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Biochemical Identification of Bacteria Collectible Cultures

Key words: *collection of microorganisms, biochemical identification, bacteriological analyzer taxon.*

Annotation: *The article presents the results of biochemical identification of taxonomic groups and subcultures bacteria collection fund microbiologically automated analyzer.*

Culture collection of microorganisms as biological gene pool of storage facilities is a national treasure in each country. The main objectives of such collections are inventory, conservation, development of methods for identification, assessment of the possible use of micro-organisms in basic research in biotechnology (1).

Quality collections determined by the number and variety of items, the presence of conditions to ensure reliable preservation of biomaterials in an unchanged form, adequate

characterization and adequate information support models in modern databases. The information needed to researchers diverse and includes information on the status of the organisms in the modern system of classification, data-producing strain, the methods of cultivation and long-term storage, the degree of hazard when working with various groups of micro-organisms (pathogenic/opportunistic human, animals and plants), and others. (2-5).

The assessment of biological diversity has now become one of the traditional methods of the state of natural ecosystems and environmental monitoring (6). To the greatest extent, this approach is developed in relation to complex multicellular biosamples (animals, plants, insects, etc.), Most of which showed a reduction in species biodiversity by increasing anthropogenic pressures on the ecosystem. (7) Relation to the same members of a microcosm presentation on Biological Diversity developed a much lesser extent, that depends on the complexity of the correct species identification (8).

To solve this problem it was proposed a set of test systems for biochemical identification with visual or automated based on the results and the definition of the kind of microorganism in the attached code directory or by using specialized computer programs (9).

Currently, the majority of the collections of our Republic are identified with the classical microbiological methods. However, in developed collections polyphasic are used under move, which is to bring together all possible data as phenotypic and genetic character, in order to obtain reliable identification (10).

In Republican collection of microorganisms are deposited a culture of bacteria including bacilli identified depositors routine biochemical tests on enzyme activity. Therefore the aim of the work is to check the collection cultures of bacteria groups, including bacilli that their taxonomic passport.

Materials and methods

Subjects: collectible subculture bacteria, including bacilli. Biochemical identification was performed on bacteriological analyzer Vitek 2 Systems using cards VITEK2 GN, GP, BSL.

Map Vitek 2 GP, GN is intended to identify the most gram positive, gram negative organisms on two analyzers Vitek Systems. This map contains 47 biochemical tests. Identification map GP, GN based on standard biochemical methods using substrates developed for assessing the used sources of carbon and nitrogen, as well as enzymatic activity.

Microorganisms evaluated for pure cultures Cultural characteristics Colony on MPA and based on microscopy of gram stained smears.

Results and Discussion

The activities of the biochemical identification of cultures to check for compliance taxonomic previously deposited bacteria including bacilli passport. For comparison we have taken 20 cultures of bacteria. DSA was used for the identification of culture, diluted in 3.0 cm3 of 0.45-0.50% aqueous solution of NaCl. The density of the culture should be 1.80-2.20

McFarland, which was measured using a calibrated densitometer Vitek 2 DENSICHEK. Performed biochemical identification of 10 plants of the genus Bacillus microbiologically automated analyzer VITEK 2 Systems using cards VITEK 2. results are shown in Table 1.

Table 1 - Biochemical identification of the genus bacillus

№	the strain name	Identification on bacteriological analyzer	Identity %
1	B-RKM 0029 *Bacillus subtilis 31*	*Bacillus subtilis*	*93*
2	B-RKM 0102 *Bacillus subtilis IKI*	*Bacillus subtilis*	*91*
3	B-RKM 0183 *Bacillus subtilis PR 28*	Bacillus pumilus	*97*
4	RKM0285 *Bacillus subtilis Zb 52*	*Bacillus subtilis*	*85*
5	B-RKM 0209 *Bacillus subtilis штамм - 65-V2*	*Bacillus subtilis*	*86*
6	B-RKM 0274 *Bacillus subtilis 33 ПП*	*Bacillus subtilis*	*96*
7	B-RKM 0275 *Bacillus subtilis 45 ПП*	*Bacillus subtilis*	*95*
8	RKM0293 *Bacillus thuringiensis AE4*	*Bacillus thuringiensis*	*95*
9	RKM0340 *Bacillus thuringiensis Pb 53*	*Bacillus thuringiensis*	*95*
10	RKM 0341 *Bacillus thuringiensis Pb 30*	*Bacillus thuringiensis*	*92*

Note: Secure identification of microorganisms counted as a percentage. Great identification of 96-99%, a very good identification of 93-95%, 89-92% good identification, acceptable identification of 85-88%.

Culture RKM0285 Bacillus subtilis Zb 52 of nameplate indicates biodekstruktorom for sewage treatment of fat and protein contaminants. Table 2 shows the biochemical identification analyzer Vitek 2 Systems RKM0285 Bacillus subtilis Zb 52.

Table 2 - Biochemical characterization of Bacillus subtilis Zb 52 analyzer Vitek 2 Systems

β- xylo zidaza	+	L-lizin	-	L-aspartate	+	Leucine	+	Phenylal anine	-	L-proline	-
β-galactosid ase	+	L-pyrrolidon e	+	α-galactosi dase	+	Alanine	+	Tyrosine	-	Beta-N-acetile	
Ala-Phe-ProArilam idaza	+	cyclodextr in	-	D-galactose	-	Glyco gene	+	Myo-inositol	+	Methil-A -D-gluco Piranizud	+
Ellman	+	methyl D-xylose	-	α-mannosid ase	-	Malta triose	+	Glycine	-	D-mannit	+
D-manose	+	D-	-	N-acetyl-	-	Palatinos	+	L-	+	β-	+

		melezitose		D-glucosesamine		e		raminose		glucozydasa	
β -mannozidaza	-	phosphorylcholine	-	pyruvate	+	α-Glucozidaza	+	D-tagatose	-	D-tregalose	+
inulin	+	D-glucose	+	D-ribose	+	Putrescching, Assimilation	-	Growth at 6,5 % NaCl	+	R to cannamicyn	-
R R to oleandomycin	-	esculin, hydrolysis	+	tetrazoliu m red	+	R to polymyxin	+				

The data obtained culture RKM183 Bacillus subtilis PR 28 was identified as a Bacillus pumilus culture by 97%. This culture corresponds to the original passport data on tribal affiliation. Other cultures as a result of identifications fully comply with nameplate data on the genus and species of the strain.

The identification process is a constant comparison of the biochemical profile of the test organism with profiles of all organisms and groups database. Calculate the amount of data that shows how the results obtained correspond to the typical reactions of each organism database. The work on the biochemical identification of 10 taxonomic group of bacteria cultures. Results of the study are shown in Table 3.

Table 3 Biochemical identification of a group of bacteria

№	the strain name	Identification on bacteriological analyzer	Identity %
1	RKM 0038 *P. vulgaris* 177	*Proteus vulgaris*	99
2	RKM 0039 *Staphylococcus aureus*т 6538	*staphylococcus aureus*	99
3	RKM 0040 *E. coli* 157	*E. coli*	99
4	RKM 0047 *Streptococcus faecium*	*Enterococcus faecium*	*86*
5	RKM 0052 *E. coli*	*E.hermannii*	95
6	RKM 0057 *Staphylococcus aureus* 209P	*Staphylococcus aureus*	95
7	RKM 0059 *Serratia marcencens 221*	*Serratia marcencens*	99
8	RKM 0289 *Dietziia maris U2.1*	*Не определен*	-
9	RKM 0418 *Pseudomonas aeruginosa G15*	*Pseudomonas aeruginosa*	*99*
10	RKM 0419 *Pseudomonas aeruginosa G24*	*Pseudomonas aeruginosa*	*98*

Note: Secure identification of microorganisms counted as a percentage. Great identification of 96-99%, is a very good identification of 93-95%, 89-92% good identification, acceptable identification of 85-88%.

Culture RKM 0289 Dietziia maris U2 identified on the analyzer with the result "is not defined." In the identification of microorganisms result of "not specified" is issued when atypical biochemical profile, or in the absence of the taxonomy in the database (Table 4).

Culture RKM 0047 Streptococcus faecium culture identification Enterococcus faecium. Enterococcus faecium is kind of enterococci, which is part of the normal microflora of the human digestive tract, as well as some mammals. According to the accepted classification of enterococci previously belonged to Class D streptococci and Enterococcus faecium were called Streptococcus faecium. Thus, in the passport information when depositing reflected earlier name Streptococcus faecium, and the analyzer shown in the identification of a new title Culture Enterococcus faecium.

Culture RKM 0052 E. coli culture was identified E.hermannii. E.hermanii - kind of enterobacteria, are part of the microflora of the gastrointestinal tract; isolated from wounds; from blood and cerebrospinal fluid. In the resultant culture E.hermanii and E. coli belong to the family Enterobacteriaceae. By culture-morphological properties of culture are alike.

Table 4 - Biochemical characterization Dietziia maris U2 analyzer Vitek 2 Systems

Ala-Phe-ProArilamidaza	-	Adonitol	+	L-pyrrolidone arilamidap	-	L arabit	-	Phenylalanine	-	B galoksidaza	-
Production H2S	-	Beta-N-acetyl	-	glyutamil arilamidaza	-	D-glucose	-	D-cellobiose	-	fermentation of glucose	-
Beta-glucozydasa	-	D maltose	+	D-mannitol	-	D-mannose	-	Beta ksilozidaza	-	βalaninarilamidaza	-
L-prolinarilamidaza	+	lipase	-	palatinose	-	tyrosine arilamizidaza	-	urease	-	D-sorbitol	-
saccharose	-	D tagatose	-	D-trehalose	-	citrate	-	malonate	-	5 keto-gluconate	-
lactate	-	A-glucosidase	-	succinate	-	BetaN	-	A galactosidase	-	phosphatase	-
α-glucosidase	-	ornithine decarboxylase	-	lizindikarboksilaza	-	L histidine	-	coumarate	-	Beta-glucuronidase	-
0/129 Persistence	-	Glu-Gly-Arg	-	L Mallat	-	Ellman	-	L lactate	-		
Ala-Phe-ProArilamidaza	-	Adonitol	+	L-pyrrolidone arilamida	-	L arabit	-	Phenylalanine	-	B galoksidaza	-

				p							
Production H2S	-	Beta-N-acetyl	-	glyutamil arilamidaza	-	D-glucosa	-	D-cellobiose	-	fermentation of glucose	-
Beta-glucozydasa	-	D maltose	+	D-mannitol	-	D-mannose	-	Beta ksilozidaza	-	βalaninarilamidaza	-
L-prolinarilamidaza	+	lipase	-	palatinose	-	tyrosine arilamizidaza	-	urease	-	D-sorbitol	-
saccharose	-	D tagatose	-	D-trehalose	-	citrate	-	malonate	-	5 keto-gluconate	-
lactate	-	A-glucosidase	-	succinate	-	BetaN	-	A galactosidase	-	phosphatase	-
α-glucosidase	-	ornithine decarboxylase	-	lizindikarboksilaza	-	L histidine	-	coumarate	-	Beta-glucuronidase	-
0/129 Persistence	-	Glu-Gly-Arg	-	L Mallat	-	Ellman	-	L lactate	-		

Thus, the comparative analysis of the results of identification collection cultures of bacteria including bacilli and routine microbiological method using bakanalizatora culture Bacillus subtilis PR 28 as Bacillus pumilus, Dietziia maris U2 was not determined analyzer, E. coli was identified as E.hermannii.

In the future we plan to identify all collection cultures analyzer Vitek 2 Systems.

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