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APPLICATION OF COMMERCIAL TEST-SYSTEMS TO IDENTIFY GRAM-NEGATIVE FACULTATIVELY ANAEROBIC BACTERIA

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Aim. To validate the suitability of commercial API 20E test-system (bioMerieux) for the identification and characterization of facultative gram-negative phytopathogenic bacterial isolates. **Methods.** Conventional microbiological methods, API 20E test-system (bioMerieux) according to the manufacturer's instructions. **Results.** The identification results for *Erwinia amylovora, Pectobacterium carotovorum* and *Pantoea agglomerans* isolates were derived from the conventional and API 20E test systems, which, were in line with the literature data for these species. The API 20E test-system showed high suitability for *P. agglomerans* isolates identification. Although not all the species of facultatively anaerobic phytopathogenic bacteria may be identified using API 20E test-system, its application will surely allow obtaining reliable data about their physiological and biochemical properties, valuable for identification of bacteria, in the course of 24 h. **Conclusions.** The results of tests, obtained for investigated species while using API 20E test-system, and those of conventional microbiological methods coincided. The application of API 20E test-system (bioMerieux) ensures fast obtaining of important data, which may be used to identify phytopathogenic bacteria of *Erwinia, Pectobacterium, Pantoea* genera.

Keywords: phytopathogenic bacteria, bacterial diseases of plants, properties of bacteria, diagnostics, test-systems.

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INTRODUCTION

At present bacterial plant diseases become ever more wide-spread and bring even more damage to agriculture. Along with such objective factors as the change in climatic conditions, the main reasons include some subjective factors: the absence of resistant cultivar, and almost complete unavailability of preparations, protecting from agents of bacteriosis [1].

One of the most relevant elements of the system of controlling the distribution of bacterial plant diseases is timely diagnostics and determination of a disease agent. It allows ensuring efficient protection of plants and mitigating the possibility of disease distribution.

As symptoms of many plant diseases of different etiology are similar, it is very important to isolate and identify the agent preferably in laboratories, ssufficiently equipped for this purpose. It is impossible to determine the real reasons of plant diseases without the microbiological analysis and to plan and implement the corresponding complex of measures to limit the distribution of the agent and to reduce the loss of agricultural crop harvest [2].

The main requirements to the methods, applied for diagnostics and identification of phytopathogenic bacteria, are accuracy and reproducibility of the obtained results as well as minimal time, resources and labor expenditures.

Modern methods, applied to identify phytopathogenic bacteria, may be divided into microbiological, immunological, and molecular-biological ones. The application of microbiological methods is often sufficient for most laboratories to conduct routine analyses in detecting and diagnosing bacterial disease agents. In addition, their advantage is the possibility to obtain in-

formation about the biological specificities of the agent and the data, required to detect infection sources. However, the application of such methods is limited by a considerable duration of analyses and the requirement to use a great number of different media and reagents. To accelerate routine microbiological analyses, many companies offer commercial kits of known microbiological tests – so called test-systems [3]. The kits of bioMerieux company are some of the most commonly used test-systems.

Diagnostic API-systems were elaborated to identify a wide spectrum of microorganisms to be used in scientific and practical laboratories [4]. However, they are mostly used to identify bacteria, pathogenic and conditionally pathogenic for humans, and those of practical value for humans.

The aim of present work was to validate the suitability of commercial API 20E test-system (bioMerieux) for the identification and characterization of facultative gram-negative phytopathogenic bacterial isolates.

MATERIALS AND METHODS

API 20E test-system (bioMerieux), containing test-plates and a set of reagents, was used to identify gram-negative facultative anaerobic rods. When used with the corresponding software of the manufacturer, this system allows identifying bacteria of *Enterobacteriaceae* family and some other gram-negative bacteria.

The possibility of using API 20E test-systems to identify phytopathogenic bacteria was analyzed taking into consideration the availability of diagnostically relevant tests for these bacterial species in the test-system and the data of the properties of bacteria, kept in the collection of live cultures of the Department of Phytopathogenic Bacteria of the D. K. Zabolotny Institute of Microbiology and Virology, NAS of Ukraine.

Table 1. Cultural and physiological properties of *E. amylovora*, *P. carotovorum*, *P. agglomerans*

Test	*E. amylovora* UKM B-1095	*P. carotovorum* UKM B-1075	*P. agglomerans P324*
The form of cells	Rods	Rods	Rods
Gram's staining	–	–	–
Motility	Mobile	Mobile	Mobile
Pigment formation	–	–	Yellow
Fermentation of glucose:			
aerobic	+	+	+
anaerobic	+	+	+
Pectinase	–	+	–
Oxidase	–	–	–

Note. (–) – negative sign; (+) – positive sign.

The following bacterial strains were used in the work:

Erwinia amylovora (Burril 1882) Winslow et al. 1920 type strain UKM B-1095 (ATCC 15580, NCPPB 683);

Pectobacterium carotovorum (Jones 1901) Hauben et al. 1999 type strain UKM B-1075 (ATCC 15713, NCPPB 312);

Pantoea agglomerans (Ewing et Fife 1972) Dehey 1989 strain P324.

Cultural-physiological and biochemical properties of phytopathogenic bacteria were studied by conventional microbiological methods [2, 5]. API 20E test-system was applied according to the manufacturer's protocol (bioMerieux, France). The results were registered after the cultivation for 24 h at 28 °C and compared against the data, obtained using classic methods.

RESULTS AND DISCUSSION

All species of *Erwinia* genus and species of *Pectobacterium* genus, which have been rather recently reclassified from *Erwinia* genus and are most frequent agents of soft rot of plants, belong to phytopathogenic gram-negative facultative anaerobic bacteria. The strains of *Pantoea agglomerans,* previously also referred to *Erwinia* genus, are now referred to the group of gram-negative facultativy anaerobic bacteria. *P. agglomerans* is present on the surface of healthy plants as a representative of epiphytic microflora and may induce the infectious process as a facultative pathogen. The representatives of the mentioned species were used to estimate the reasonability of applying API 20E test-system to identify phytopathogenic bacteria (Table 1).

The diagnostics of bacteriosis agent also requires the determination of pathogenic properties of the isolates and the confirmation of their participation in pathogenesis after artificial infection of plants.

Recently such commercial test-kits, as API 20E, API 20Ne, Microbact NE, BIOLOG GN, have been used to study biochemical and physiological properties of microorganisms more frequently [3]. These

test-systems are easy to use; they ensure faster and more accurate identification of a larger number of bacterial strains.

API 20E test-system, designed to identify bacteria of *Enterobacteriaceae* family, is based on 21 standardized biochemical tests. This system is a plate (a strip) of 20 microwells, containing dehydrated substrates (Figure). The cell suspension of the investigated microorganism in physiological solution and required reagents are introduced to each well in sterile conditions in accordance to the manufacturer's instructions. The registration of results is performed 24 h later, noting the change in the color of the medium in accordance with the table of registering the results.

The microbiological tests, presented in API 20E test-system, include such diagnostically relevant tests for

The general layout of API 20E test-system

Erwinia, Pectobacterium, Pantoea species as tests for the presence of arginine dihydrolase, phenylalanine deaminase, urease, utilization of citrates, mannitol, rhamnose [6].

The advantage of API 20E system is the velocity of conducting rather a large number of biochemical tests as well as their standardization which ensures the possibility of comparing results, obtained in dif-

Table 2. Physiological and biochemical properties of *Erwinia amylovora*

Test/enzyme	*E. amylovora* UKM B-1095		*E. amylovora* according to the literature data [6]
	Using API 20E	By conventional methods	
β-galactosidase	−	n/d	a/d
Arginine dihydrolase	−	n/d	−
Lysine decarboxylase	−	n/d	−
Ornithine decarboxylase	−	n/d	−
Utilization of citrates	−	−	a/d
Formation of H_2S	−	−	−
Urease	−	−	−
Tryptophane deaminase	−	n/d	−
Formation of indole	−	−	−
Formation of acetoin (reaction of Voges-Proskauer)	+	+	a/d
Gelatinase	−	+	+
Utilization:			
of D-glucose	+	+	+
of D-mannitol	−	−	−
of inositol	−	−	−
of D-sorbite	+	+	+
of L-rhamnose	−	−	−
of D-sucrose	+	+	a/d
of D-melibiose	+	+	+
of amygdaline	−	−	−
of L-arabinose	−	−	var.
Reduction of nitrates	−	−	−

Note. Here and in Tables 3, 4 (−) − negative sign; (+) − positive sign; var. – variable feature; a/d – absent data; n/d – not defined.

ferent laboratories. API-systems are widely used to characterize and identify microorganisms of different groups [4].

Tables 2–4 present the results of studying the properties of *E. amylovora* UKM B-1095, *P. carotovorum* UKM B-1075 and *P. agglomerans* P324 using the mentioned test-system. In most cases they corresponded to the characteristics of these bacterial species, mentioned in the literature. There was also an observed agreement between the results, obtained by conventional methods and using API 20E test-system.

It should be noted that some authors indicate the inconsistency of results, obtained while using conventional microbiological methods and API test-systems [3]. We observed some differences while studying the ability of strains *E. amylovora* UKM B-1095 and *P. agglomerans* P324 to dilute gelatine (Table 2, 4). According to the literature data, these bacterial species are capable of diluting gelatine, however, contrary results were registered while using API 20E test-system. The use of the conventional method to study this feature demonstrated that *E. amylovora* UKM B-1095 and *P. agglomerans* P324 dilute gelatine, but it occurs during long-lasting cultivation.

The software, provided by the manufacturer of API 20E test-system, was used to confirm that *P. agglomerans* P324 belongs to *P. agglomerans* species with rather a high similarity level. The strain *P. carotovorum* UKM B-1075 was also defined as *P. agglomerans*, but with 40 % similarity level.

API 20E test-system is primarily designed for clinical use; it contains tests, relevant for the identification of gram-negative bacteria of clinical and dietary origin. However, recently its application has been spread to other spheres of microbiology as well [4, 7].

Table 3. Physiological and biochemical properties of *Pectobacterium carotovorum*

Test/enzyme	*P. carotovorum* UKM B-1075		*P. carotovorum* according to the literature data [6]
	Using API 20E	By conventional methods	
β-galactosidase	+	n/d	a/d
Arginine dihydrolase	–	n/d	– (5 % +)
Lysine decarboxylase	–	n/d	– (5 % +)
Ornithine decarboxylase	–	n/d	– (5 % +)
Utilization of citrates	+	+	a/d
Formation of H_2S	–	–	+
Urease	–	–	–
Tryptophane deaminase	–	n/d	–
Formation of indole	–	–	–
Formation of acetoin (reaction of Voges-Proskauer)	–	–	a/d
Gelatinase	+	+	+
Utilization:			
of D-glucose	+	+	+
of D-mannitol	+	+	+
of inositol	–	–	var.
of D-sorbite	–	–	+
of L-rhamnose	+	+	+
of D-sucrose	+	+	a/d
of D-melibiose	+	+	+
of amygdaline	+	+	+
of L-arabinose	+	+	+
Reduction of nitrates	–	–	–

There are rare literature data, testifying to successful application of API 20E test-system to study the pro-perties of bacteria, associated with plants. For instance, API 20E test-system was used to characterize and identify strains of *Erwinia amylovora*, isolated from chokeberry tree and wild strawberry [8]. Also test-systems API 20Ne and API 20E were used to study bacteria, isolated from grass and previously referred to *Pseudomonas graminis* species. A high similarity level was obtained for these strains (98.5 %) and *P. chlororaphis* [9]. Vantomme *et al*. [10] studied the possibilities of API 20E test-system for express-diagnostics and comparison of *E. amylovora* strains, isolated in different countries, and demonstrated the efficiency of using this test-system to identify *E. amylovora* [10].

The impossibility to identify phytopathogenic bacteria with high similarity level, using the test-system and software to it, is related to the absence of the data about phytopathogenic and plant-associated gram-negative facultative anaerobic bacteria in the database. However, the application of this test-system to determine the bacteriosis agent provides for fast obtaining of results, promoting the identification of bacteria.

CONCLUSIONS

It was shown that the results of tests, obtained for the investigated species of *Erwinia amylovora, Pectobacterium carotovorum* and *Pantoea agglomerans* using API 20E test-system and conventional microbiological methods, coincided. The application of API 20E test-system (bioMerieux) ensures fast obtaining of important data, which may be used to identify phytopathogenic bacteria of *Erwinia, Pectobacterium, Pantoea* species.

Table 4. Physiological and biochemical properties of *Pantoea agglomerans*

Test/enzyme	*P. agglomerans* P324		*P. agglomerans* according to the literature data [6]
	Using API 20E	By conventional methods	
β-galactosidase	+	n/d	a/d
Arginine dihydrolase	–	n/d	–
Lysine decarboxylase	–	n/d	–
Ornithine decarboxylase	–	n/d	–
Utilization of citrates	+	+	a/d
Formation of H_2S	+	+	+
Urease	–	–	–
Tryptophane deaminase	+	n/d	+
Formation of indole	–	–	–
Formation of acetoin (reaction of Voges-Proskauer)	–	–	a/d
Gelatinase	–	+	+
Utilization:			
of D-glucose	+	+	+
of D-mannitol	+	+	+
of inositol	–	–	–
of D-sorbite	+	+	var.
of L-rhamnose	+	+	+
of D-sucrose	+	+	a/d
of D-melibiose	+	+	+
of amygdaline	+	+	+
of L-arabinose	+	+	+
Reduction of nitrates	+	+	+

Застосування комерційних тест-систем для ідентифікації грам-негативних факультативно анаеробних фітопатогенних бактерій

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Мета. Оцінити можливість використання комерційної API-системи для ідентифікації і вивчення властивостей факультативно анаеробних грам-негативних бактерій. **Методи.** Загальноприйняті мікробіологічні методи, тест-система API 20E (Biomerieux) за інструкцією виробника. **Результати.** Набір мікробіологічних тестів API 20E містить діагностично значущі тести для характеристики факультативно анаеробних фітопатогенних бактерій видів *Erwinia amylovora, Pectobacterium carotovorum* і *Pantoea agglomerans*. Властивості бактерій *E. amylovora, P. carotovorum, P. agglomerans*, визначені з використанням тест-системи і класичними методами, збігаються з даними літератури для цих видів. Із застосуванням програмного забезпечення виробника тест-системи, з достатньо високим рівнем подібності вдалося ідентифікувати бактерії виду *P. agglomerans*. Хоча не всі види факультативно анаеробних фітопатогенних бактерій можна ідентифікувати за допомогою тест-системи API 20E, її застосування, без сумніву, дозволяє отримати достовірні і важливі для ідентифікації бактерій дані щодо їхніх фізіолого-біохімічні властивостей протягом 24 год. **Висновки.** Результати тестів, одержані для досліджених видів за використання тест-системи API 20E і класичними мікробіологічними методами, збігаються. Застосування тест-системи API 20E (Biomerieux) дозволяє швидко отримати важливі дані, які можна використати для ідентифікації фітопатогенних бактерій родів *Erwinia, Pectobacterium, Pantoea.*

Ключові слова: фітопатогенні бактерії, бактеріальні хвороби рослин, властивості бактерій, діагностика, тест-системи.

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