4.6 Potential use of endophytes in the pharmaceutical industry

Abstract Endophytes are microorganisms, usually fungi or bacteria less commonly algae and viruses, that live in plant tissues without causing disease symptoms in their host. It is estimated that there are over one million endophytic fungal species, but because of their habitat, they have been discovered relatively recently and therefore poorly studied. (Gupta, Chaturvedi, Kulkarni, & Van Staden, 2019) It is estimated that less than 1% of all endophytic species are currently known. When observing the mutual relationships of plants with endophytes, most researchers take the view that such associations are commensal (Ayswaria, Vasu, & Krishna, 2020). Nowadays, endophytic microorganisms are considered to be a potential source of compounds - secondary metabolites. Endophyte bioactive compounds can be used in the pharmaceutical industry. Endophytes are mainly used in the production of antibiotics, antioxidants, various enzymes, anti-inflammatory, antimicrobial, antiparasitics and antifungal drugs, immunosuppressors, and also as anticancer agents. The interest in biotechnology is great, as the application of secondary metabolites of endophytes is possible in the cosmetic industry, agricultural complex, textile production, and food industry besides pharmaceuticals. The relevance of this topic and its further research on the use of already available and the discovery of new bioactive components of endophytic microorganisms can help scientists in resolving the problems of resistance of some pathogenic strains to modern approaches in antibiotic therapy. The potential uses are great, as endophytes can be extracted from numerous plants worldwide. The properties and characteristics of extracted endophytes will vary due to their geographical location and environmental conditions. Besides the wide variety of endophytic microorganisms for production, an important factor is the ability to use the same fungus, bacterium, or algae to synthesize a significant number of different active compounds. These compounds are interesting because they can manifest their action in several directions. In this article we considered several options for classifying endophytic microorganisms, listed the possible applications in the pharmaceutical industry, also considered the most used bioactive compounds from the Streptomyces

genus actinobacteria, in addition, we reviewed substances with anti-tumor activity, which are now used to treat cancer of various human organs and are available as drugs for preparing injection solutions, metabolites of endophytes equally found their application. The aim of this work was to describe the modern classification of endophytes and show their potential use in antibiotic drugs as active agents in cancer treatment and their use as sedative drugs.

Keywords: Anti-bacterial agents; antineoplastic agents; bacteria; endophytes; fungi.

Introduction Endophytes are microorganisms (most commonly bacteria and fungi, less commonly algae and viruses) that live in plant tissues without causing harm to their host plant. They are an important part of the plant micro-ecosystem (Harrison, & Griffin, 2020). Over evolution, microorganisms have established a symbiotic relationship with plants, on which the metabolic products of microorganisms can depend, which can be used in the production of medicines. A detailed study of endophytes has highlighted biologically active compounds that can be used as:

- anti-cancer agents;
- immune suppressors;
- enzymes;
- in anti-inflammatory drugs;
- antimicrobials;
- antiparasitic drugs;

In addition, their neuroprotective, antioxidant and insulinomimetic properties were noted. The ubiquitous distribution of endophytic microorganisms offers opportunities for their use in drug biotechnology. Endophytes can serve as the basis for biopreparations, which will reduce the need for chemical compounds in the future (Yerkhova, & Katynska, 2021)

Aim The purpose of this article is to describe different endophytes and their use in medicinal products.

Methods of work include description and synthesis.

The main part The term endophytes applies to a community of microorganisms that have chosen living plant tissues as their habitat, and they generally do not exhibit pathogenicity to their host plant (Farahat, 2020). Unlike rhizosphere and phyllosphere representatives of plant-microbe symbiosis, endophytic microorganisms can enter a closer relationship with the host plant. In general, these microorganisms bring certain benefits, in some cases they can strongly influence the phenotype of the plant. Besides affecting the plant phenotype, endophytes also modulate phytohormone levels, produce vitamins and improve nutrient supply. It was also noted that in the presence of endophytes, the plant more easily tolerates biotic stress, resistance to pathogenic microflora, to destruction by insects and herbivores rises (Gouda, Das, Sen, Shin, & Patra, 2016).

The taxonomic composition of an endophytic bacterial community can have great biodiversity within the same plant species. Microbial diversity indicators mainly depend on abiotic and biotic factors, which also include:

- soil conditions;
- biogeography;
- plant species;
- the interaction between microbes and their host.

Through the vastness of the endophyte group (bacteria, fungi, algae and viruses) there are two variants of classification.

The first method of classification includes general information from biology and genetics, and not an unimportant point in this systematization is their ability to transfer from one host to another. Based on this system, a division is made into two categories: systemic and non-systemic (transitional) endophytes.

The second method of classification applies strictly to fungal endophytes. Based on this nomenclature, endophytes are divided into 4 classes. These groups are classified according to the part of the plant colonized (solely the shoots, solely the above-ground or only the underground part of the plant or the entire plant) and the area of the plant tissue. It is also possible to divide these 4 classes into 2 groups - clavicipitaceae

endophytes (class 1) and non-clavicipitaceae endophytes (classes 2, 3, and 4) (Jia, Chen, Xin, Zheng, Rahman, Han, & Qin, 2016)

Endophytes throughout their life cycle can act as producers of a large range of compounds that can be used in the pharmaceutical industry as lead compounds in the development of new drugs. Today, the bio-prospecting of secondary metabolites that can be used in pharmaceutical production is becoming increasingly important.

To date, many secondary metabolites have been found and classified into groups for convenience — alkaloids, quinones, benzopyranones, phenolic acids, flavanoids, saponins, steroids, terpenoids, tannins, xanthones, tetralones, etc.

When extracting biologically active compounds from endophytes, all kinds of circumstances must be considered, such as climatic conditions of the environment of the habitat of the plant, time of collection and even geographical location. Endophytes are a treasure trove of medicinal compounds that may in the future become an inexhaustible source for the production of a wide range of drugs (antimicrobial, anti-arthritic, antidiabetic, antitumor and immunosuppressant drugs). Discovery of new and study of already known bioactive compounds and secondary metabolites can help in overcoming the resistance of pathogenic microorganisms to drugs (Singh, Kumar, Singh, & Pandey, 2017). The problem of antimicrobial resistance is mainly caused by irrational prescribing, self-medication, overuse of drugs. At the same time, the demand for antibiotics is increasing due to a rapidly growing population, with an aging population, which is pulling increasing cases of infectious diseases and chronic diseases (WAAAR: World Alliance Against Antibiotic Resistance).

In the studies of different plants, it has been found that in each plant species, several species of endophytes can be present. (Newman, & Cragg, 2020) Among different endophytes, actinomycetes are known for their 52.73% share in the production of antibiotics, 50% of which are used in the treatment of human diseases.

The most commonly used producer of antibiotics is Streptomyces actinobacteria genus. The main compounds with descriptions of their activity and plants from which they were isolated are presented in Table 1.

Table 1 Antibiotic substances produced by *Streptomyces* actinobacteria genus

Strain Producer	**Active compound**	**Active on**	**The host plant**
Streptomyces NRRL 30562	Munumbicins	Broad spectrum *Bacillus anthracis, Streptococcus pneumoniae, Enterococcus faecalis, Staphylococcus aureus, S.aureus, E.faecalis.*	*Kennedia nigricans*
Streptomyces NRRL 30566	Cockadamucins	Wide spectrum of activity	*Grevillea pteridifolia*
Streptomyces HKI 0595	Xiamycin A	Electoral activity as for HIV	*Bruguiera gymnorrhiza*
	Xiamycin B	Antibacterial, Also applicable for methicillin-resistant *Staphylococcus aureus* and vacomycin-resistant *Enterococcus faecium*	*Kandelia candel*
	Indolespin		
	Sespenin		
Streptomyces MSU-2110	Coronamycin	Antimicrobial, it also shows its activity in relation to *P.falciparum*	*Monstera sp*
Streptomyces Tc022	Actinomycin D	Antifungal Antitumor	*Alpinia*

References: Hur, Jang, & Sim, 2021; Finocchiaro, 2020; Pfaffenbach, Bakanas, O'Connor, Herrick, & Sarpong, 2019; Pratiwi, Hidayat, Hanafi, & Mangunwardoyo, 2020.

Besides antibiotic activity, endophytes also have antitumor activity. One of the best known compounds is paclitaxel (Taxol). It was first found in the endophyte fungus Taxomyces andreanae (the chasian plant is the Pacific yew Taxus brevifolia). Paclitaxel is a potent mitotic inhibitor. Now on the market there is a drug Taxol of the same name, which is used for treatment of ovarian cancer, breast cancer (if there are affected lymph nodes after standard therapy), metastatic breast cancer, non-small-cell lung cancer. Also recommended for: Kaposi's sarcoma in AIDS patients, transitional cell bladder cancer, leukemia, squamous cell head or neck cancer, esophageal cancer.

In addition to paclitaxel, vinblastine, which is produced by the endophytic fungus Curvularia verruculosa found in Catharanthus roseus, is also widely used as a pro-tumour drug compound. Vinblastine is a chemotherapeutic agent that is used as a mitosis inhibitor in chemotherapy. Vinblastine binds to the protein tubulin and thus inhibits the formation of microtubules. During cell division, microtubules ensure that the corresponding chromosome pairs of newly formed cells are separated. As a result, the alkaloid prevents cell division. They also block the synthesis of DNA and RNA (Parthasarathy, Shanmuganathan, & Pugazhendhi, 2020). On the pharmaceutical market, the drug is marketed under the names Vinblastine and Welbe. In fact, the list of compounds that exhibit antitumor activity based on endophyte metabolites could go on, but so far only vinblastine and taxol have entered serial production.

In addition to its antimicrobial and antitumor activity, the compound hypericin has become widely used. Hypericin is a bright red chemical belonging to the category of naphthodiandrons. Hypericin has several pharmacological effects such as antiviral, anticancer and antidepressant (Verebová, Beneš, & Staničová, 2020). In addition, recent evidence suggests that it can be used in photodynamic therapy or photochemotherapy as a diagnostic or therapeutic agent (Vigneshwari, Rakk, Németh, Kocsubé, Kiss, Csupor, & Szekeres,2019). Although hypericin has been known for over 60 years, its mechanism of action has not yet been elucidated. Hypericin was first isolated from Hypericum perforatum. In subsequent

studies it was found possible to cultivate it from the endophytic strain Thielavia subthermofila. Now hypericin can be found in sedative medicines, antidepressants and used to treat diseases of the nervous system (Gelarium hypericum, Deprivit, Sedaton, etc.).

Discussion and conclusions Nowadays, endophytes unfortunately remain an understudied group of microorganisms. With new research, endophytes have proven to be reliable renewable sources for the industrial synthesis of bioactive compounds. These substances can be used in many pharmaceutical preparations effective against a wide range of pathogens.

With the help of biologically active substances produced by endophytamas it is possible to bypass the resistance of some pathogens. (Martínez-Romero, Aguirre-Noyola, Bustamante-Brito, González-Román, Hernández-Oaxaca, Higareda-Alvear, & Servín-Garcidueñas, 2020) The range of applications of endophytes is very wide and they can be used as anticancer agents (taxol, vinblastine), immune suppressors, enzymes (vidyl hydrolases, lyases, oxidoreductases and transferases), anti-inflammatory drugs (cineol), antiparasitic drugs, antifungal drugs (Actinomycin D), antiviral drugs (various alkaloids), antimicrobial drugs (Coronamycin). In addition to pharmaceutical applications, secondary metabolites of endophytes can be used in cosmetics, agriculture and food industries.

Results Generally speaking, the pros of using endophytes in industrial production include:

- There's a tremendous variety of species;
- Prevalence;
- The ability to synthesize multiple drug compounds from the same genus of microorganisms. A striking example is the genus *Streptomyces* actinobacterium, which can produce many active compounds that can exhibit a wide range of activity against many pathogens;
- Thanks to endophytes it is possible to bypass antibiotic resistance, e.g. for methicillin-resistant *Staphylococcus aureus* and vacomycin-resistant *Enterococcus faecium*.

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