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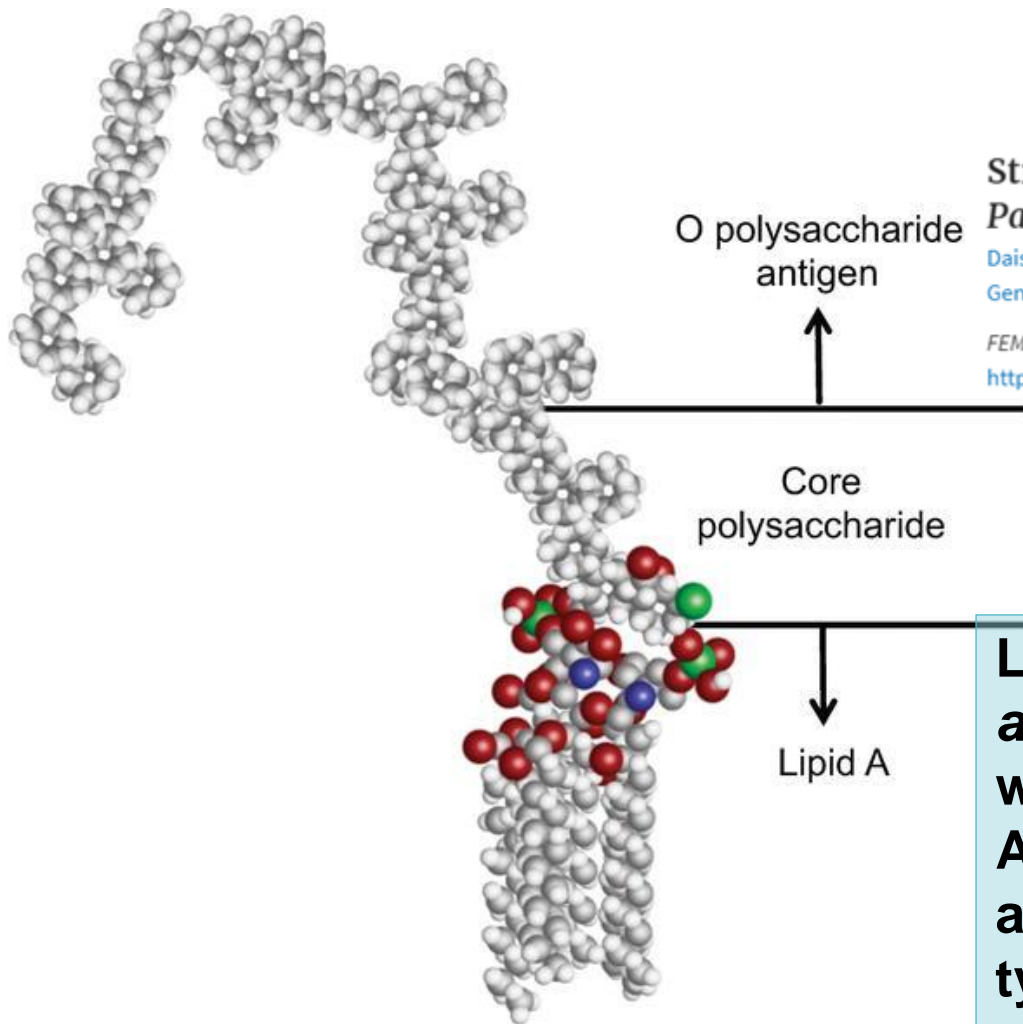
LIPOPOLYSACCHARIDES OF *PANTOEA* AGGLOMERANS P1a, P324 AND 8488: BIOLOGICAL PROPERTIES, THE O-SPECIFIC POLYSACCHARIDES AND LIPIDS A STRUCTURE

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Structural characterization of lipid A obtained from *Pantoea agglomerans* lipopolysaccharide ^{FREE}

Daisuke Tsukioka, Takashi Nishizawa, Toshio Miyase, Kazuo Achiwa, Takuya Suda, Gen-Ichiro Soma ✉, Den'ichi Mizuno

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<https://doi.org/10.1111/j.1574-6968.1997.tb10335.x>



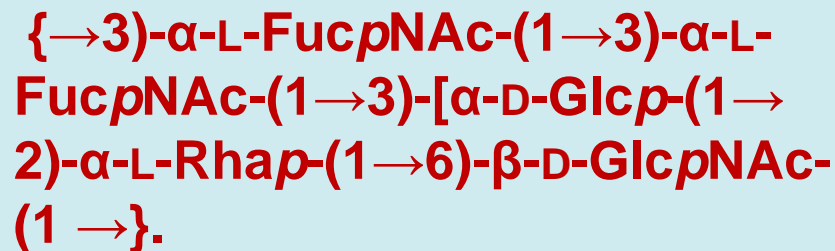
Lipopolysaccharide of *Pantoea agglomerans* is constructed with at least two kinds of lipid A of different levels of acylation. One is of the same type as that of *Escherichia coli* with **hexa-acyl lipid A** and the other is the *Salmonella minnesota* type with **hepta-acyl lipid A**.

Structure of the O-specific polysaccharide chain of the lipopolysaccharide of *Enterobacter agglomerans*

Yannis Karamanos^{a, 1}, Ossarath Kol^a, Jean-Michel Duszeski^a, Gérard Strecker^a, Bernard Fournet^{a, 2}, René Zalisz^b



The OPS isolated from the LPS of *E. agglomerans* was found to have the pentasaccharide repeating-unit:



Characterization of the O-antigen polysaccharide derived from *Pantoea agglomerans* IG1 lipopolysaccharide

Masahito Hashimoto^{a, 1}, Rune Satou^a, Mami Ozawa^a, Hiroyuki Inagawa^{b, c, d}, Gen-ichiro Soma^{b, c, d}



The polysaccharide is composed of linear tetrasaccharide repeating units, consisting of glucose and rhamnose, where 40% of one of the rhamnose residues is substituted with glucose:

$$\rightarrow 2)\text{-}\alpha\text{-L-Rhap}\text{-}(1\rightarrow 6)\text{-}\alpha\text{-D-Glcp}\text{-}(1\rightarrow 2)\text{-}[\beta\text{-D-Glcp}\text{-}(1\rightarrow 3)]_{0.4}\text{-}\alpha\text{-L-Rhap}\text{-}(1\rightarrow 2)\text{-}\alpha\text{-L-Rhap}\text{-}(1\rightarrow$$

Note
The structure of the O-specific polysaccharide of the lipopolysaccharide from *Pantoea agglomerans* strain FL1


Alessio Cimmino^{a, c}, Guido Marchi^b, Giuseppe Surico^b, Anna Hanuszkiewicz^c, Antonio Evidente^a, Otto Holst^{c, d}




A neutral OPS consisting of d-rhamnose was obtained of the LPS of the *Pantoea agglomerans* strain FL1. The chemical repeating unit of the polymer was identified as a linear tetrasaccharide of the structure:

$$\rightarrow 2)\text{-}\alpha\text{-D-Rhap}\text{-}(1\rightarrow 2)\text{-}\beta\text{-D-Rhap}\text{-}(1\rightarrow 3)\text{-}\alpha\text{-D-Rhap}\text{-}(1\rightarrow 2)\text{-}\alpha\text{-D-Rhap}\text{-}(1\rightarrow$$


The **aim** of the present work was isolation, chemical characterization, and studies of functional and biological activities of the *P. agglomerans* P1a, P324 and 8488 LPS, as well as elucidation of the O-specific polysaccharides (OPS) and lipid A structure of this strains.




Isolation, purification and chemical identification (monosaccharide and fatty acid composition) of *P. agglomerans* LPSs




Investigation the sensitivity of bacteria to polymyxin B



Study the biological properties of LPS (toxicity, pyrogenicity, adhesion)



Investigation of LPS functional activity (antigenicity)

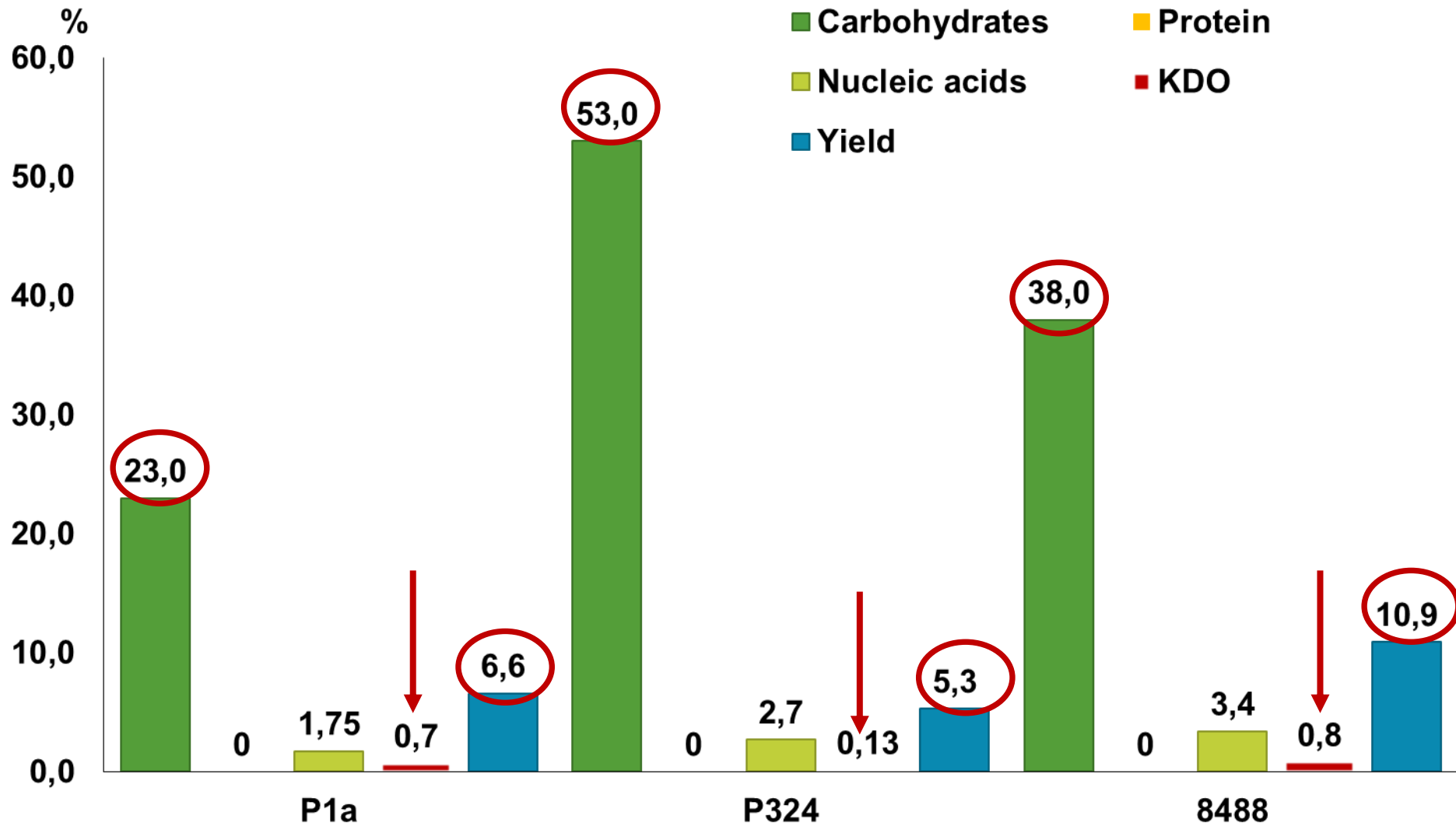


Identification the structure of the OPS and lipid A LPSs

***Pantoea agglomerans* P1a, P324** were isolated from wheat (Kherson region, Ukraine) and **8488** - from oat (Romania). The cultures were obtaining from the collection of phytopathogenic bacteria.

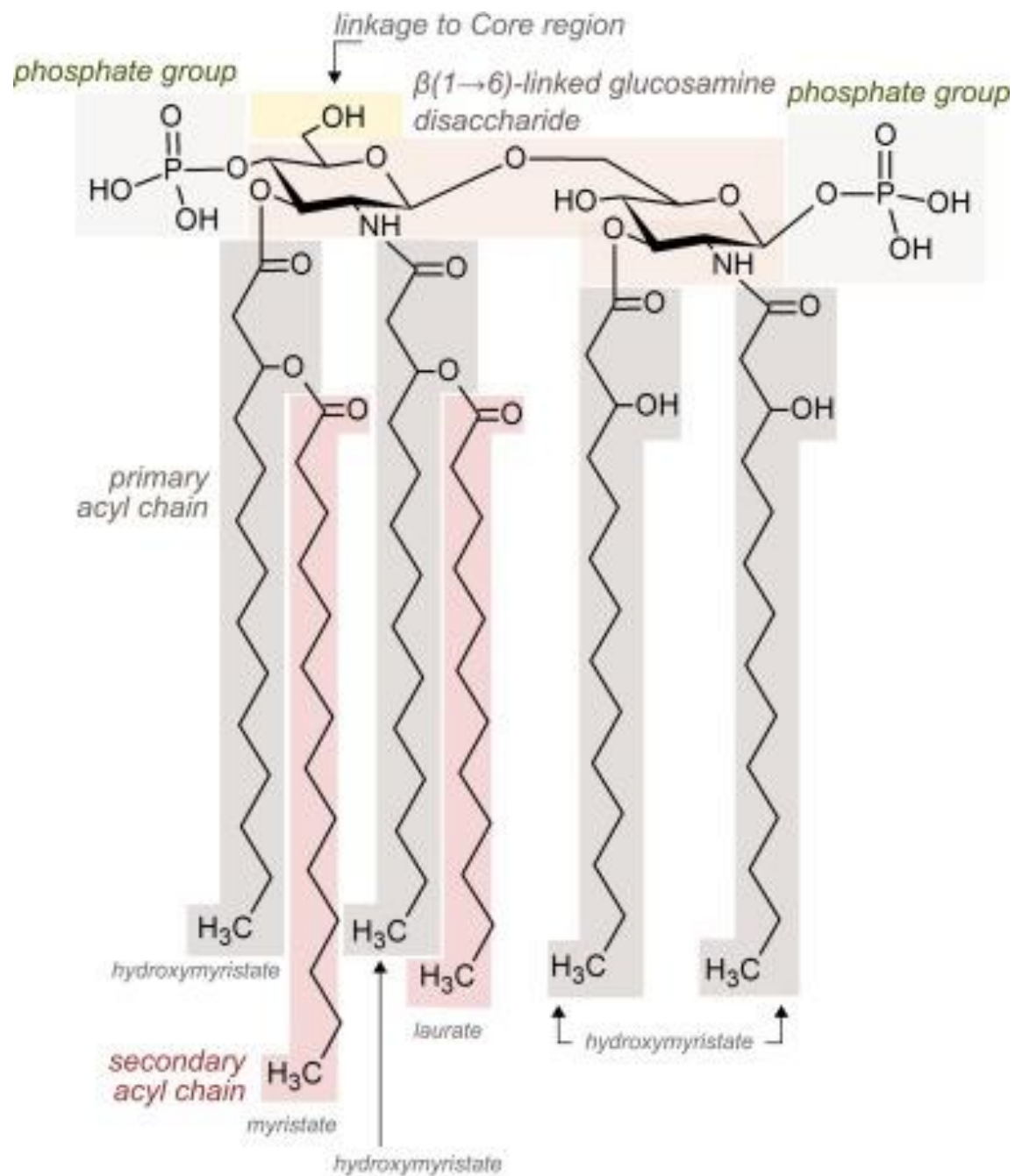
Chemical composition of the studied LPS

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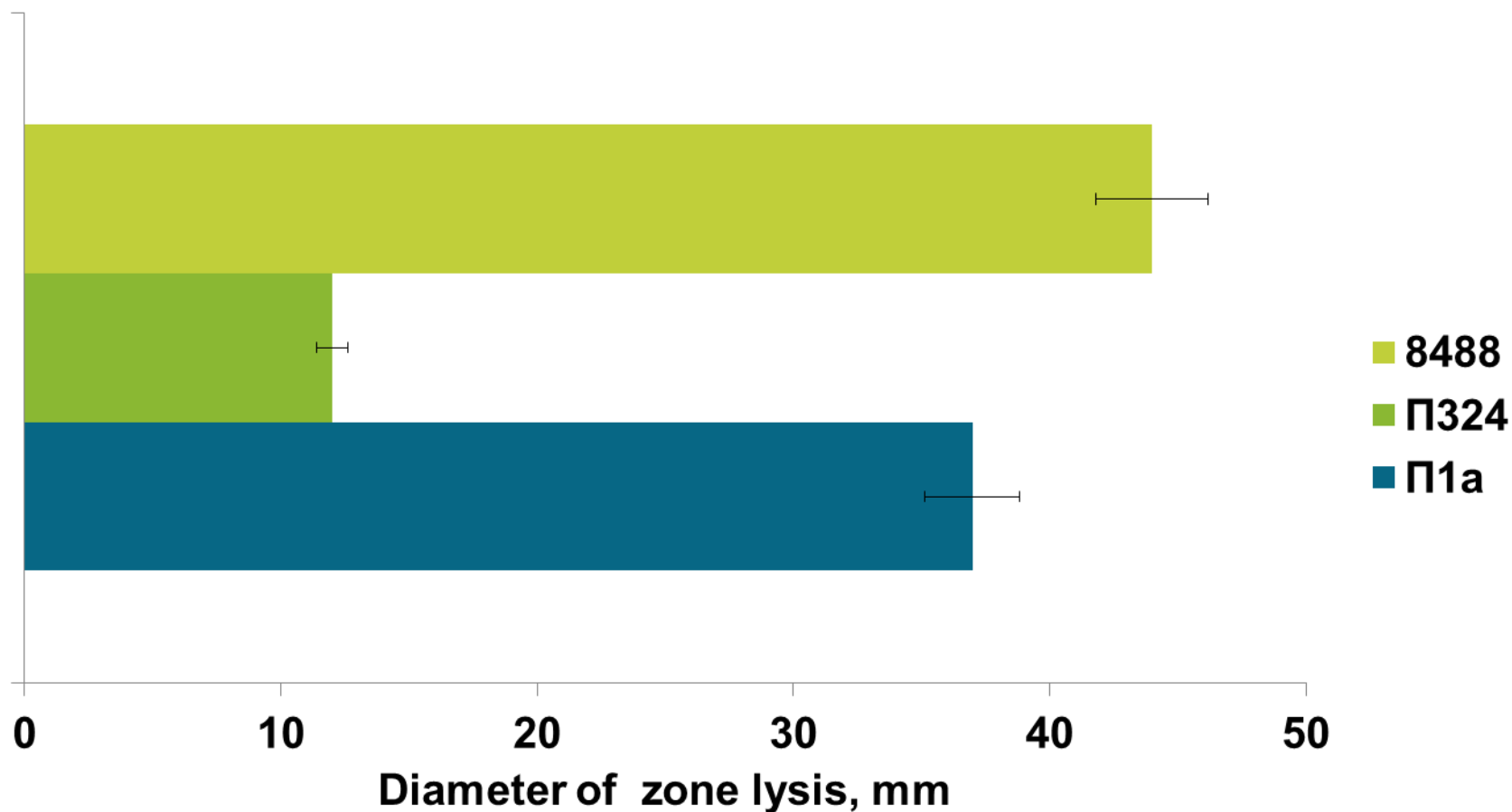


Monosaccharide and fatty acid composition of ⁶ *P. agglomerans* P1a, P324 and 8488 LPSs.

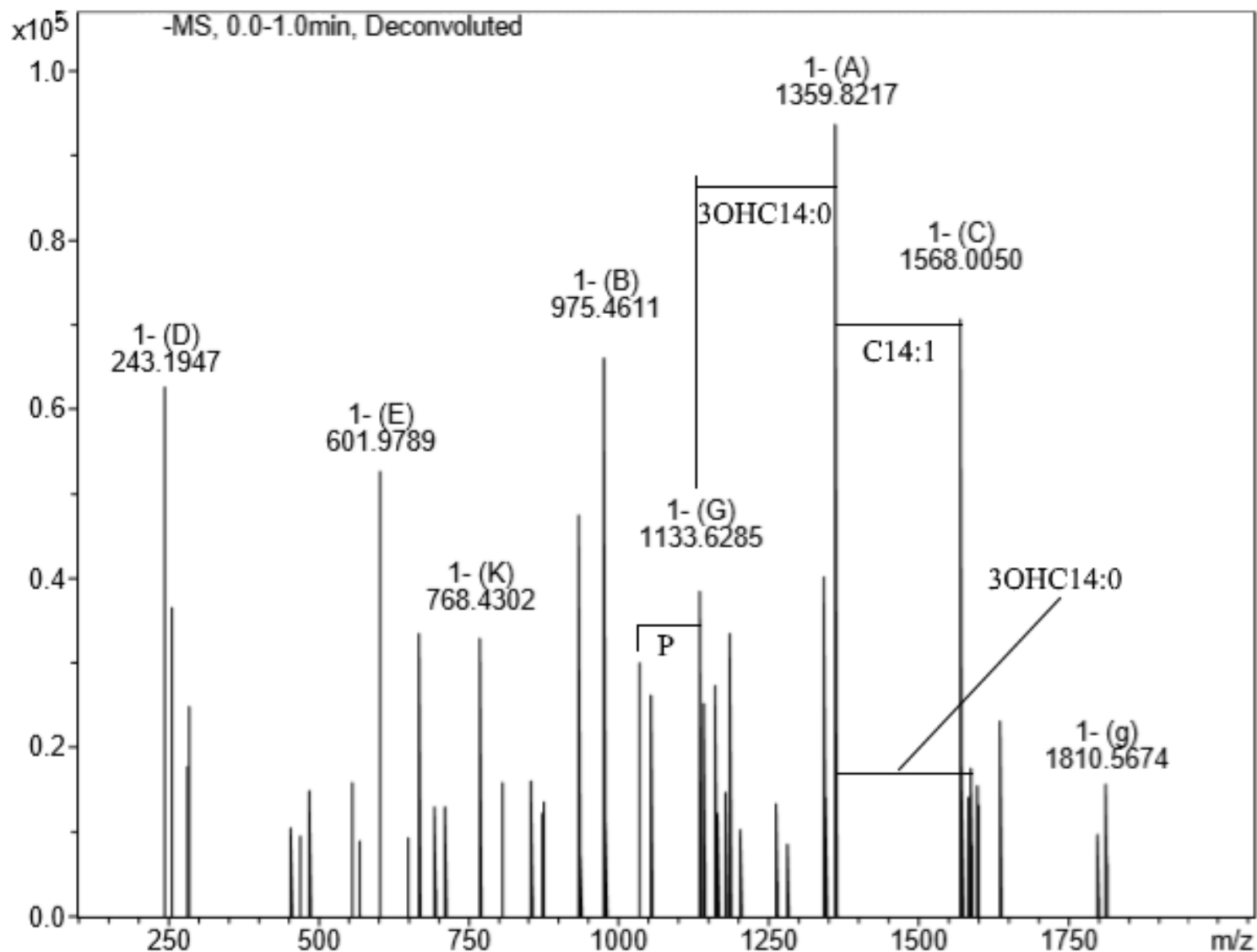
Components	% of the total sum of peak areas		
	P1a	8488	P324
Monosaccharide			
Man	22.05	30.9	-
Fuc	4.53	25.9	29.0
Rha	45.8	21.9	13.0
Glu	14.13	12.8	41.2
Gal	9.17	2.9	4.6
Rib	-	2.8	25.2
Hep	4.32	2.8	12.3
Fatty acid			
C12:0	14.73	31.5	15.0
C13:0	-	-	2.2
2(OH)C14:0	-	3.8	-
3(OH)C14:0	53.1	34.9	38.93
C14:0	-	12.9	21.6
C16:1	14.9	-	8.74
C16:0	17.27	16.9	4.63
cisC18:1	-	-	3.0
transC18:1	-	-	3.4
C18:0	-	-	2.5



Determination of the sensitivity of the microbial culture to polymyxin B



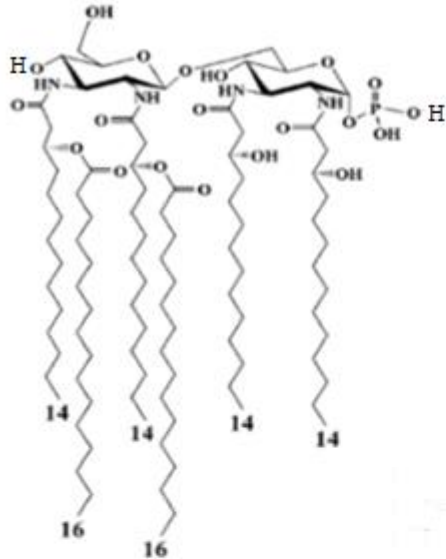
Part of mass spectrum of *P. agglomerans* lipid A.



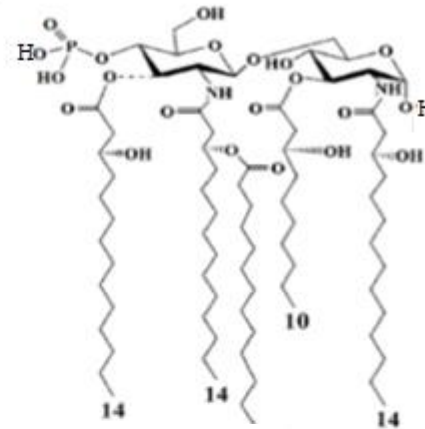
Structure of *P. agglomerans* P1a, P324 and 8488 lipids A

10

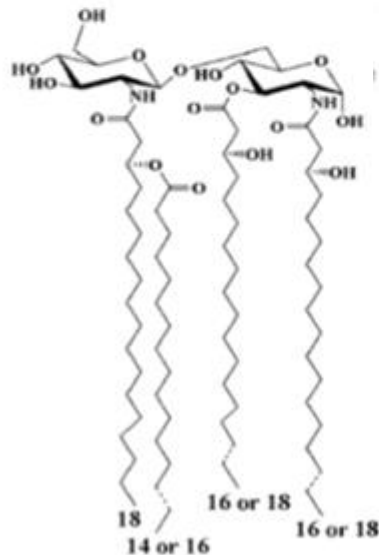
a



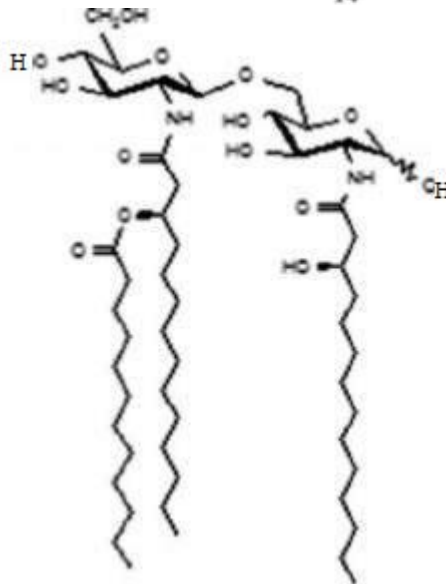
b



c



d



Types of lipid A:
a – hexaacyl;
b – pentaacyl;
c – tetraacyl;
d - triacyl .

Types of *Pantoea agglomerans* lipid A structures

P1a

1) Hexaacyl

4 residues 3(OH)C14:0
2 residues C12:0

2) Tetracyl

3 residues 3(OH)C14:0
1 residue C12:0

P324

1) Hexaacyl

4 residues 3(OH)C14:0
1 residue C12:0
1 residue C18:0

2) Tetracyl

3 residues 3(OH)C14:0
1 residue C12:0

8488

1) Hexaacyl

4 residues 3(OH)C14:0
1 residue C14:0
1 residue C12:0

2) Pentaacyl

3 residues 3(OH)C14:0
1 residue C12:0
1 residue C14:1

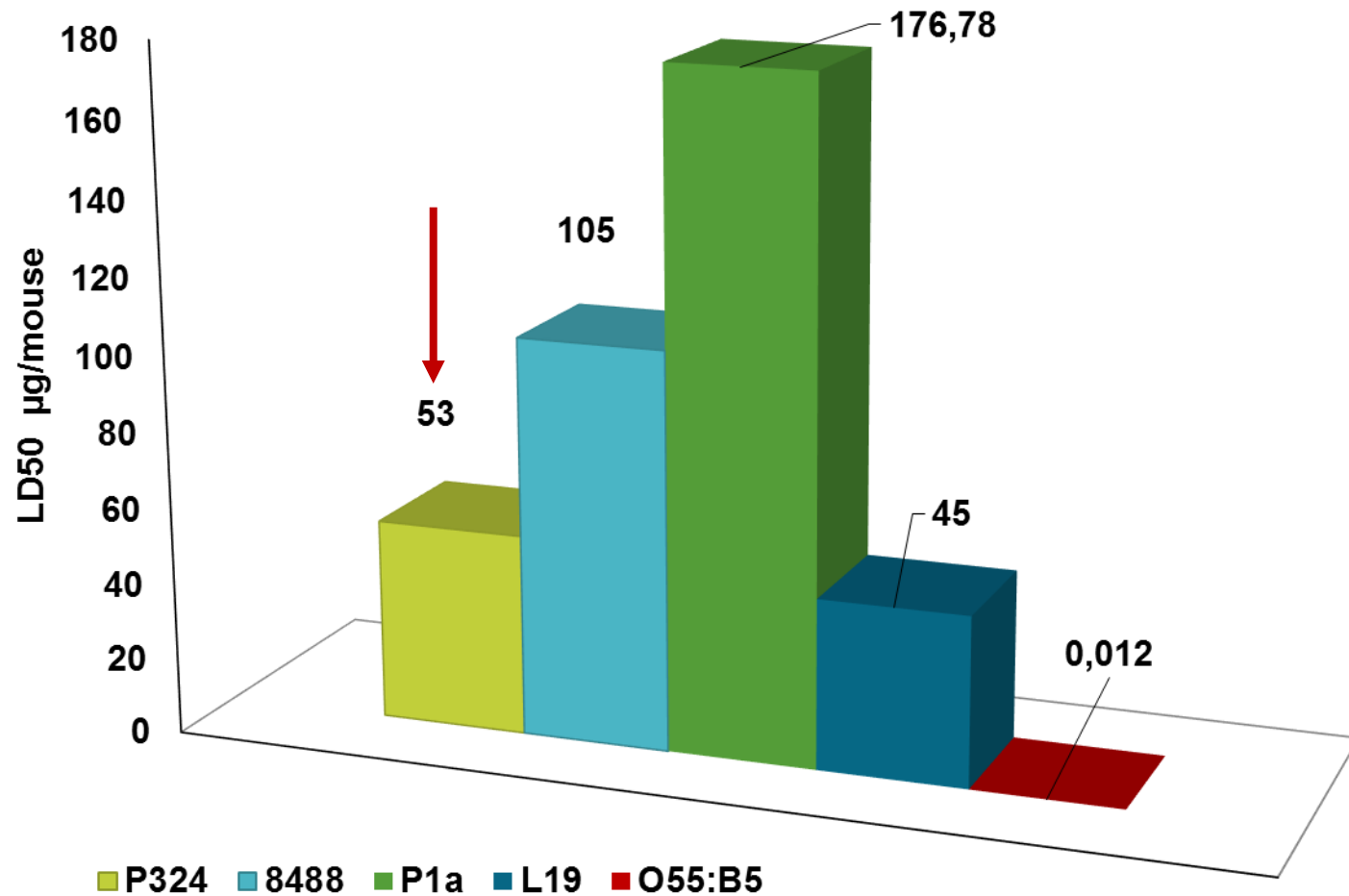
3) Tetracyl

3 residues 3(OH)C14:0
1 residue C12:0

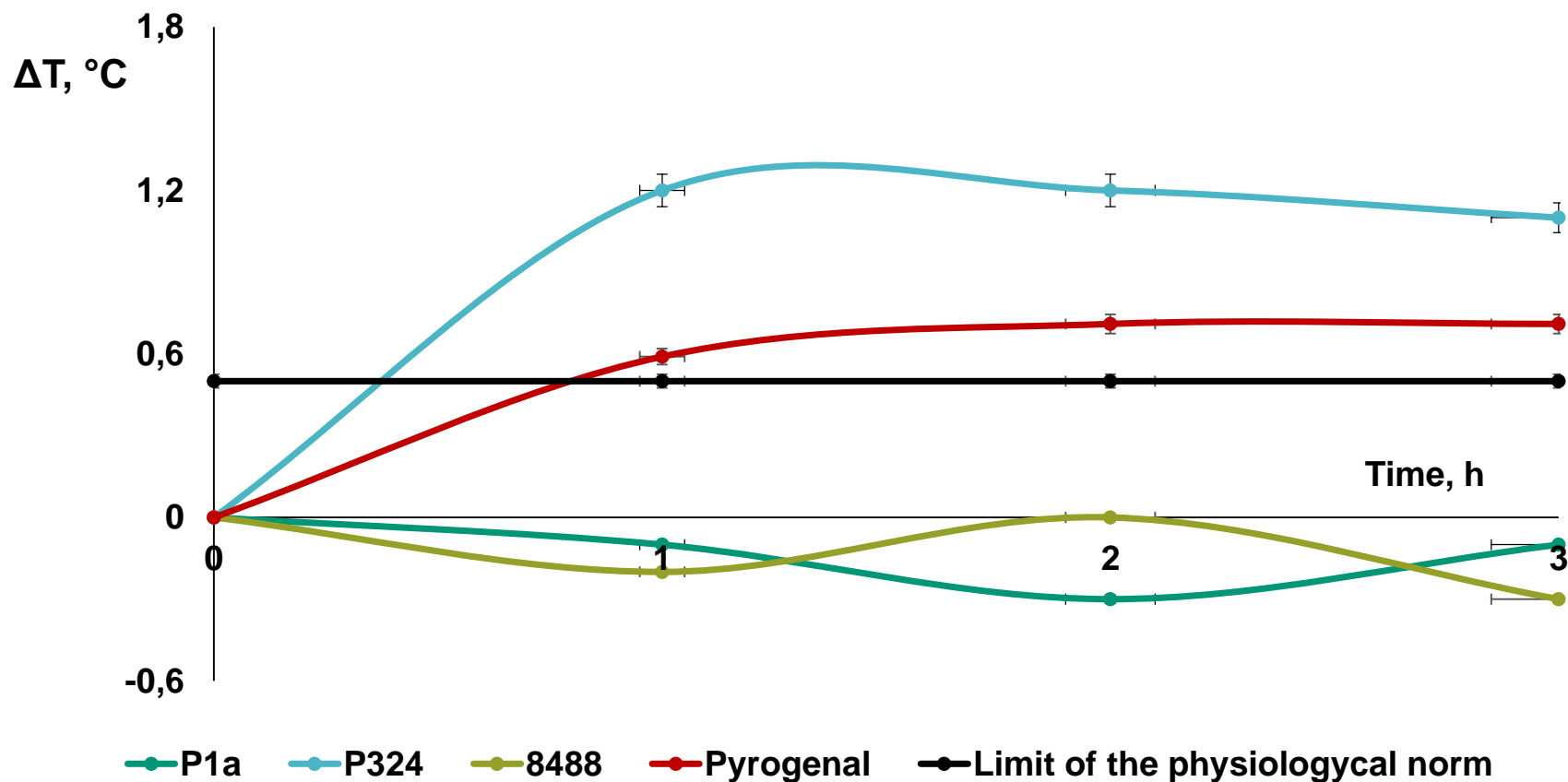
4) Triacyl

2 residues 3(OH)C14:0
1 residue C12:0

Toxicity of *Pantoea agglomerans* P1a, P324, 8488 and *E.coli* L19,O55:B5 LPSs



Pyrogenicity of *Pantoea agglomerans* P1a, P324 and 8488 LPSs



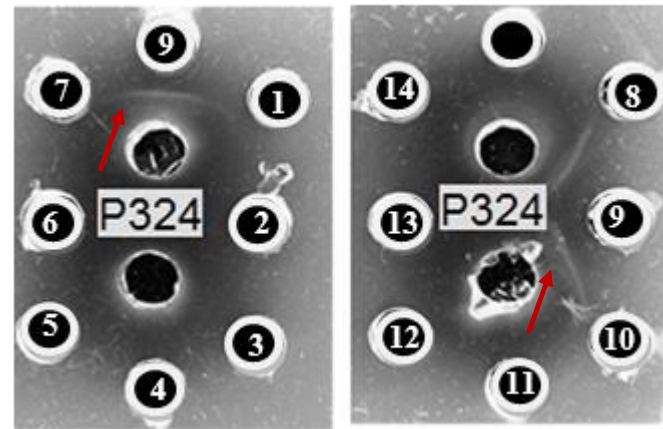
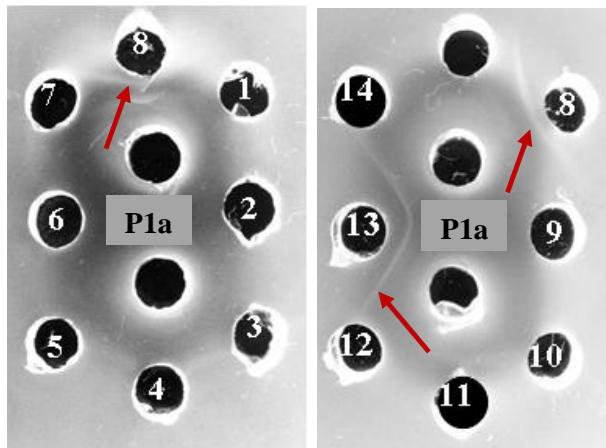
OPS structures *P. agglomerans* P1a (a), P324 (b) and 8488 (c)

$\rightarrow 3)-\alpha-D\text{-Manp}-(1\rightarrow 4)-\beta-D\text{-Fucp}-(1\rightarrow 4)-\alpha-D\text{-Fucp}-(1\rightarrow$
(a)

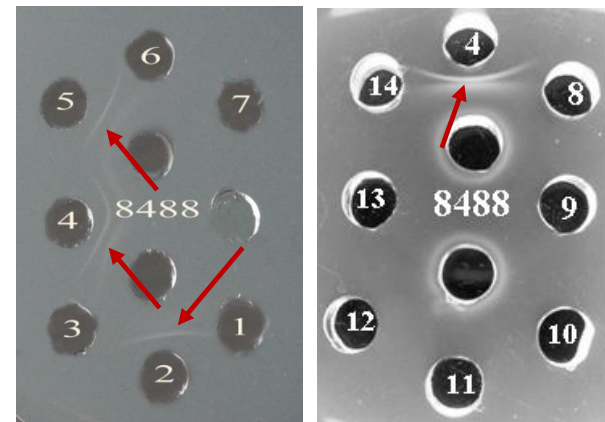
$\rightarrow 3)-\alpha-L\text{-Rhap}-(1\rightarrow 4)-\alpha-D\text{-Glc}p-(1\rightarrow$
(b)

$\rightarrow 3)-\alpha-L\text{-Rhap}-(1\rightarrow 6)-\alpha-D\text{-Manp}-(1\rightarrow 3)-\alpha-L\text{-Fucp}-(1\rightarrow 3)-\beta-D\text{-GlcNAcp}-(1\rightarrow$
(c)

The reaction of double immunodiffusion in agar by Ouchterlony with O-antiserum against *P. agglomerans* P1a, P324 and 8488 with LPSs from other *P. agglomerans* strains



LPS from *P. agglomerans* 7960a (1), 7969 (2), 8456 (3), 8488 (4), 8490 (5), 8606 (6), 8674 (7) P1a (8), P324 (9), 7406 (10), 7604 (11), 9637 (12), 9649 (13), 9668(14).



Conclusions

1. Lipopolysaccharides of *P. agglomerans* P1a, P324 and 8488 were purified and chemically characterized. The investigated LPSs were heterogeneous in both monosaccharide and fatty acid composition.

2. The assumption on the absence of 4-amino-4-deoxy-L-arabinose as a substitute in lipids A of *P. agglomerans* P1a, P324 and 8488 LPSs, based on the data of bacterial sensitivity to polymyxin, was confirmed by HR ESI mass spectrometry lipids A in which were not identified any substitutes in the position of the C-4' glucosamine. Lipid A samples were found to be represented by hexa-, penta-, tetra- and tri-acylated species.

3. The LPS of tested strains showed a relatively low level of the toxic activity compared with other representatives of *Enterobacteriaceae* family. Assay of pyrogenicity showed that that the LPSs of *P. agglomerans* P1a and 8488 were not pyrogenic. While LPS of *P. agglomerans* P324 was more pyrogenic than the pharmaceutical preparation «pyrogenal».

4. The OPS of three *P. agglomerans* P1a, P324 and 8488 strains are represented by linear tri-, di- and tetrasaccharide repeating units, respectively.

5. O-antiserum against *P. agglomerans* P1a, P324 and 8488 didn't showed cross-reactivity with the LPSs of these strains. The O-antiserum against *P. agglomerans* P1a indicates a serological cross-reaction only with LPS of *P. agglomerans* 9649. At the same time, O-antiserum against *P. agglomerans* 8488 exerted a serological cross-reactivity with the LPS of two other *P. agglomerans* 7969 and 8490 strains. These data testify that these strains contain common antigenic determinants in the OPS of their LPS, that is, they belong to one of the same serogroup.

6. The structural and serological data indicate immunochemical heterogeneity of *P. agglomerans* strains and may be used in creation of serological classification schemes of *P. agglomerans* strains.



Thank you for attention!