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Review

Beneficial and harmful roles of bacteria from the *Clostridium* genus*

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Bacteria of the *Clostridium* genus are often described only as a biological threat and a foe of mankind. However, many of them have positive properties and thanks to them they may be used in many industry branches (e.g., in solvents and alcohol production, in medicine, and also in esthetic cosmetology). During the last 10 years interest in application of *C. botulinum* and *C. tetani* in medicine significantly increased. Currently, the structure and biochemical properties of neurotoxins produced by these bacterial species, as well as possibilities of application of such toxins as botulinum as a therapeutic factor in humans, are being intensely researched. The main aim of this article is to demonstrate that bacteria from *Clostridium* spp. are not only pathogens and the enemy of humanity but they also have many important beneficial properties which make them usable among many chemical, medical, and cosmetic applications.

Key words: *Clostridium* spp., biotechnological applications, medical and cosmetic importance, pathogenicity

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INTRODUCTION

Bacteria of the *Clostridium* genus are anaerobic, Gram-positive, rod-shaped, endospore-forming bacteria. They have been known for a very long time. The first information about them is presented in *Epidemics III* —— the book described by Hippocrates in 430–370 BC. Hippocrates reported on *C. histoliticum* as a cause of gas gangrene (Mayr, 1969). The main symptom of this disease is swelling of the skin (Nigel *et al*., 2001). Other diseases caused by *C. tetani* were described by Charles Bell in his book. The information about other diseases caused by *C. tetani* appeared in *Essays on the Anatomy and Philosophy of Expression* in 1824 (Mayr, 1969). However, bacteria from the *Clostridium* genera were recognized as a separate starter culture were observed only in the 19th century by Louis Pasteur. He was the first to recognize that bacteria can exist and grow without oxygen. His discovery was a huge revelation in his time. Pasteur called such microorganisms *Vibrion butyrique* because of the main product of their fermentation pathway and introduced the word "anaerobic" to name life without oxygen. *V. butyrique* was renamed 20 years later as *C. butyricum* by another scientist — Adam Prażmowski (Dürre, 2001).

Bacteria from the *Clostridium* genera are commonly present in natural environment, e.g. they live in dust, soil, water, bottom sediments and in human and animal alimentary canals (Moriishi *et al*., 1996). They are mostly known as pathogenic microorganisms. However, they also play an important role in many fields of industrial metabolite production. Bacteria from *Clostridium* ssp. are able to ferment divers organic compounds and produce large amounts of gases (carbon dioxide, hydrogen, methane), organic acids (lactic, acetic, butyric, and fumaric acids), and also solvents (butanediol and propanediol) (Buckel *et al*., 2005). They are also used in medicine and esthetic cosmetology. In the past 10 years an interest in application of *C. botulinum* and *C. tetani* in medicine significantly increased. Currently, the structure and biochemical properties of neurotoxins produced by these bacteria, as well as the possibilities of application of such botulinum toxins as a therapeutic factors in humans, are being intensely researched (Jankovic & Hallet, 1994; Brin, 1997; Bigalke & Shoer, 2000; Schiavo *et al*., 2000; Kreydon *et al*., 2000; Rosetto *et al*., 2001; Dressler, 2000). In the literature one can also find information that bacteria from *C. sporogenes*, *C. butyricum*, *C. perfingens*, *C. acetobutylicum* and *C. botulinum* species can be employed in bacteriocin production (Clarke *et al*., 1975; Barber *et al*., 1979; Eklund *et al*., 1988).

In the bacterial world, in one family there are positive and negative bacteria for human and animal species. It is more interesting when the same bacterial strain plays a double role and is both: harmful and beneficial. This article is a review of some possibilities of applications for the bacteria from *Clostridium* genera in biotechnological processes (in the chemical and energy industry), in medicine and cosmetic, and about pathogenicity of these bacteria.

THE USE OF BACTERIA OF THE *CLOSTRIDIUM* GENUS IN THE CHEMICAL INDUSTRY

Bacteria of the *Clostridium* genus are able to employ intense fermentation metabolism (Jiang *et al*., 2009; Masset *et al*., 2010). They are able to produce wide range of metabolites, including: 1,3-propanediol (Biebl *et al*., 1998; Kubiak *et al*., 2012), acetic acid, butyric acid, formic acid (Wu & Yang, 2003; Song, 2011), ethanol, butanol, acetone (Jones & Woods, 1986; Ezeji *et al*., 2003), carbon dioxide (Khanal *et al*., 2004), hydrogen and ammonia (Levin *et al*., 2006; Ren *et al*., 2007; Skonieczny & Yargeau, 2009; Oh *et al*., 2009; Beckers *et al*., 2010). The

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Abbreviations: ACh, acetylcholine; BoNT, botulinum neurotoxin; BoNT-A/BTX-A, botulinum toxin type A; BDEPT, bacterial directed enzyme prodrug therapy; COBALT, combination of bacteriolytic therapy; LD50, lethal dose; *C., Clostridium;* 1,3-PD, 1,3-propanediol; *V., Vibrion; S., Salmonella*

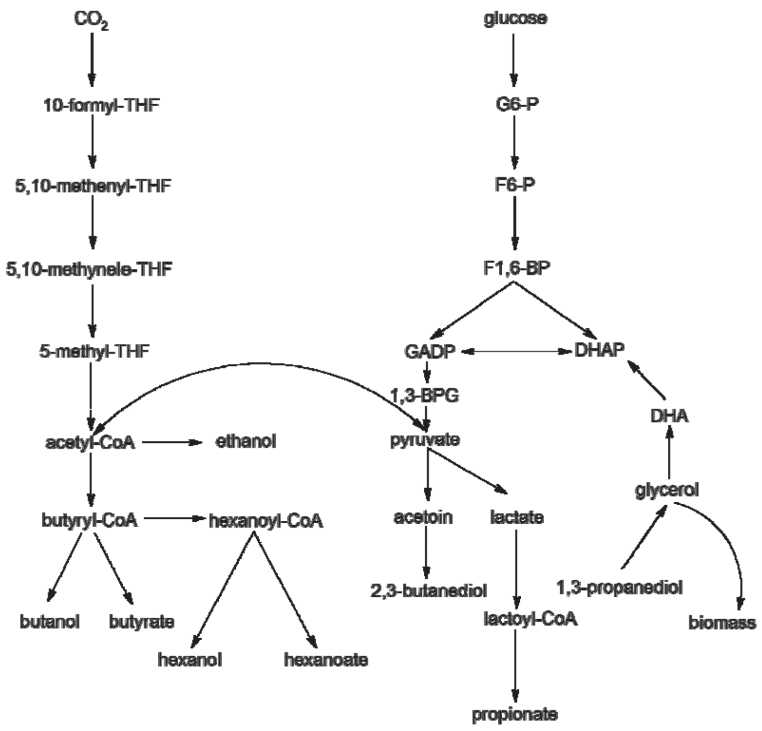

Figure 1. Metabolic pathways of bacteria from the *Clostridium* genera

metabolic pathway of production of metabolites by the *Clostridium* genus bacteria is shown in Fig. 1.

Production of 1,3-propanediol

1,3-propanediol (1,3-PD) is a three-carbon diol which consists of two hydroxyl groups on the primary carbon atoms, with the formula $C_3H_8O_2$. 1,3-PD is also known as a trimethylene glycol, 1,3-dihydroxypropane, propane-1,3-diol. 1,3-PD is an important chemical by-product in polymer production (Igari *et al.*, 2000; Drożdżyńska *et al.*, 2011). 1,3-PD is one of the oldest known fermentation products. This diol was identified in 1881 by August Freund, as a product of glycerol fermentation by *C. pasterianum*. In the past, 1,3-PD was produced only via chemical methods (Igari *et al.*, 2000). Nowadays, an attractive alternative for chemical synthesis is a microbial conversion of 1,3-PD from renewable resources (Nakamura *et al.*, 2003; Mu *et al.*, 2006). A number of microorganisms can grow on glycerol as the sole carbon and energy source and produce 1,3-PD. A large group of these microorganisms are bacteria of the *Clostridium* genus. This group includes: *C. diolis*, *C. acetobutylicum*, *C. perfingens*, *C. butyricum*, C. *pasteurianum* (Biebl *et al.*, 1998; Hao *et al.*, 2008; Kubiak *et al.*, 2012). *C. butyricum* is the best wild strain to produce 1,3-PD (Wilkens *et al.*, 2012). This strain is characterized by high productivity and little nutrition requirements (Buckel, 2005; Drożdżyńska *et al.*, 2011).

Bacteria of the *Clostridium* genus are also able to synthesize 1,3-PD with the use of metabolic engineering. Metabolic engineering of the microbial production of 1,3-PD concerns native producers of 1,3-PD (*C. butyricum*) as well as heterologous hosts (*C. acetobutylicum*) – microorganisms which are able to form 1,3-PD via genetic manipulations (Celińska, 2010; Leja *et al.*, 2011).

Production of organic acids

Organic acids are low-molecular weight compounds which are found in all organisms and which are characterized by the possession of one or more carboxyl groups (Jones, 1998). Organic acids are among the candidate replacements for in-feed antibiotics. Some of acids (lactic, acetic) have a long history of use in food preservatives (Skrivanova *et al.*, 2006). Bacteria of the genus *Clostridium* (*C. butyricum*, *C. beijerinckii*, *C. populeti*, *C. propionicum*, *C. thermobutyricum*, *C. tyrobutyricum)* are able to synthesize organic acids: succinic acid, acetic acid, butyric acid and propionic acid (Johns, 1952; Luers *et al.*, 1997; Zhu *et al.*, 2002; Willims *et al.*, 2003). Butyric acid is widely used in the chemical industry (for the synthesis of polymers of butyryl), pharmaceutical, and especially in the food industry to strengthen butter notes of flavor in food products (Zigova *et al.*, 1999; Wu & Yang, 2003; Liu *et al.*, 2006). Butyric acid is produced mainly by oxidation of butyraldehyde obtained from oxosynthesis of propylene (Wu & Yang, 2003). Propionic acid is widely used as an antifungal agent in food, beer and as an intermediate in the synthesis of herbicides, cellulose acetate (propionate plastics), solvents and pharmaceuticals (Kośmider *et al.*, 2010). In contrast to propionate production in *Propionibacterium*, which is produced through decarboxylic acid pathway, propionate in *C. propionicum* can be produced through the acrylate pathway (Tracy *et al.*, 2012). There are two major areas of commercial application of acetic acid today: food-grade vinegar and chemically synthesized industrial acetic acid. Among the bacteria of the *Clostridium* genus, *C. thermoaceticum* is able to synthesize acetic acid from xylobiose (Nakamura *et al.*, 2011). Biosynthetic routes often have product-specific advantages over chemical synthesis, which are important for extending and adding value to products.

Production of solvents

Solvents are liquids which form a homogeneous system (solution) with the substances dissolved in them. It is well known for almost 100 years that bacteria can produce solvents by fermentation. In the 1920s biotechnological production of solvents has been replaced by petrochemical methods. In the 1960s fermentation which used bacteria of the *Clostridium* genus was completely eliminated from industry. *Clostridium* ssp. were eliminated because the process was characterized by low productivity, low yield and high cost of the product recovery. Nevertheless, this method cannot be forgotten altogether. Currently, we can see an internationally growing concern for the environment and the need to gain independence from the petrochemical industry. This tendency led to an increased interest in the production of solvents from natural raw materials (e.g. corn, soy, molasses, wood hydrolysates etc.). A lot of research in this area has made the process competitive (Jones *et al.*, 1982; Dabrock *et al.*, 1992; Qureshi *et al.*, 2000).

Bacteria of the *Clostridium* genus are able to synthesize solvents such as acetone, butanol and ethanol. This

group includes: *C. acetobutylicum*, *C. pasteurianum*, *C. beijerinckii*, *C. saccharoperbutylacetonicum* and *C. saccharobutylicum*, *C. sporogenes* (Johnson *et al*., 1997; Keis *et al*., 2001; Bankar *et al*., 2012; Sun & Liu, 2012; Tracy *et al*., 2012; Gottumukkala *et al*., 2013). *C. beijerinckii* is able to produce isopropanol in addition to butanol (George *et al*., 1983; Chen & Hiu, 1986). Instead, *C. pasterianum* ferments hydrocarbons to butanol, acetone, carbon dioxide and hydrogen (Heyndrickx *et al*., 1991; Dabrock *et al*., 1992).

For the production of solvents, acetylo-CoA and butyrylo-CoA are the key intermediate factors to obtain ethanol and butanol. Acetyl-CoA is the key intermediate factor to produce acetone (Harris *et al*., 1999; Kasap, 2002).

THE USE OF BACTERIA OF THE *CLOSTRIDIUM* GENUS IN THE ENERGY INDUSTRY

Energy is the source of all human activity. In nature, energy is never lost but it is changing its form. There are many different ways in which energy can be stored, converted and amplified for our use (Demirbaş, 2001).

Production of hydrogen

Hydrogen is the element which is a very efficient source of energy, the energy equal to $33Whg^{-1}$. Hydrogen is regarded as one of the most important sources of energy. It can be also converted into energy in combustion engines, fuel cells and as a component of rocket fuel. Hydrogen is produced mainly by chemical methods (Chin *et al*., 2003). Currently, 90% of total production is obtained either from methane or electrolysis of water. However, the ability of using microorganisms in hydrogen production has been intensly investigated. Among others, direct and indirect biophotolysis, photofermentation and dark fermentation have been examined (Levin, 2006; Lo *et al*., 2010). Among promising solutions for biological hydrogen production there is hydrogen fermentation. Among numerous groups of microorganisms which are able to produce hydrogen, bacteria of the *Clostridium* genus, especially *C. acetobutylicum* and *C. butyricum*, are typically used (Chin *et al*., 2003; Chen *et al*., 2005).

The reaction of molecular hydrogen formation is as follows (Khanal *et al*., 2004):

$$C_{12}H_{22}O_{11} + 5\ H_2O \rightarrow 4\ CH_3COOH + 4\ CO_2 + 8\ H_2$$
$$C_{12}H_{22}O_{11} + H_2O \rightarrow 2\ CH_3CH_2CH_2COOH + 4\ CO_2 + 4\ H_2.$$

However, in this group of microorganisms there are also strains which are able to produce hydrogen not only from glycerol , but also from xylose — *C. tyrobutyricum* (Liu *et al*., 2006) and from cellulosic substrates — *C. thermocellum* (Levin *et al*., 2006).

Biomass conversion

Biomass is a term used for all organic material that stems from plants (McKendry, 2002a). Biomass is the oldest energy source used by humans. Traditionally, biomass has been utilized through direct combustion and this process is still widely used in many parts of the world. Historically, biomass has been dispersed, labor intensive and land intensive source of energy. Biomass differs from other alternative energy sources in that this resource is diversified. Biomass can include wastes, standing forests and energy crops. It can be converted to energy through a number of conversion processes (Demirbaş, 2001). Conversion of biomass to energy can employ two main technologies — thermochemical and biological (McKendry, 2002b).

Many microorganisms are able to utilize a variety of carbohydrates for the conversion of lignocellulose biomass to bioenergy. Pectinolytic bacteria of the *Clostridium* genus seem to be especially attractive in this aspect of biomass conversion. In contrast to the hyperthermophilic microorganisms (*Caldicellulosiruptor kristjanssonii*, *Anaerocellum thermophilum*), bacteria of the *Clostridium* genus are less thermophilic. This group of bacteria includes numerous species that utilize crystalline cellulose, as well as hemicellulose, as growth substrates (Blumer-Schuette *et al*., 2008). *C. flavum*, *C. laniganii* during fermentation of pectins loosen the plant tissue and allow fast separation of the cellulose fibers (Lanigan, 1959). These groups of microorganisms are of great importance to biomass degradation.

SOME APPLICATIONS OF THE *CLOSTRIDIUM* GENUS IN MEDICINE AND COSMETICS

Over the past few years botulinum neurotoxin (BoNT) has transformed from a cause of life-threatening affliction to a medical therapy. In 1978, Dr. Alan Scott was the first to use BoNT-A in humans for treatment of strabismus. Nowadays, after elucidating the pharmacological mode of the botulinum toxin action, it has become possible to use it in a wide spectrum of health disorders (Mahajan & Brubaker, 2007).

There are seven types of immunologically distinct serotypes of botulinum neurotoxin (BoNT): A, B, C1, D, E, F, and G. Toxins produced by clostridial bacteria are high-molecular-weight protein complexes. Each toxin is antigenically distinct, but they have similar molecular weights of 150 kD, as well as structures. All the toxins consist of a light and heavy chain linked by a disulfide bond. BoNT acts by blocking the release of acetylcholine (ACh) at the neuromuscular junction and it results in flaccid paralysis of the treated muscle (Johnson & Bradshaw, 2001; Majid, 2010; Tsui, 1996; Wheelera & Smith, 2013).

Botulinum toxin is effective in the treatment of some pain syndromes, e.g. BoNT can selectively weaken painful muscles by interrupting the spasm pain cycle. BoNT-A is well tolerated in the treatment of chronic pain disorders in which pharmacotherapy can cause side effects, such as migraines, chronic lumbar pain, tension headaches and myofascial pain. The reduction in the consumption of analgesics and length of action of three to four months per dose represent other advantages of its use (Colhado *et al*., 2009).

Types A and B of the toxins can also be used in the treatment of strabismus, blepharospas, nystagmus, vocal tics and stuttering, various manifestations of tremor, facial muscle contraction and many other dystonias, including cervical focal dystonias.

Additionally, injections of the botulinum toxin are among the latest means of therapy in treatment of neurological diseases (spasticity, in particular cerebral palsy, Parkinson's disease, Tourette's syndrome), gastroenterological diseases (achalasia), urological diseases (detrusor-sphincter dyssynergia, detrusor instability, lower urinary tract dysfunction), ophthalmological (strabismus) or dermatological diseases (hyperhidrosis, facial flushing). Treatment of muscle hyperactivity by injecting BTX-A into selected muscles produces dose-dependent chemical denervation resulting in reduced muscular activity (Barnes *et al*., 2011; Friedmana & Potulska, 2001; Graham *et al.,* 2000; Lin, 2007; Sutcliffe *et al*., 2005; Tsui, 1996). For example, in a recent study of 30 patients with

Tourette's syndrome and phonic tics, injection of the botulinum toxin improved symptoms in 93% and helped to improve the patients' quality of life (Sutcliffe *et al.*, 2005).

Moreover, not only metabolites of bacteria of the *Clostridium* genus, but also whole bacterial cells are used for medical therapy. Several studies have demonstrated that they can be used as probiotic agents against internal hemorrhages caused by enterohaemorrhagic *Escherichia coli*. Furthermore, they inhibit the growth of *C. difficle, S. typhimurium* and *Vibrio* spp (Nigel *et al.*, 2001; Ranade, 1989).

In recent years, there have been reports on the use of natural and engineered non-pathogenic bacterial species as potential antitumor agents, either to deliver tumoricidal molecules or to provide direct tumoricidal effects. Currently, the interest in alternative methods of cancer treatment is high because traditional cancer therapies have limited effectiveness due to poor penetration that reduces the dose present throughout tumors and due to the lack of selectivity to cancer cells which results in causing damage to normal tissue (Minton *et al.*, 1995; Barbé *et al.*, 2006; St Jean *at al.*, 2008; Patyar *at al.*, 2010).

Bacteria have the potential to overcome these limitations by actively targeting all tumor regions and delivering therapeutic payloads. The high specificity for cells of solid tumors is based on hypoxic tumors regions. Normal tissues are well oxygenated which prevents germination and growth of anaerobic bacteria whereas the hypoxic tumor regions allow the germination of spores and vegetative cell proliferation. Live, attenuated or engineered non-pathogenic bacterial species are capable of multiplying selectively in tumors and inhibiting their growth (Mellaert *at al.*, 2006; Patyar *at al.*, 2010; Wei *at al.*, 2008). Research on *C. novyi* (attenuated strain) showed significant anti-tumor effects, but these experiments also led to death.

The use of combination of bacteriolytic therapy (COBALT) also resulted in anti-tumor effects, but still was not devoid of animals' death. *C. novyi* has been investigated in conjunction with chemotherapy, radiotherapy and radioimmunotherapy.

Bacteria are also able to produce and secrete proteins, which can be combined with targeting in order to apply a focused therapy. Using proteins with different functionality, there are three methods of bacterial treatment that can be adjusted: enzymatic drug activation, controlled cytotoxicity and biomolecule secretion (St Jean *at al.*, 2008).

First strategy, called bacterial directed enzyme prodrug therapy (BDEPT) involves the tumor-specific location of bacteria to locally activate systemically administered 'prodrugs' within the tumor. BDEPT is a two-step therapy. First, recombinant spores carrying the genetic information about recombinant proteins are injected into the patient's body and they are targeted to the site of the tumor where the enzyme is expressed. Next, once the levels of enzyme expression are optimal, prodrugs are administered and are converted to a cytotoxic drug by the expressed enzyme strictly at the tumor site (Lehouritis *et al.*, 2013). The last strategy involves producing biologically active molecules to induce a physiological response. Studies have shown that *Clostridum* can be designed to produce therapeutically significant levels of a cytokine, interleukin-2, which causes T-cell-mediated neoplastic death.

Over the past decade, facial cosmetic procedures have become more common place in maxillofacial and oral surgery and dentistry. One of the most often requested procedures is a treatment with botulinum toxin type A (BoNT-A), which is known under several names such as Botox, Dysport.

The most common cosmetic procedure performed by intramuscular injection of BoNT is aimed to reduce facial wrinkles. Facial wrinkles are formed primarily due to intensive work of leading muscles, wrinkling one's brows and longitudinal muscle. Treatment of facial wrinkles involves injecting into the muscle different doses of the botulinum toxin, which reduces facial muscle activity. A significant improvement in facial skin tension is observed in approximately 90% of patients. The first effect of such a therapy is observed after 1–4 days, and a maximum after 2–3 weeks. The final effect is obtained after 3–6 months of therapy. Another procedure carried out with the use of botulinum toxin is correction of Masseteric hypertrophy. Masseteric hypertrophy usually results from anatomical asymmetry of the jaw, habitual asymmetric use of the jaw, excessive chewing of gum, clenching during exercise or sleep, and congenital malformations. The results of treatment with intramasseter injections of botulinum toxin have been encouraging and satisfying to patients (Jaspers *et al.*, 2011; Majid, 2010).

Additionally, the botulinum toxin is used in treatment of glabellar lines, frontalis muscle, commuter lines, smile orbital resin and masseter muscle hypertrophy. Recently, the therapeutic utility of the botulinum toxin in the treatment of excessive sweating was reported (Sutcliffe *et al.*, 2005).

Pathogenicity

Many bacteria of the *Clostridium* genus have adverse effects on food products and human health. The *Clostridium* genus includes 35 species of pathogens producing exotoxins, including *C. botulinum, C. perfringens, C. tetani, C. barati, C. haemolyticum, C. novyi, C. septicum, C. chauvoei and C. difficile* (Moriishi *et al.*, 1996). The most well-known pathogen of the *Clostridium* genus is *C. botulinum* that causes botulism. Botulism is caused by a botulinum neurotoxin (BoNT) produced from the anaerobic, spore-forming bacterium *C. botulinum* (Shapiro *et al.*, 1998). The most common cause of poisoning in humans are neurotoxins type A, B, and E (Bielec & Modrzewska, 2007; Wheeler & Smith, 2013). BoNT is a very strong poison, for example it is 10^{12} more lethal than sodium cyanide. The estimated LD50, for humans (assuming a body weight of 70 kg), extrapolated from experiments on animals, has been estimated to be approximately 70 μg by oral administration, 0.09–0.15 μg to intravenous administration and 0.7–0.9 μg by inhalation (Majid, 2010; Tsui, 1996). The disease can be divided into six types — foodborne botulism, wound botulism, infant botulism, adult intestinal toxemia botulism, inhalation botulism and iatrogenic botulism (Cherington, 1998; Bielec & Modrzewska, 2007). All forms of botulism produce the same distinct clinical syndrome. Botulism leads to paralysis that usually starts with the muscles of the face and then spreads towards the limbs. In severe forms, it leads to paralysis of the breathing muscles and causes respiratory failure, which may lead to death (Aureli *et al.*, 2008; Sobel, 2005).

Foodborne botulism is due intoxication caused by consuming food contaminated with the botulinum toxin. However, wound botulism is caused by contamination of wound with *C. botulinum* spores and subsequent germination of these spores and production of

toxin in the anaerobic milieu of an abscess. The toxin is partially absorbed into the blood (Mahajan & Brubaker, 2007; Sobel, 2005).

Infant botulism occurs in infants between 1 week and 11 months of age and is a combination of infection and intoxication, including ingestion of *C. botulinum* spores, germination within the gastrointestinal tract and *in vivo* production of toxin (Caya *et al*., 2004; Jagoda & Renner, 1990). In part due to the immaturity of the child's intestinal flora and in part due to a relatively low production of clostridial-inhibiting bile acid as compared to the adult gastrointestinal tract, this factor promotes development of the disease. A significant risk factor for the development of infant botulism is honey consumption, because 15% to 25% of honey products harbor botulinum spores. Therefore, children under one year of age should not eat honey at all (Caya *et al*., 2004; Moriishi *et al*., 1996; Bielec & Modrzewska, 2007).

Adult intestinal toxemia botulism is caused by the colonization of the colon by *C. botulinum* producing BoNT *in situ* and its absorption into the blood. The emergence of this form of botulism is caused by the presence of earlier lesions in the gastrointestinal tract: disturbance of bacterial flora composition after antibiotic therapy, a condition after a surgical operation or chronic gastrointestinal inflammatory bowel diseases (Bielec & Modrzewska, 2007).

Iatrogenic botulism is an extremely rare form of the disease that is caused by intake of BoNT A in the form of injection. The symptoms are usually limited to generalized muscle weakness, slight drooping of eyelids, double vision or dryness of mucous membranes in the mouth. Inhalation of botulinum toxin does not occur naturally in the natural environment. We all know the case from 1962, where in one of the laboratories in Germany three employees were infected. All those who became ill were exposed to an aerosol containing BoNT A. All of them had symptoms similar to foodborne botulism (Bielec & Modrzewska, 2007).

Like *C. botulinum*, bacteria of the C. *tetani* and *C. sordellii* species are dangerous for human health. Infections of those bacteria cause acute diseases and pose difficult clinical challenges. *C. tetani* causes tetanus. *C. tetani* usually enters the body through a wound and spores start to germinate. Toxins are produced and disseminated via blood and lymphatics. Tetanus is characterized by generalized rigidity and convulsive spasms of skeletal muscles.

C. sordellii infections are usually fatal. Most commonly, these infections occur after trauma, childbirth, and routine gynecological procedures, but they have recently been associated with medically induced abortions and injection drug use (Aldape *et al.,* 2006).

C. difficle causes nosocomial infections associated with food. Strains that exhibit disease produce two different protein toxins: a strong cytotoxin and an enterotoxin causing diarrhea; they are released by the vegetative cells (Bielec & Modrzewska, 2007).

C. perfingens causes gas gangrene and is the cause of severe food poisoning, associated with the release of enterotoxin by vegetative cells. In the development of gas gangrene other species of *Clostridium* are involved, including *C. novyi* and *C. septicum* (Stackebrandt & Hippe, 2001).

CONCLUSIONS

The *Clostridium* genera consist of many bacterial species with a wide range of physiological properties, thanks to which they may be used in many branches of the industry. Despite the fact that a lot of *Clostridium* species are pathogenic, some of them are used in medicine, in cancer treatment, and in cosmetology to remove wrinkles. Moreover, enzymes isolated from these bacterial cells are commonly used in modern biotechnology.

The important issue is to demonstrate that bacteria from *Clostridium* spp. are not only pathogens and the enemy of humanity but they also have many beneficial and important properties which make them usable among other chemical, medical, and cosmetic applications.

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