as *Georgenia muralis* [3].

The strain was isolated from farmland soil from Shandong province in China [3]. Characteristic feature of this genus is new murein type, L-Lys—Gly—L-Glu—L-Glu (A4α) in the peptidoglycan of a cell wall.

Valuable markers in chemotaxonomy are polar lipids i.e. phospho- and glycolipids. Phospholipids are broad and important markers in actinomycete taxonomy [5]. The early observations showed that polar lipid profile was useful in the differentiation of actinomycetes and allied taxa giving the reference glycolipid

patterns. Studies of our group revealed some specific glycolipids markers f. ex. in *Propionibacterium* [9], *Rothia* [8], *Saccharopolyspora* [2] and many other genera [6].

The aim of the study was to establish a polar lipid profile of *Ruania* and to compare it with other representatives of actinobacteria. Additionally, isolation, purification and chemical characteristics of major lipid compounds were performed.

Materials and methods

Ruania albidiflava PCM 2644^T (PCM – Polish Collection of Microorganisms) was originally obtained from Chinese partners. Strains used in comparative experiments: *Micrococcus luteus* PCM 525^T, *Rothia dentocariosa* PCM 2249^T, *Propionibacterium propionicum* PCM 2431^T and *Oerskovia xantineolytica* PCM 2385^T.

Ruania was grown on Rich medium [3] on a rotary shaker under aerobic conditions at 37 °C for 48 h. *Micrococcus luteus*, *Rothia dentocariosa* and *Oerskovia xantineolytica* were cultivated on medium 79 [6] but *Propionibacterium propionicum* according to [9].

After cultivation biomass was centrifuged (6000rpm, 20 min) and washed twice with phosphate buffer (PBS).

The wet biomass was extracted twice with chloroform-methanol (2:1 v/v) [7]. The obtained lipid samples were analyzed by thin layer chromatography using specific spray reagents: vanillin, orcinol, ninhydrin and Dittmer & Lester reagent for phosphorus [7].

Major lipids were isolated using column adsorption chromatography and purified by TLC and high performance liquid chromatography [9].

Fatty acid, neutral sugar and methylation analyses were performed by GLC-MS [9].

MALDI-TOF mass spectra were performed in the positive ion mode with DHB matrix on Kratos Kompact-SEQ instrument.

Results and discussion

After 48 h incubation of *R. albidiflava* cells in submerged culture the biomass was obtained with yield 11.8 g from 1l of medium. Morphology of colonies and Gram staining smears are presented in Fig. 1.

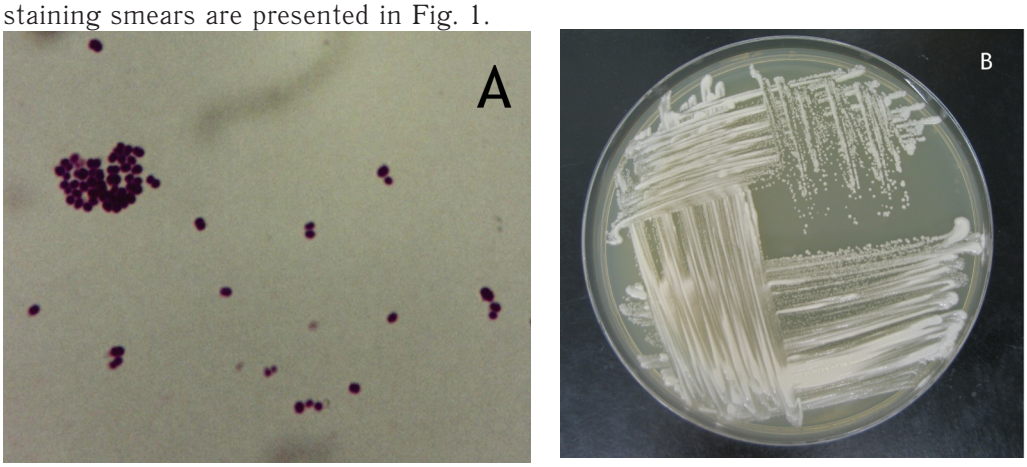


Fig. 1. *Ruania albidiflava*
A – Gram stain of cells from Rich medium (48h submerged culture, magnification 1000x; B – colonies on nutrient agar (72h, 28 °C)

Chloroform-methanol extract of bacterial biomass of *R. albidiflava* was obtained with yield of 0.32%. The polar lipids of *Ruania* were phospatidylglycerol, diphosphatidylglycerol and two unknown lipids labelled G1 and G2. TLC analysis of these compounds showed that they were reactive with vanillin, orcinol reagent and Dittmer and Lester reagent, so these lipids contained sugar and phosphorus (Fig. 2). No reaction with ninhydrin was reported (Fig. 2).

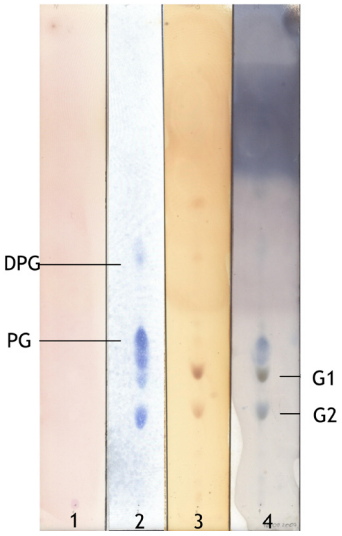


Fig. 2. TLC chromatogram of lipid extract from Ruania albidiflava
Abbreviation: G1, G2 — major lipid compounds, DPG — diphosphatidylglycerol, PG — phosphatidylglycerol. Solvent system: chloroform — methanol — water (65:25:4 v/v/v), detection: 1 — ninhydrin regent, 2 — Dittmer and Lester reagent, 3 — orcinol reagent, 4 — vanillin reagent.

Comparative TLC showed that glycolipid profile of *Ruania* differed from previously reported in *Rothia*, *Arthrobacter*, *Micrococcus* and *Propionibacterium*, but was similar to *Oerskovia xantineolytica* which belongs to *Micrococcineae* suborder (Fig. 3).

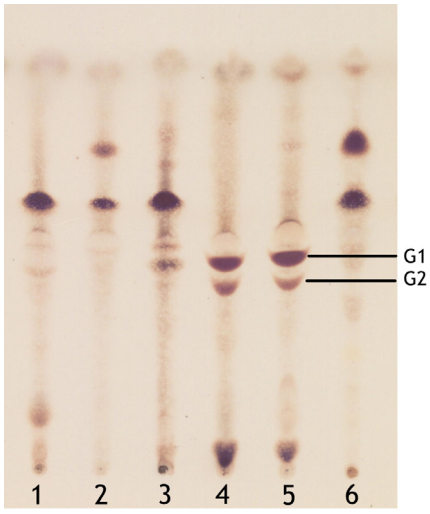
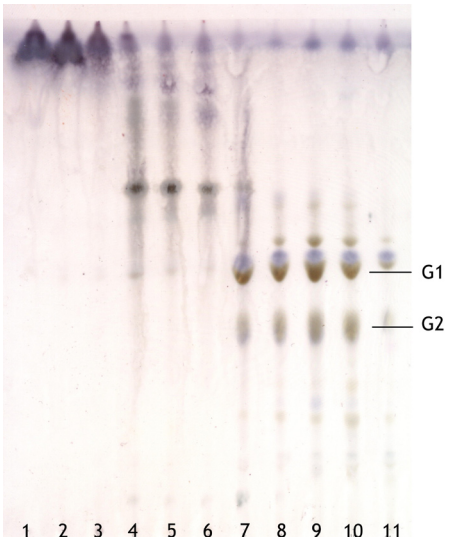


Fig. 3. TLC chromatogram of glycolipids from *Actinobacteria*
1 — *Micrococcus luteus*, 2 — *Arthrobacter globiformis*, 3 — *Rothia dentocariosa*, 4 — *Oerskovia xantineolytica*, **5** — *Ruania albidiflava*, 6 — *Propionibacterium propionicum*. Solvent system like on Fig. 2, detection: orcinol reagent.

Glycolipids G1 and G2 were separated and purified by different chromatographic methods. The crude lipid extract of *R. albidiflava* was separated on a column of silica gel eluted with solvents: chloroform, acetone and methanol (Fig. 4).



The methanol fractions were partially purified by preparative TLC. Pure glycolipids were obtained by HPLC.

Fig. 4. TLC of *Ruania albidiflava* lipid fractions from column chromatography eluted subsequently with chloroform, acetone and methanol

1-3 — chloroform fractions, 4-6 — acetone fractions, 7-10 — methanol fractions, 11-crude lipid extract. Solvent system as on Fig. 2, detection: vanillin reagent.

GLC-MS analysis of sugar part of major glycolipids revealed the presence of glycerol, inositol, mannose and galactosamine in both compounds (Fig. 5). Methylation analysis showed that mannose and galactosamine were in terminal positions.

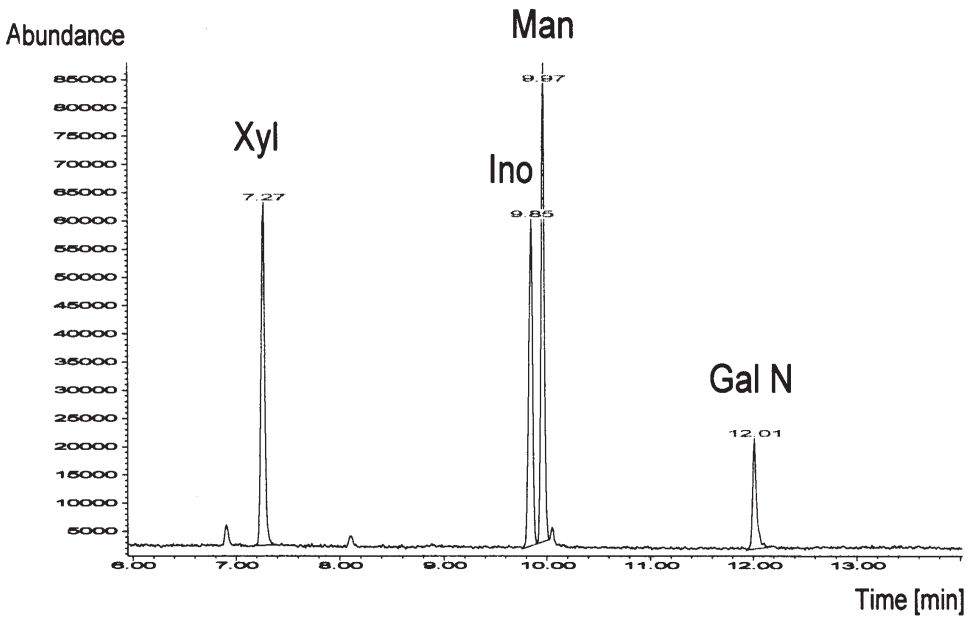


Fig. 5 GLC/MS chromatogram of the sugars present in glycolipid G1 of *R. albidiflava*

Peak 1 — (Rt=7.27 min) xylose represents internal standard, 2 — (Rt=9.85 min) inositol, 3 — (Rt=9.97 min) mannose, 4 — (Rt=12.01) galactosamine.

Fatty acids compositions of both glycolipids were similar and were mainly *iso* and *anteiso* branched pentadecanoic acids (Tab.). Similar results were obtained for whole cell fatty acids analysis in *Ruania albidiflava* [3].

Table

Fatty acids composition of major lipid compounds of *R. albidiflava*

Fatty acids	Retention time Rt [min]	GL 1 (%)	GL 2 (%)
n $C_{14:0}$	5.40	14.4	13.9
iso $C_{15:0}$	6.29	26.6	20.4
anteiso $C_{15:0}$	6.40	30.8	43.1
n $C_{15:0}$	6.76	3.1	tr
iso/anteiso $C_{16:0}$	7.62	6.3	4.0
n $C_{16:0}$	8.10	15.0	18.6
n $C_{17:0}$	9.02	3.8	tr

MALDI-TOF analysis of intact glycolipids revealed that molecular mass of G2 was lower than G1. On MALDI spectra four major peaks were seen, differed by 14 mass units, that was connected with heterogeneity of fatty acids (Fig. 6). The difference in molecular mass between G2 and G1 indicates probably loss of one fatty acid residue.

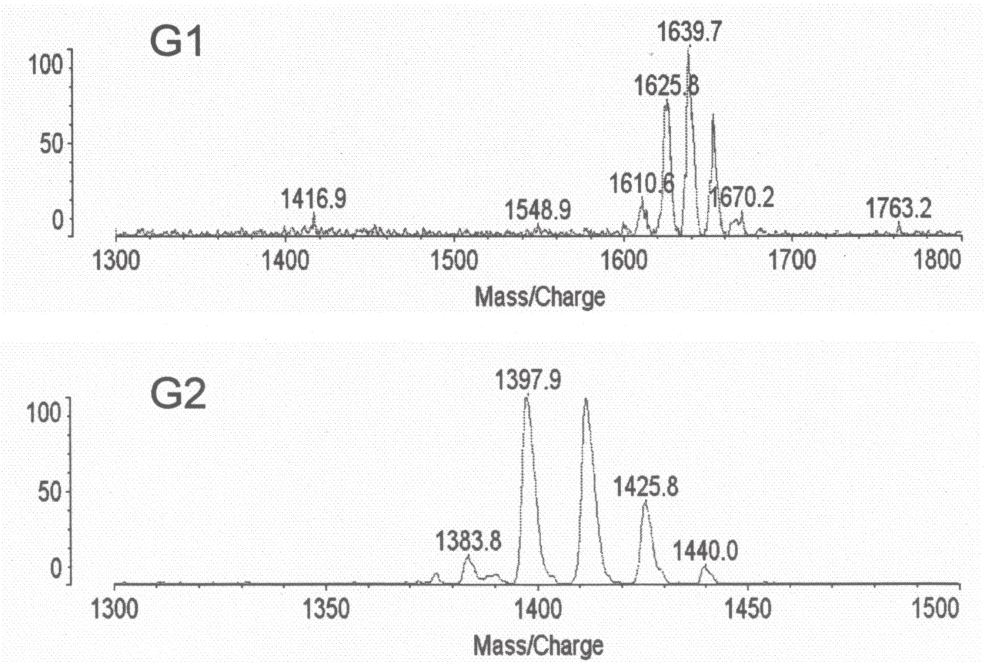


Fig. 6 MALDI-TOF mass spectra of glycolipid G1 and G2 of *Ruania albidiflava*
Kratos Kompact-SEQ instrument, linear positive mode, DHB matrix.

Lipid compounds of *R. albidiflava* appeared to be related to phosphatidylinositol mannosides (PIM). PIM are abundant in some actinobacteria f. ex. in *Mycobacterium* and are involved in the interactions of mycobacteria with host cells [11, 1].

Ruania albidiflava represents characteristic lipid profile consisted of two unknown lipid compounds, both belonging to glycerophospholipids.

REFERENCES

1. de Libero G., Lucia M., Recognition of lipid antigen by T cells // Nature Reviews. — 2005. — 5. — P. 484–496.

2. Gamian A., Mordarska H., Ekiel I., Ulrich J., Szponar B., Defaye J. Structural studies of the major glycolipid from *Saccharopolyspora* genus // Carbohydr. Res. — 1996. — 296. — P. 55–67.

3. Gu Q., Paściak M., Luo H., Gamian A., Liu Z., Huang Y. Ruania albidiflava gen. nov., sp. nov., a novel member of the suborder *Micrococcineae* // Int. J. Syst. Evol. Microbiol. — 2007. — 57. — P. 809–814.

4. Hamada M., Iino T., Tamura T., Iwami T., Harayama S, Suzuki K. Serinibacter salmoneus gen. nov., sp. nov., an actinobacterium isolated from the intestinal tract of a fish, and emended descriptions of the families *Beutenbergiaceae* and *Bogoriellaceae* // Int. J. Syst. Evol. Microbiol. — 2009; 0: ijs.0.011106-0v1-ijs.0.011106-0.

5. Lechevalier M.P., Stern A.E., Lechevalier H.A. Phospholipids in the taxonomy of actinomycetes. in: Actinomycetes, eds.: K.P. Schaal, G. Pulverer // Zentbl. Bakteriol. Hyg. 1 Abt Suppl 11. — Stuttgart: Gustav Fischer Verlag, 1981. — P. 111–116.

6. Mordarska H. Taxonomic value of glycolipids of actinomycetes and allied taxa // The Actinomycetes. — 1985/1986. — 19. — P. 11–32.

7. Mordarska H., Paściak M.: A simple method for differentiation of *Propionibacterium acnes* and *Propionibacterium propionicum* // FEMS Microbiology Letters. — 1994. — 123. — P. 325–329.

8. Paściak M., Holst O., Lindner B., Mierzchała M., Grzegorzewicz A., Mordarska H., Gamian A. Structural and serological characterization of the major glycolipid from *Rothia mucilaginosa* // Biochim. Biophys. Acta. — 2004. — 1675. — P. 54–61.

9. Paściak M., Holst O., Lindner B., Mordarska H., Gamian A. Novel bacterial polar lipids containing ether linked alkyl chains. The structures and biological properties of the four major glycolipids from *Propionibacterium propionicum* PCM 2431 (ATCC 14157T) // J. Biol. Chem. — 2003. — 278. — P. 3948–3956.

10. Stackebrandt E., Rainey F.A., Ward-Rainey N.L. Proposal for a new hierarchic classification system, *Actinobacteria* classis nov // Int. J. Syst. Bacteriol. —1997. — 47. — P. 479–491.

11. Vergne I. and Daffй M. Interaction of mycobacterial glycolipids with host cells // Frontiers in Bioscience. — 1998. — 3. — P. 865–876.

М. Пащак[1], Э. Каспров[1], Й. Хуанг[2], А. Гамиан[1]

[1]Институт иммунологии и экспериментальной терапии имени Людвика Хиршфельда, Польская Академия наук, ул. Рудольфа Вейгля, 12, 53-114, Вроцлав, Польша; тел.: + 48 71 370 99 22, e-mail: pasciak@iitd@pan.wroc.pl

[2]Институт микробиологии, Академия наук Китая, Бейинг, 100101, Китай

ПОЛЯРНЫЕ ЛИПИДЫ *RUANIA ALBIDIFLAVA* – НОВОГО ПРЕДСТАВИТЕЛЯ ПОДПОРЯДКА *MICROCOCCINEAE*

Реферат

Ruania albidiflava представляет собой новый вид и род подпорядка *Micrococcineae* в классе *Actinobacteria*. Целью исследований было изучение профиля полярных липидов *Ruania* и сравнение его с профилями других представителей актинобактерий. Дополнительно было проведено выделение, очистка и изучение химических характеристик основных липидных соединений. Выделение основных липидов осуществляли методом колонково-адсорбционной хроматографии, а очи-

щали методами тонкослойной хроматографии и высокоэффективной жидкостной хроматографии. Полярные липиды *Ruania* были представлены фосфатидилглицеролом, дифосфатидилглицеролом и двумя неизвестными липидами, содержащими сахар и фосфор. Газово-жидкостная хроматография—масс-спектрофотометрия и метиляционный анализ сахаров большинства гликолипидов выявили наличие глицерола, инозитола и терминальных остатков маннозы и галактозамина. Жирные кислоты были представлены в основном разветвленными изо- и антиизо- С15:0. Сравнительная тонкослойная хроматография показала, что гликолипидный профиль *Ruania* отличался от ранее изученных профилей *Rothia, Arthrobacter, Micrococcus* и *Propionibacterium*, но был схож с таковым у *Oerskovia*, принадлежащих к подпорядку *Micrococcineae*.

К л ю ч е в ы е с л о в а: бактериальные липиды, гликолипиды, *Ruania,* химические маркеры.

М. Пащак[1], Е. Каспров[1], Й. Хуанг[2], А. Гаміан[1]

[1]Інститут імунології і експериментальної терапії імені Людовіка Хіршфельда, Польська Академія наук , вул. Рудольфа Вейгля, 12, 53-114, Вроцлав, Польща
тел.: + 48 71 370 99 22, e-mail: pasciak@iitd@pan.wroc.pl
[2]Інститут мікробіології, Академія наук Китаю, Бейінг, 100101, Китай

ПОЛЯРНІ ЛІПІДИ *RUANIA ALBIDIFLAVA* – НОВОГО ПРЕДСТАВНИКА ПІДПОРЯДКУ *MICROCOCCINEAE*

Реферат

Ruania albidiflava являє собою новий вид і рід підпорядку *Micrococcineae* у класі *Actinobacteria*. Метою досліджень було вивчення профілю полярних ліпідів *Ruania* і порівняння його з профілем інших представників актинобактерій. Додатково було проведено виділення, очищення та вивчення хімічних характеристик основних ліпідних сполук. Виділення основних ліпідів здійснювали методом колонково-адсорбційної хроматографії, а очищення методами тонкошарової хроматографії і високоефективної рідинної хроматографії. Полярні ліпіди *Ruania* були представлені фосфатидилгліцеролом, дифосфатидилгліцеролом та двома невідомими ліпідами, які містять цукор та фосфор. Газо-рідинна хроматографія-масспектрометрія та метиляційний аналіз цукру більшості гліколіпідів виявили наявність гліцеролу, інозитолу та термінальних залишків манози і галактозаміну. Жирні кислоти були представлені в основному розгалуженими ізо- та антиізо- С15:0. Порівняльна тонкошарова хроматографія показала, що гліколіпідний профіль *Ruania* відрізнявся від раніше вивчених профілів *Rothia, Arthrobacter, Micrococcus и Propionibacterium,* але був подібним до таких у *Oerskovia*, що належать до порядку *Micrococcineae*.

К л ю ч о в і с л о в а: бактеріальні ліпіди, гліколіпіди, *Ruania,* хімічні маркери.