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Distribution, biological characteristics and the national economic importance of bacteria of the <u>Bacillus</u> <u>subtilis</u> mesentericus group.

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The aerobic sporophytic bacteria <u>Bacillus subtilis</u>-<u>Bac. mesentericus</u> are true cosmopolitans. They are encountered everywhere; they can exist in the most variegated conditions: ground dust, river slime, vegetal residue, lake, river, and sea water, various types of soil, food products and even the organisms of insects, people and animals as well. (1-15). The greatest quantity of bacteria belonging botthe <u>subtilis-mesentericus</u> group is taken from soils. They are found in soils at various depths, independent of the degree of aeration, although higher soil layers are richer in these microorganisms.(16). The ground water level affects the growth of this group of bacteria: active multiplication of <u>Bac. subtilis</u> and <u>Bac. mesentericus</u> is observed in soils with deep ground water levels, while the type <u>Bac. cereus</u> dominates in soils with shallow ground water (35-84 cm) (17).

Hay and potato bacilli in natural conditions, namely in soils, have various cell forms. Along with the most common rod-shaped cells, cells with the appearence of reduced dimension <u>cocci</u> occur. The latter lead an independent existence and under proper conditions take on the rod-shaped form belonging to the given organism.(18). The greatest quantity of spores of these bacteria is observed in the soil in the autumn-winter period, October to February (16).

Various ecological-geographic zones and types of soil are characterized by unique regularities in the quantitative and qualitative reservoirs of bacteria of the hay-potato bacillus group. Northern soils are generally poor in bacillus forms (19). <u>Bac. subtilis</u> and <u>Bac. mesentericus</u> are distributed mostly in the brown and grey-earth soils of the dry steppes, where they amount to nearly 10% of the total number of bacilli (2). Southern breeds have a higher white stemperature optimum and are distinguished by a higher biosubtilis and <u>Bac. mesentericus</u> play an important role in thermogenesis connected with the dialysis of the micro-

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organisms. Thus, for example, in manure, composts and straw these bacteria develop fully and can attain a dominating position amidst the other microflora (20, 2, 21). Thermophilic strains of these bacteria with an optimal temperature for growth of 40-55° are extracted from natural sources (22-24). Dowben and Weidenmueller (25) obtained separate variants of potato bacillus which are able to grow at temperatures of 63-72°. The authors established that cells of the thermophilic variants contain 3.5-4.5 times more protein than cells which grow at a temperature of 37°. However, if cells adapted to growth at high temperatures are cultured at 37°, then in just 2-3 generations they lose the ability to grow at elevated temperatures.

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Plant cover influences the distribution of these bacteria. Thus, for example, the potato bacillus appears in great quantity in the soil under alfalfa, but in the same soils under cotton the quantity is significantly less (6).

The addition of fertilizers also has significance for the multiplication of spore bacilli. A high level of phosphoric fertilizers increases the quantity of the rhizospheric microflora <u>Bac. mesentericus</u>, <u>Bac. mycoides</u>, <u>Bac. megaterium</u>, and others (26). Afrikyan (5) has shown a regularity in the distribution of the bacteria group <u>subtilis-mesentericus</u> and <u>Bac. mycoides</u>: as the bacteria <u>Bac. subtilis and Bac. mesentericus</u> increase in the soil, the quantity of <u>Pac. mycoides</u> decreases. The author connects this regularity with the antagonistic mutual relations which exist between these bacteria. However such a difference in the qualitative reservoir of bacillus forms is probably explained by their living conditions, especially when nitrogenous.

Bacteria of the hay-potato bacillus group take an active part as ammonificators, along with other spore bacilli, in processes of mineralizing organic substances in the soil. But, unlike many other bacilli, they are included in these processes mainly in the latest stages in the decay of organic compounds (27). These bacteria easily make use of the more accessible forms of introgen, for example ammonium compounds, while <u>Bac. mycoides</u> requires some aminoacids for its development (28,29). The significant distribution of hay and potato bacillus in soils with proven agricultural capability may be connected with this. In soils of the <u>taiga</u> and sod-podzol, where the process of mineralization is hindered, active multiplication of these bacteria is not noted, and their quantity does not exceed 1% (4,6,2,21). There are significanly more bacteria of the <u>subtilis-mesentericus</u> group in cultivated soils than in virgin soils. In connection with this it is suggested that they be regarded as indicators of soil nitrogen regime improvement (30,31).

Along with easily accessible nitrogen sources, these bacteria make use of such more inert compounds as urea, hippuric acid (32), and molecules of humus substances (33,34). The destructive activity of the bacteria of the hay-potato bacillus is observed in the decay of peat compounds (35).

The hay bacillus in a synthetic medium with the mineral compounds of nitrogen (NH4)<sub>2</sub>SO<sub>4</sub> and KNO<sub>3</sub> at first in its development makes use of NH4<sup>+</sup> and then NO<sub>3</sub><sup>-</sup> (36). An ability of the potato bacillus selectively to utilize certain amino-acids has been observed. (37). Thus, these bacteria grow in a synthetic nutrient medium with aspartic acid, asparagine, glutamic acid, glutamine, proline and analine, which serve simultaneously as sources of nitrogen and carbon, and do not grow due to histidine, tryptophan, cysteine and some other amino-acids. In addition the use of separate amino-acids produces the creation of other amino-acids in the medium, and other amino-acids do not develop in the culture liquid only when analine and glutamic acid are assimilated. In a medium with arginine the potato bacillus is able to create urea (38). Sorokin (39) has concluded from research on the potato bacillus that biosynthesis of amino-acids can take place even due only to inorganic sources of nitrogen and carbon in a simple mineral medium of ammonium salt, sodium bicarbonate and water with no organic compounds added.

Bacteria of the hay and potato bacillus group are able to assimilate simple carbohydrates -- mono- and disaccharides, certain polysaccharides -- dextrin and starch, organic acids and alcohols, and also complex high-molecular carbohydrates such as pectin and xylan which go into vegetal tissue substances. Strains of potato and hay bacilli which decompose pectic substances play an important role in flax and hemp soaking processes (40-44). V.L. Omeljanskij (45) has pointed out this property of aerobic sporophytic bacteria. In contrast with causes of anaerobic decomposition of pectic substances, aerobic bacilli oxidize all products of hydrolysis of pectic substances, although not with the same speed. Galactose and acetic acid oxidize more rapidly; xylose, arabinose and galactomic acid more slowly. The hay bacillus, according to some writers' data (46,48), is able to decompose xylan or hemicellulose, which takes second place after cellulose in its distribution in nature among other carbohydrates. The creation of xylanase or hemicellulase by the hay bacillus in laboratory experiments is induced by adding xylan or D-xylose to the medium. However, other writers emphasize that the synthesis of xylanase complex does not depend on the presence or absence of xylan or xylose in the medium, i.e. they have a constitutive character (49). Additionally, it is stated that a temperature of 35° is more favorable for the synthesis of these ferments than a temperature of 25°. From many works it is known that <u>Bac. subtilis and Bac. mesentericus</u> are not able to assimilate lignin and cellulose.

The ways that carbohydrates are assimilated by these microorganisms have been quite well studied (50-52). Depending on the conditions of culturing, the potato and hay bacilli are able to oxidize substrates using gaseous oxygen or to cause fermentation which produces organic acids, alcohols and gas. Thus, Bac. subtilis and Bac. mesentericus in anaerobic conditions will ferment glucose with the creation of 2-3-butylene glycol, acetic acid, ethyl alcohol, glycerine and carbonic acid. Beyond that, traces of formic and succinic acid appear. In aerobic conditions the creation of glycerine does not take place (53). <u>Bac. subtilis</u>, str. Marburg, developing in a full-value nutrient medium in anaerobic conditions, causes a homofermentative lactic fermentation (54). In aerobic conditions only acetic acid, acetoin, and carbonic acid are formed. Glycolysis takes place according to the diagram of Embden-Meirhof (55). In anaerobic as well as in aerobic conditions the glycolitic way of dissimilating glucose by hay bacillus cells takes preference over the hexosemonophosphate way. Thus, according to the data of Goldman and Blumenthal (5), even in aerobic conditions <u>Bac</u>. <u>subtilis</u> cells, str. Marburg C4, make use of 60 to 70% of the glucose according to the diagram of Embden-Meirhof, the rest by the hexose-monophosphate way.

Chardiner in 1903 (cited by Loginova et al, 23) found lactic and acetic acids among other products of fermentation of starch by <u>Bac. subtilis</u>. In Waksman's monograph (56) there is a reference to a work in which it is indicated that <u>Bac. mesentericus</u> ferments starch with the formation of  $CO_2$ , formiate and valeric acid. M.M. Rotmistrov and A.E. Karpov (57) showed the ability of the potato bacillus to ferment in a potato medium with the formation of a significant quantity of gas, butyric and acetic acids, and ethyl and butyl alcohols.

In prolonged culturing in anaerobic conditions <u>Bac</u>. <u>mesentericus</u> Trevisan sharply lowers its catalysis activity (by 40 times) and loses its ability to grow in agar media in aerobic conditions (58).

Eac. subtilis contains a full set of cytochromes -a, b, c (59). The type of cytochrome system depends on the feeding conditions and the stage of culturing. (60,61). The various mechanisms for electron transfer that appear in these bacteria determine the variability of their physiological features when cultured in various conditions. Thus, Bac. subtilis cells when raised in a compound tryptonyeast medium with glucose is characterized by active respiration and a simultaneous ability for fermentation, and the cells which grew in a synthetic medium lose the ability to ferment, but oxidize glucose in the respiration process. In addition qualitative changes in the set of ferments (aldolases, glyceraldehyde phosphate dehydrogenases, etc.) have been shown (60). Therefore, the various ways for assimilating carbon and the great possiblities for assimilation favor the wide distribution of these bacteria in nature.

Bacteria of the <u>subtilis-mesentericus</u> group belong to the most widely distributed types of bacteria not only in soil, but also in water (21,62,8,13). The indicated microorganisms, along with representatives of the <u>Pseudomonas</u>, <u>Micrococcus</u> and other families, take an active part in the decomposition of dead plankton, playing a large role in cleaning stored water. Therefore, the dialysis of these bacteria in nature is highly variegated. They take part in processes of mineralization of organic substances and the formation of humus, on which the fertility of soils is based and which favors their cultivation; the trophism of stored water depends on their work as well.

When separated from soils of various ecologicalgeographical zones the bacteria of the hay-potato bacillus group show great variability in their morphologicalphysiological features. Natural variants, which have attained the name of ecological-geographical variant, are identified only with great difficulty with current definers. They differ from one another in their biological properties and often appear close to other types of the genus Bacillus -- Bac. pumilis, Bac. brevis, Bac. mycoides and others (63-65,18). The same culture, for example Bac. mesentericus, when sowed on MPA often creates typical and atypical forms: folded, mesentery shaped, dry spreading on the agar surface and also flat, smooth, and scum. There can be up to 10 types of potato bacillus colonies in agar media. Various forms have been described in detail by M.O. Krasil'nikov and displayed in photographs in his monograph "Soil microorganisms and higher growth" (18). In experimental conditions M.O. Krasil'nikov showed that the same culture of Bac. mesentericus has different morphological features depending on soil properties. Thus,

in podzols variants of the type Bac. cereus and Bac. subtilis were obtained, while in chestnut soils were variants of the type Bac. lichenformis and Bac. subtilis. In black-earth soils forms of the most variegated types were created. Later writers (66) obtained mycoid-like variants of potato bacillus when cultured in sterile samples of sod-podzolic soils. In addition, changes of certain biochemical features often used for identifying these bacteria were observed. Bac. mycoides, which is quite clearly differentiated by cultural features from the potato bacillus, was shown to have natural variants close to potato and hay bacilli in their features. In particular, a variant with folded colonies, reminiscent of Bac. mesentericus colonies, was taken from samples of southern soils (63). Atypical grainy, folded and smooth variants of <u>Bac</u>. <u>mycoides</u> have been described.(27, 18). The grainy form is often found in rich soils -- in podzols, grey earth, chestnut soils, Transvolga soils (67). A variant of Bac. mycoides similar to Bac. subtilis was uncovered during studies of dissociation (68).

In experimental conditions, acted on by various factors (low temperatures, amount of medium, bacteriophages, phytoncides, UV-radiation, anaerobic culturing conditions, magnetic field), variants of potato bacillus are formed which are close to other independent types of bacilli in their biological features (69-78,57,2,79-84). Of special interest are the works of M.M. Rotmistrov and A.E. Karpov (57), who by culturing Bac. mesentericus in a potato mash obtained anaerobic variants of this culture close to representatives of the Clostridium family in their taxonomic features. At the same time these authors observed the formation of aerobic rough-surface variants, in a number of features close to Bac. mesentericus Trevisan, in such anaerobic bacilli as C1. buturicum. The authors admit the possibility of such changeability in actual ecological conditions in nature and emphasize that the study of the influence of extremal factors, for example aerobiosis and anaerobiosis, may permit putting together truer propositions regarding obligatory and tolerant forms.

Thus an unusually wide changeability is observed in the spore bacteria group <u>subtilis-mesentericus</u>, appearing both in natural and in laboratory conditions. The problem of natural changeability in microorganisms is attracting the attention of researchers more and more. Microbiologists, biochemists and geneticists of various positions are attempting to explain the cause of this phenomenon. The theory of mutation with later selection has the most adherents (85). Facts obtained in experimental studies of natural changeability (dissociation) of certain sporophytic bacteria make it

possible to propose that bacteriophages may have a part in this process (86).

If one takes into account the conflicting data relative to the diagnostic features of sporophytic bacteria, the absence of a single nomenclature system, insufficient information about the degree of polymorphism of bacillus types and about their ecological changeability and their genetic mechanisms, the difficulties in creating a system for the natural evolution of sporophytic bacteria become obvious.

Some writers are in favor of a numerical taxonomy for the genus <u>Bacillus</u> -- a classification that unites organisms on a basis of gretest similarity which is established by the study of a great quantity of features (87-89). Thus Lemille and coauthors (89) used 64 biochemical indicators to compare various types of sporophytic bacteria and as a result united <u>Bac. subtilis</u> and <u>Bac. pumilis</u> in one subgroup as most similar, establishing as well a clear difference between these types based on certain features. However it has to be noted that the data of the numerical taxonomy for sporophytic bacteria may not always correlate; other authors use different methods of grouping. A responsible evaluation of the features used to classify and to unify an actual group of microorganisms is needed.

Microorganisms belonging to the Bac. subtilits - Bac. mesentericus group have characteristic polyfermentation properties. Their ferments contain an assortment of enzymes of various classes. Oxidoreductases (catalase and peroxidase) have been displayed in these bacteria (90,91). Hay and potato bacilli have a broad spectrum of dehydrogenases: they dehydrogenate sugars more actively, amino-acids less actively, and organic acids weakly (92). Transferases, in particular aminotransferase (93) and aldolases (94,60) have been shown. <u>Bac. subtilis</u> and <u>Bac. mesentericus</u> have a specially developed system of hydrolases -- ferments which catalyze the reaction of hydrolytic decomposition of molecules. Among the hydrolases found in these bacteria are: proteases, including elastases; glucosidases -*d*-amylase, saccharoses, levansaccharose and others; amidases -- urease, 1-asparaginase; esterases -- lipases, phosphatases, in particular ribonuclease and desoxiribonuclease and others (95,96,94,92,97-99,21,100-110). Bac. subtilis, str. Marburg, synthesizes peptidases during spore formation (111). Some of these are related to aminopeptidases. Other strains of Bac. subtilis contain the ferment &-lactamase (112,113) and form hyaluronidase (114). In hay bacillus spores there are also found ferments of

the classes oxidoreductase, transferase, liase, isomerase and ligase (115).

<u>Bac. subtilis</u> is the first microorganism which has been shown to be able to lysize other bacteria (114). From a hay bacillus culture ferments were obtained which lysize isolated cell membranes of the hay bacilli themselves -- autolysines. These ferments attack the cell walls of other microorganisms as well -- bacteria, saprophytic fungi and yeasts (116-119).

<u>Bac. subtilis</u> and <u>Bac. mesentericus</u> have found uses in industry as producers of proteolytic ferments (120-131).

Ferment preparations, for example subtilisin and BPN-protease, which decompose casein, hemoglobin, ovalbumin and gelatin have been obtained from cultures of <u>Bac.</u> <u>subtilis</u> (131,104). Proteolytic ferment preparations from <u>Bac.</u> <u>mesentericus</u> have attained the names mesenterin GR and mesenterin GK. They have been shown to be active with respect to all milk and blood proteins and also able to decompose elastin and fibrin (125,132,133).

Bacterial proteases and anylases are used in the textile industry for desizing viscose, cotton and acetate yarns. They are also used in silk processing to remove sericene from the remaining protein (134). Proteolytic complexes of ferments of <u>Bac.</u> mesentericus find application in cheese-making. These ferments act not only to curdle milk but also to partially hydrolyze proteins while the cheese is maturing (135-137,133,138). Proteolytic ferments of Bac. mesentericus are also used to regenerate triacetate film (139) and, in the leather industry, to dehair and soften hides (140-142). Abroad, especially in Japan, proteases find application in the cosmetics industry in the preparation of cremes and lotions. These ferments are also used as additives to detergents (143,144). In Denmark the firm "New Industry" puts out an alkaline proteinase for washing whites (142). It is possible to foresee the use of proteolytic ferments in agriculture. Adding these ferments to animals' feed rations reduces the consumption of feed and significantly increases animal growth (142).

Through various chemical and physical factors mutants of hay and potato bacillus with high proteolytic activity have been obtained (139,145-149). Thus, under the influence of ethylene-imine and etacidin the proteolytic activity of isolated variants of <u>Bac</u>. <u>mesentericus</u>, str. 34, was raised by 20-40% compared with the initial strain (94).

Bac. subtilis and Bac. mesentericus are among the most active producers of amylase. «-Amylase of these bacteria hydrolyzes starch with the formation of glucose, maltose. and dextrins. The amylases of Bac. subtilis have been studied in detail and widely used (95,150-152). The amylases of Eac. subtilis are divided into two groups, one of which hydrolyzes carbohydrates (starch, dextrin, glycogen, amylose, amylopectin) 30 to 40% and has gotten the name rarefier or dextrinizer. Amylase belonging to the second group hydrolyzes the substrate 50 to 60% and is called a saccharifier (152). In production conditions a ferment preparation containing a-amylase is obtained by various metho methods, both depth and surface methods (153,154). The greatest output of amylase using depth culturing of Eac. <u>subtilis</u> was observed in media with maltose, dextrin and starch (155,156). The broad complex of amylolytic ferments of <u>Bac. subtilis</u>, <u>Bac. mesentericus</u> permits their use in industry for hydrolyzing starch (76,157-160,94,161). In alcohol production cultures of <u>Bac.</u> subtilis, <u>Bac</u>. mesentericus can be utilized to rarefy starchy mashes before boiling, and also to replace the fungus culture at the stage of saccharifying the starch raw material. These bacterial cultures are distinguished from fungal ones by their more thermostable anylase and greater persistence at an elevated temperature during growth (162).

Accordingly, ferments of bacteria of the hay-potato bacillus group take part in variegated intermolecular reactions such as oxidation-reduction, hydrolysis, amidation of transamination, deamination, and decarboxylizing among others.(163,164).

The presence of a great quantity of ferments will stimulate the ability of these bacteria to transform various substrates. There has recently appeared much data in the literature about sporophytic bacteria's ability to assimilate and transform such natural and synthetic carboniferous compounds, poorly accessible to the bacterial cell, as steroids, rubber, phenylamines, dyes, resins, synthetic polyvinyl alcohol fibers, and nitroanilin (165,166,134,167-170).

Aerobic sporophytic bacteria are able to assimilate petroleum carbohydrates and grow in mineral media with scant paraffin and low boiling point n-alkanes  $C_6-C_{10}$ (170,171). Aerobic sporophytic bacteria of the genus <u>Bacillus</u> which use carbohydrates as the sole source of carboniferous nutrition have been separated from petroleum-saturated soil samples from various regions of the Soviet Union. Among these strains is Eac. subtilis Cohn.

which is in second place after <u>Bac</u>. <u>circulans</u> sp. <u>thermo-philus</u>. It is interesting to note that, when cultured in protein-free saline media with carbohydrates of the paraffin and olefin types and also with individual n-alkanes from C11 to C16, these bacteria synthesize proteinases which hydrolyzes casein (172).

The ability of the hay and potato bacillus to decompose various organic substances permits these bacteria to be recommended as bacterial additives for improving and quickening treatment processes for everyday solid wastes (173, 174). The effectiveness of the use of such bacterial additives is proven not only by laboratory experiments but also by the operational experience of the waste processing plant "DANO" in Warsaw (175). While they have been in use waste treatment processes have significantly quickened.

Thus, on the one hand, the bacteria convert complex compounds to simpler ones as a result of which nitrogen, carbon and other elements can be used in new biological cycles. But, on the other hand, although the ability to decompose molecules and vita is necessary for the bacteria themselves, it can cause harm to the national economy. The question of the harmfulness of these bacteria will be examined in this survey separately.

Along with the catabolic function, a biosynthetic dialysis is characteristic for the hay and potato bacillus. Some strains of <u>Bac. subtilis</u> and <u>Bac. mesentericus</u> synthesize vitamins of the <u>B</u> group (biotin, thiamine, pyridoxine, nicotinic and pantothenic acids), paraaminobenzoic acid, and heterauxin (18,93). Hay and potato bacilli are able to produce vitamins in synthetic saline media. It is suggested that these bacteria have an important role in the accumulation of biotic substances in the soil (6). Potato and hay bacilli form various amino-acids in media (tryptophan, aspartic and glutamic) and glutamic polypeptide (176-178, 164). The authors emphasize that the accumulation of aminoacids is observed in the soil as well.

A method for obtaining glutamic acid by the activity of <u>Bac. subtilis</u> has been described (179,180).

The potato bacillus can synthesize humus-like substances, among which are amino-acids, characteristic of natural humic acids (91,181). It is entirely possible that the indicated microorganisms take part in the creation of soil humus. Since 1969 a new branch of microbiological synthesis has been developing in Japan -- a processing of certain purines which are used in the food industry as flavorings. Auxotrophic mutants of hay and potato bacilli have been used to obtain separate purine compounds, for example inosinic acid and xanthine (182,183).

Sporophytic bacteria of the subtilis-mesentericus group are quite active antagonists of various bacteria, actinomyces and fungi (184-187,18,3,112,99,188). Their antagonistic properties are well known with respect to Azotobacter, Bac. mycoides, and the like. They are distinguished as well by the broad spectrum of their activity on phytopathogenic microorganisms of the families Erwinia, Pseudomonas, Verticillium and Fusarium. Important differences in the antagonistic properties of hay and potato bacilli have been shown (189). <u>Bac. mesentericus</u> is characterized by a broader spectrum of antimicrobe activity. Many strains of potato bacillus retard the development of gramnegative and grampositive bacteria and fungi (190). Cultures of Bac. mesentericus, isolated from rhizospheres of agricultural plants, from petroleum-carrying soils and filtration fields, showed themseves to be antagonistic towards Candida albicans, Staphylococcus aureus and Proteus vulgaris. A raw antibiotic with highly selective activity on Candida albicans was obtained from the strain Bac. mesentericus 614 (190). According to Sorokin's data (191), the hay bacillus shows antagonism toward St. aureus, Salmonella pullorum and Escherichia coli.

It is known that the overwhelming majority of antibiotics derived from bacteria are obtained from microorganisms of the <u>Pac. subtilis</u> - <u>Bac. mesentericus</u> group. More than 30 antibiotics from these bacteria have been described (192,193). The antibiotics subtilin, bacitracin, bacillomycin and others have a ruinous effect on staphylococci, streptococci, diphtheria and tuberculosis bacteria, the intestinal amoeba, <u>Clostridium botulinum</u>, on dermatophytes, candidi, saprophytic and phytopathogenic mycelium fungi. Some of these have found use in medicine, agriculture and industry.

A strain of <u>Bac</u>. <u>mesentericus</u> 614 has been described the culture liquid of which has an antivirus action (99). <u>Bac</u>. <u>subtilis</u> and <u>Bac</u>. <u>mesentericus</u> suppress the development of nematodes and are characterized by entomopathogenic properties (194,195). Their entomocidal action is explained by their high phospholipase and protease activity. D.G. Zatula (196,197) has described a strain of <u>Pac</u>. <u>mesentericus</u> AB the culture liquid of which is able to deprive tumoral cells of transplantability while the cells preserve their immunogenic properties. On this basis it has been shown to be possible to obtain antitumor vaccines with a marked prophylactic action.

However bacteria of the subtilis-mesentericus group also play a negative role in human life. Hay and potato bacilli can have pathogenic features (198,199). Belen'kij and Popova connect their toxicity with a marked hemolysitic action (200). Akopjan and Afrikjan confirmed these data. having shown hemolysins in the majority of strains investigated (201). The role of Bac. subtilis has been established, together with certain close types of bacillus, in the appearence of mastitis in cows, sheep and cattle abortions, and also deformities of the visceral organs of domestic fowl (202). Cox and coworkers (203) have described septicemia caused by Bac. subtilis in a patient that had been operated on. Bac. subtilis, and in several cases Bac. subtilis together with other microorganisms, for example Spirillum sp. Pseudomonas aeruginosa and others, was found in the blood of patients with malignant tumors and other serious illnesses which were treated with chemical preparations and hormones (204).

Many strains of <u>Bac</u>. <u>subtilis</u>, isolated from food products and inactivated by heating, had toxic effect on mice when introduced inside the peritoneum (205). On the basis of cited investigations the authors concluded that herbal ferments, for example trypsin, play an important role in raising the toxicity of microorganisms of the genus <u>Bacillus</u>. They suggest a possible role of these bacteria in food poisoning.

Recently it has been shown that certain strains of potato and hay bacillus (<u>Bac. subtilis</u> var. <u>niger</u> and <u>Bac.</u> <u>mesentericus</u> var. <u>niger</u>) produce toxic substances which cause deformities of the central and peripheral nervous system (206-208). Filtrates of these bacteria contain neurotoxic substances which bring about encephalomyelitis in animals, resembling the post-vaccination complications after using a neurotissue vaccine in humans and the experimental allergenic encephalomyelitis in animals (209). According to several reports the products of hay and potato bacillus metabolism affect nitrogen exchange in the animal's brain and are characterized by an inhibiting action on DNA synthesis and on the immunological reactions of the macroorganism (210-212). Immunizing and anti-inflammatory properties of the somatic antigen from <u>Bac</u>. <u>subtilis</u> have been described in the literature (213). The author observed non-specific defense reactions in 20,000 mice against various grampositive and gramnegative bacteria, especially against  $\underline{E. \ coli}$ . The mechanism of the defensive activity consists of stimulation of PEC phagocytes and antigen intervention in the process of homeostasis.

From a filtrate of <u>Pac.</u> <u>subtilis</u> culture liquid Japanese researchers (214,215) have obtained a substance of lipoprotein nature which enters into the structure of cytoplasmic membranes of vegetal cells. This substance affects the dialysis of the heart in angina pectoris, reduces the Ca content in rabbit blood serum, prevents the separation of Ca with urine and in large measure increases the phosphorus level in serum. The introduction into rabbits of this substance, tagged with J<sup>131</sup>, showed that it is selectively accumulated in mammalian organs which take part in Ca exchange.

There has been an impairment in gas exchange in the lungs of workers who have been directly involved in making ferment preparations from <u>Bac.</u> <u>subtilis</u> (216).

In this way the saprophytic spore bacteria <u>Bac. subtilis</u> and <u>Bac. mesentericus</u>, very widely distributed in the surrounding environment, may in real conditions be dangerous to humans and animals. Taking into account the possibility that these bacteria may be in the organism, for example in the gastro-intestinal tract, and bearing in mind their harmful effects, further study of the toxic metabolites of these bacteria is necessary, and also clarifying the factors which favor their formation.

Products of metabolism of individual strains of <u>Bac</u>. <u>subtilis</u> and <u>Bac</u>. <u>mesntericus</u> have been shown to inhibit seed germination and plant development. The main role in the toxicosis of sod-podzol soils belongs to these bacteria (18,217,218). Products of the life-dialysis of the toxic strains of bacilli affect not only the quantity but also the quality of harvest, lowering the content of common nitrogen, phosphorus and sugars in the green mass.

Potato bacillus is known as a cause of disease in fruit trees: it brings about browning of the fruit and spotty leaves in apricot, peach, banana and other trees (219,220,29,221,222). <u>Bac</u>. <u>mesentericus vulgatus</u> causes bacteriosis in corn sprouts and pumpkin flowers and also brings about a bacterial spottiness in sugar beets (223-226). <u>Bac</u>. <u>subtilis</u> and <u>Bac</u>. <u>mesentericus</u> influence the development of root rot of perennial legume grasses in the non-black earth arid zone, and also bring about potato tuber rot (227,

228). Cases where these bacteria deformed the leaves of feed legumes, sorghum and tobacco and also immature tomatoes in the Crimea have been described (229). From deformed fiber-flax plants with symptoms of wilt, Lebedeva (230) obtained a 99.9% culture of Bac. mesentericus. The majority of writers connect the phytogeniticity of these bacteria with their ability to separate proteolytic and pectolytic ferments, in particular protopectinase, and consider that this feature is strengthened when there is insufficient moisture, high temperature, and so on. Some writers regard the indicated bacteria as conditionally pathogenic and view them as transitional between saprophytic and phytopathogenic bacteria (230). Gorlenko and coauthors showed in 1953 that separate strains of Bac. mesentericus when grown on vegetal substrates and certain media acquire phytopathogenic features (231). It is also interesting to note that the transition to "parasitism" is accompanied by a change in the form of the colonies and in the size of the cell (229). It is necessary to take the fact into account that the spores of these bacteria have been observed for a long time in dessicated shape and their pathogenic properties are not apparent in this form. It should be emphasized that the phytopathogenic bacteria which deform agricultural plants may be dangerous to humans and animals, especially since they develop in plants which in raw form are used in food. The toxic products of the life-dialysis of these bacteria can accumulate in vegetal tissues and harmfully affect the animal organism (232).

Bac. subtilis and Pac. mesentericus cause harm to food industries: dairy-lactic, yeast, pastry, sausage, wine processing and others (233,234,71,235-237). According to the data of some writers (235), the hay bacillus and forms close to it cause dessert wines to spoil, in as much as their spores can withstand pasteurization and are distinguished by their high persistence in alcohol. Medvins'ka (7) and Rogacheva (233,234) include the potato bacillus among the microorganisms harmful to the sugar and canning industries and emphasize that these bacteria spoil sugar syrups, molasses, and also, developing in cans, can cause them to bulge.

In flour-milling and bread-making industrial practice <u>Bac. mesentericus</u> is well known as the cause of the potato disease which is known as "draw" (TJAGUCHA tr.) (2).

There is information in the literature about decomposition of wool fiber by sporophytic bacteria, in particular <u>Bac. subtilis</u> and <u>Bac. mesentericus</u>. These microorganisms can cause the ammonification of keratin, the wool fiber

protein (134,238). Synthetic polyvinyl alcohol fibers (biolan CC, methylan, iodin) also undergo the activity of <u>Bac. mesentericus</u> (166). The potato bacillus can change the external appearance of the fiber, its integrity, and additionally it can actually change its own properties itself. <u>Bac. mesentericus</u>, along with certain fungi, can spoil dye films on various surfaces (239). As a source of carbon and other elements these microorganisms use various resins which go into the composition of organic dyes (polyvinylacetate, styrenebutadiene, acrylate), due to which their viscosity changes (240,241). They are also able to partially decompose rubber (165).

Thus, bacteria of the hay-potato bacillus group can be viewed both as useful and as harmful microorganisms. An important role in biogenic cycling of elements belongs to these bacteria. In nature they perform the valuable function of synthesis-decomposition of organic substances, complete the mineralization of organic residue and form biologically active substances which have effects on living organisms. The polyfermentative dialysis of these bacteria opens perspectives for their practical use.

A deepened knowledge of the biology of these microorganisms, especially in connection with their ecology, discovery of the genetic bases of their mutability and an orderly systematization will unquestionably yield important results which will have practical significance as well as theoretical.

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