REVIEW DOI: 10.5504/BBEQ.2011.0116 A&EB
AGRICULTURE AND ENVIRONMENTAL BIOTECHNOLOGY

BIOACTIVE METABOLITES PRODUCED BY MICROORGANISMS COLLECTED IN ANTARCTICA AND THE ARCTIC

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ABSTRACT

Survival of microorganisms in the Antarctic and Arctic conditions of life requires of the relevant genera and species special adaptability and resistance against stressors such as lack of substrate, UV-radiation, low temperatures for a long time and short-term intense heat during the Antarctic and Arctic summer. Therefore genetic adaptation of Antarctic and Arctic organisms to stress factors would be expected. All this suggests in a global aspect synthesis of new metabolites with unique structures and specific biological activity. Antarctic and Arctic polar regions are considered as a huge reservoir of microorganisms with versatile antimicrobial potential. Our studies are motivated by an interest in the functional role played by natural products in the ecological interactions of the polar strains with the other members of the microbial community. This overview presents some of the most important secondary metabolites (antibiotics, alkaloids, high carbon amino acids, nitro compounds, diketopiperazines etc.) produced by Antarctic and Arctic microorganisms. The secondary metabolites biosynthesis probably plays an important role in the adaptation and survival of microorganisms in the ice deserts of Antarctica and the Arctic.

Biotechnol. & Biotechnol. Eq. 2011, **25**(4), Suppl., 1-7

Keywords: Antarctica, the Arctic, bioactive metabolites, secondary metabolites, antibiotics, alkaloids

Introduction

Antarctica is a particularly interesting biosphere due to the special climatic conditions and the distance to other continents. Antarctica is the coldest, driest, windiest and most inaccessible continent on earth, with inherent difficulties for scientific research, which make otherwise practical experiments impossible. Only about 2% of continental Antarctica is ice-free (6). Antarctica is the coldest place on Earth because the coldest natural temperature ever recorded on the planet was −89.2 °C and it was measured in this continent. For comparison, this is 11 °C colder than subliming dry ice. Antarctica is an icy desert with little precipitation. Temperatures of the South Pole reach a minimum of between −80 °C and −90 °C in the interior in winter and a maximum of between 5 °C and 15 °C near to the coast in summer. Sunburn is often a health problem because the snow surface reflects almost all of the ultraviolet rays falling on it (27). East Antarctica is colder than its western counterpart because of its higher elevation. Only cold-adapted plants and animals survive there, including penguins, seals, nematodes, tardigrades, mites, many types of algae and other microorganisms, and tundra vegetation. The climate of Antarctica does not allow extensive vegetation. A combination of extreme low temperatures, poor soil quality, lack of moisture and lack of sunlight inhibit plant growth. As a result, plant life is limited to mostly mosses and liverworts. The flora of the continent largely consists of lichens, bryophytes, algae and fungi (3). Growth generally occurs in the summer and only for a few weeks at most. More than 200 species of lichens are found to be in Antarctica (3).

The Arctic is a region located at the northernmost part of the Earth. The Arctic consists of the Arctic Ocean and all or parts of Canada, Russia, Greenland, the United States, Norway, Sweden, Finland and Iceland. The Arctic region consists of a vast, ice-covered ocean, surrounded by treeless permafrost. The Arctic‘s climate is characterized by cold winters and cool summers. Precipitation mostly comes in the form of snow. Strong winds often stir up snow, which creates the illusion of continuous snowfall. Average winter temperatures can be about −40 °C, and the coldest recorded temperature is approximately −68 °C. Coastal Arctic climates are moderated by oceanic influences, having generally warmer temperatures and heavier snowfalls than the colder and drier interior areas. The Arctic region is a unique area among Earth‘s ecosystems (2). Life in the Arctic includes organisms living in the ice (19), zooplankton and phytoplankton, fish and marine mammals, birds, land animals, plants, and human societies. Arctic vegetation is composed of dwarf bushes, herbaceous plants, lichens and mosses, which all grow relatively close to the ground, forming tundra.

Antarctica is colder than the Arctic for two reasons. First, much of the Antarctic continent is more than 3 kilometres (2 mi) above sea level, and temperature decreases with elevation. Second, the Arctic Ocean covers the north polar zone: the ocean‘s relative warmth is transferred through the icepack and prevents temperatures in the Arctic regions from reaching the extremes typical of the land surface of Antarctica (27).

The first reports about Antarctic microflora were published by Tsiklinsky in 1908 (26), who collected data during the French Antarctic Expedition in 1903-1905. Interesting information

about the bacterial microflora of Antarctica was also gathered by Darling and Siple (7). They isolated 178 bacterial cultures from ice, water, plant remains and soil. Based on the preferred temperature of their environment, microorganisms are divided into three groups: psychrophiles (optimal growth temperature at 15 °C or lower and minimum temperature for growth at 0 °C); psychrotrophs (psychrotolerants), which grow at 5 °C or lower but show higher (20-28 °C) optimal growth temperatures, and thermophiles that grow at 45-90 °C (22). Besides these three groups, some authors distinguish also a fourth group, the so-called extreme thermophiles, thriving under optimum temperatures of 90-110 °C (4). In the microbial ecosystems of Antarctica and the Arctic psychrophilic and psychrotrophic organisms play a major role in the biodegradation of organic matter.

Bioactive secondary metabolites produced from Antarctic and Arctic microorganisms

The survival of microorganisms in the Antarctic and Arctic conditions of life requires of the relevant genera and species special adaptability and resistance against stressors such as lack of substrate, UV-radiation, low temperatures in a long time and short-term intense heat during the Antarctic and Arctic summer. Therefore genetic adaptation of Antarctic and Arctic organisms to stress factors would be expected. All this suggests in a global aspect synthesis of new metabolites with unique structures and specific biological activity.

Antarctica and the Arctic polar regions are considered as a huge reservoir of microorganisms with versatile antimicrobial potential (23).

Cladosporium cladosporioides is a fungal plant pathogen that affects wheat. It is also isolated from Antarctica (1). *Cladosporium cladosporioides* is the source of the series of chemical compounds known as calphostins and isocladosporin (16, 18). The known calphostins include calphostin A, B, C, D and I. The calphostins are inhibitors of protein kinase C. The most potent member of the series is calphostin C.

In 1992 was published the discovery of new species from the genus *Sphingobacterium* which produces sphingolipids and sphingophospholipids (25). The species is *Sphingobacterium antarcticus* and is psychrotrophic.

Acyl-thiazolylcarboxamides had been found before only in the few bacitracins from *Bacillus subtilis* and *Bacillus licheniformis*. The recently described simple derivative bacillamide (**Fig. 1a**) from another *Bacillus* sp. showed a reasonable activity against the dinoflagellate *Cochlodinium polykrikoides* with an LC_{50} value of 3.2 µg/ml (17).

A new sulphur-containing natural alkaloid named microbiaeratin (**Fig. 1b**) was isolated from the culture filtrate of *Microbispora aerata* strain imbas-11A. The organism was isolated from penguin excrements, collected on the Antarctic Livingston Island. A low antiproliferative and cytotoxic effect of microbiaeratin was determined with L-929 mouse fibroblast cells, K-562 human leukemia cells (GI_{50} >50 µg/ml) and HeLa human cervix carcinoma (CC_{50} >50 µg/ml). Microbiaeratin did not show antimicrobial and microalgal activity (13).

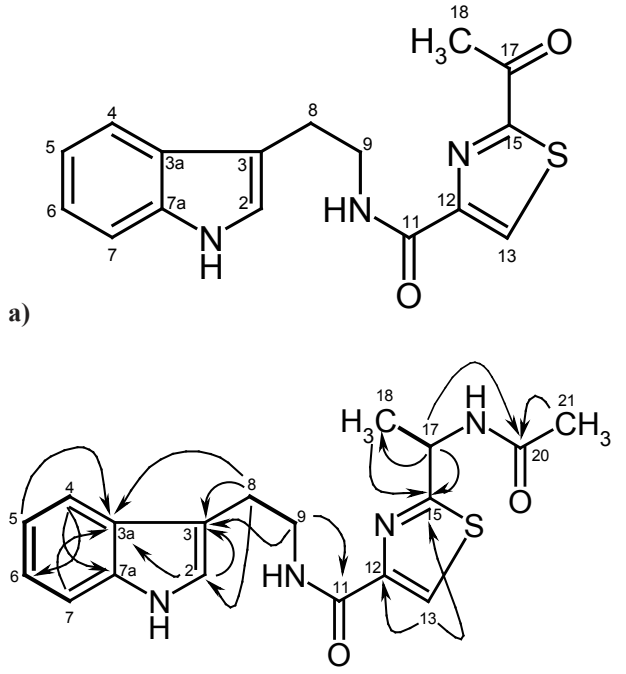

Fig. 1. Structure of thiazolylcarboxamides microbiaeratinin (bacillamide) (**1a**) and microbiaeratin (**1b**) isolated from antarctic microorganisms.

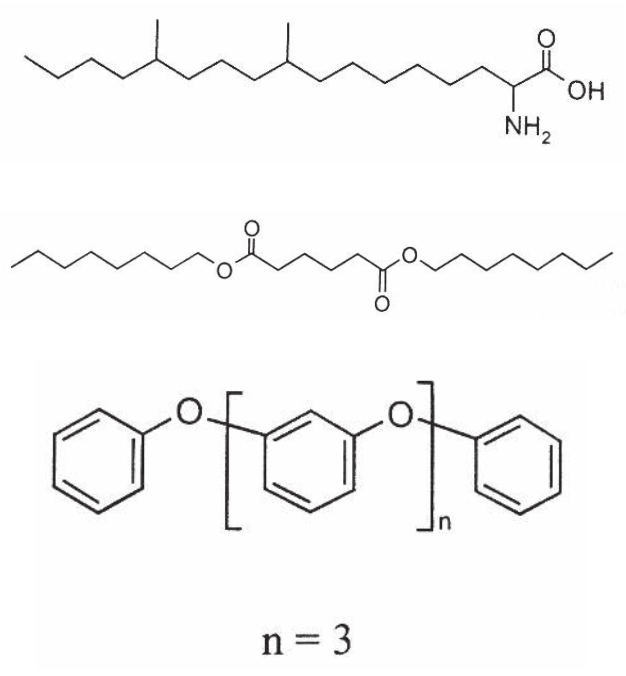

Fig. 2. Structure of bioactive substances, isolated from the Antarctic strain *Streptomyces* sp. 1010: 2-amino-9,13-dimethyl heptadecanoic acid (**2a**), hexanedioic acid dioctyl ester (**2b**), polyphenylether (**2c**).

The antarctic strain *Streptomyces* sp. 1010 (14) contained the novel 2-amino-9,13-dimethyl heptadecanoic acid (**Fig. 2a**) and additionally the polyphenylether (**Fig. 2c**) and hexanedioic acid dioctyl ester (**Fig. 2b**).

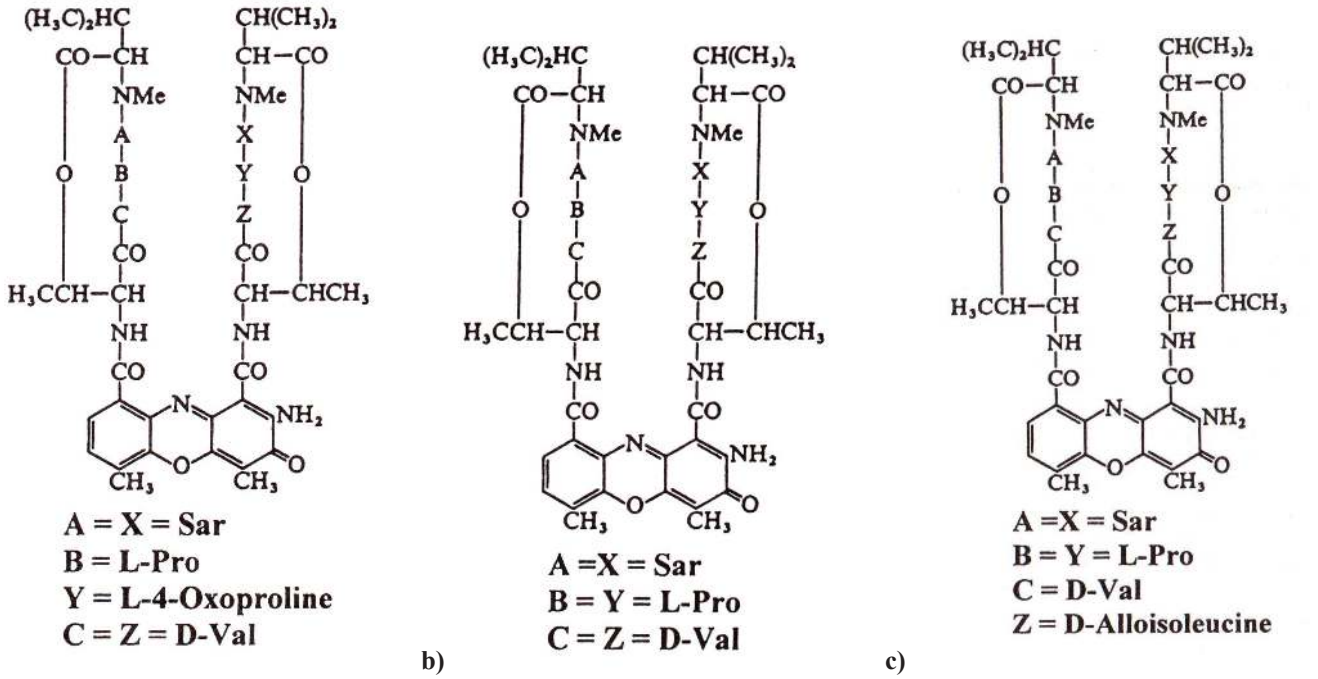

Fig. 3. Structure of actinomycines, isolated from strain *Streptomyces flavovirens* 6[7]: actinomycin X_2 (**3a**), actinomycin D=C_1 (**3b**), actinomycin C_2 (**3c**).

Streptomyces flavovirens 6[7], isolated from soil samples in the region of Livingston Island, Antarctica, produced antibiotics with antimicrobial and antitumour activity, which belonged to the actinomycins (**Fig. 3a, Fig. 3b** and **Fig. 3c**). They are a group of closely related peptide antibiotics. The chromophore 3-amino-1,8-dimethyl-2-phenoxazone-4,5-dicarboxylic acid is connected with two cyclopentapeptides (15).

The compound N-acetyltryptamine (**Fig. 4**) is a natural product directly isolated from the culture broth of the antarctic strain *Microbispora aerata* subsp. *nov.* imbas-11A and belongs to the family of the indole alkaloids (12).

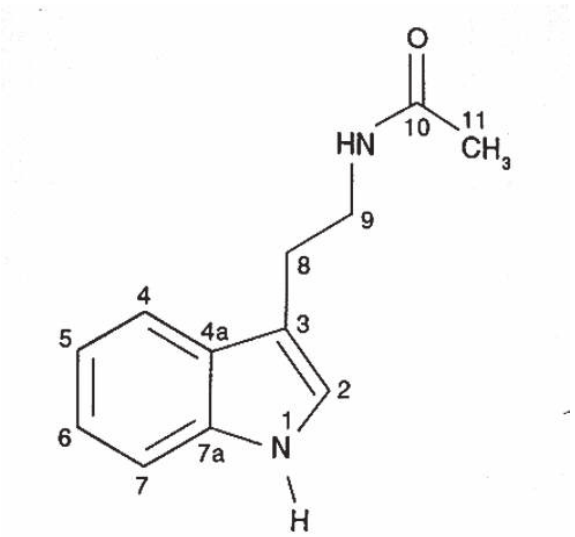

Fig. 4. Structure of N-acetyltryptamine.

Penicillium islandicum has world-wide distribution, including in the polar regions. Its known metabolites include: islandicin (**Fig. 5a**), emodin (**Fig. 5b**), endocrocin (**Fig. 5c**), skyrin (**Fig. 5d**), flavoskyrin (**Fig. 5e**), rubroskyrin (**Fig. 5f**), chrysophanol (**Fig. 5g**), roseoskyrin, iridiskyrin, simatoxin (8, 9) and five hepatotoxins: luteoskyrin (**Fig. 5h**), cyclochlorotine (**Fig. 5i**), islanditoxin, erythroskyrin (**Fig. 5j**) and rugulosin (**Fig. 5k**) (9). The most toxic are luteoskyrin and cyclochlorotine, the latter causing liver cirrhosis and fibrosis. Luteoskyrin and rugulosine produce liver necrosis, affect DNA polymerase, inhibit energy transfer and uncouple oxidative phosphorylation in mitochondria, and erythroskyrin causes paralysis and hepatic damage with nephrotic changes and injury to the lymphatic system (5).

From *Penicillium griseofulvum,* collected in Greenland, were isolated as secondary metabolites griseofulvin (**Fig. 6a**), fulvic acid (**Fig. 6b**), mycelianamide (**Fig. 6c**), roquefortine C (**Fig. 6d**) and roquefortine D (**Fig. 6e**), chanoclavine I (**Fig. 6f**) and elymoclavine (**Fig. 6g**) with antimicrobial activity (11).

Penicillium coprobium, collected in the Arctic, produces the secondary metabolites styrene (**Fig. 7a**), 1-undecene (blackberry smell) (20), patulin (**Fig. 7b**), pyripyropens, cyclopiamin, roquefortine C, meleagrin (**Fig. 7c**) and neoxaline (**Fig. 7d**) (10, 11).

Twenty-five aromatic nitro, dinitro and trinitro compounds were isolated in low yields of less than 1 mg/l from a *Salegentibacter* sp. strain T436 derived from Arctic pack ice (**Fig. 8**). Their structures were elucidated by MS and NMR techniques. Seven of these compounds, namely, 2-hydroxy-3-(4'-hydroxy-3'-nitrophenyl)-propionic acid methyl ester,

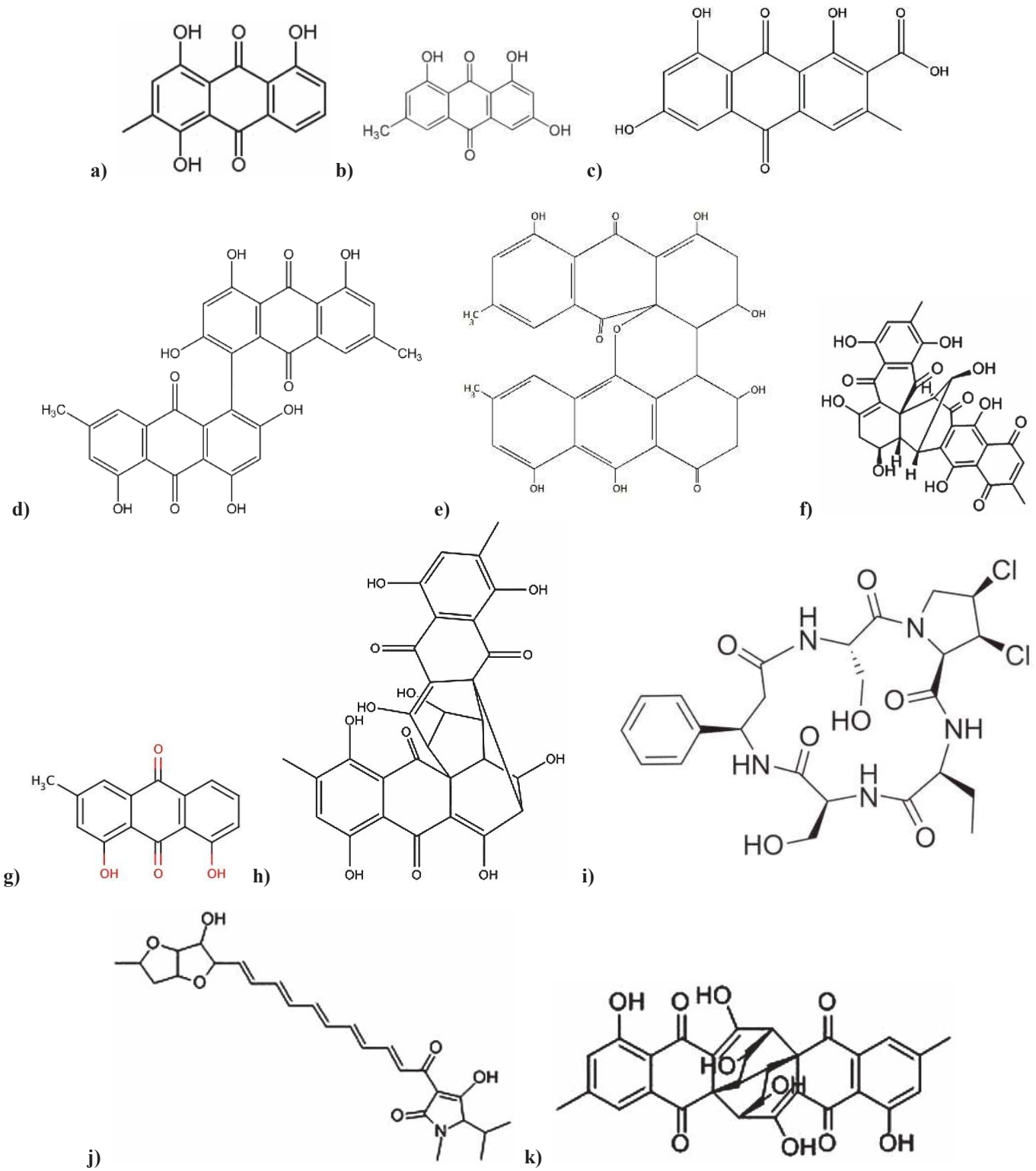

Fig. 5. Structure of bioactive substances isolated from *Penicillium islandicum*: islandicin (**5a**), emodin (**5b**), endocrocin (**5c**), skyrin (**5d**), flavoskyrin (**5e**), rubroskyrin (**5f**), chrysophanol (**5g**), luteoskyrin (**5h**), cyclochlorotine (**5i**), erythroskyrin (**5j**), rugulosin (**5k**).

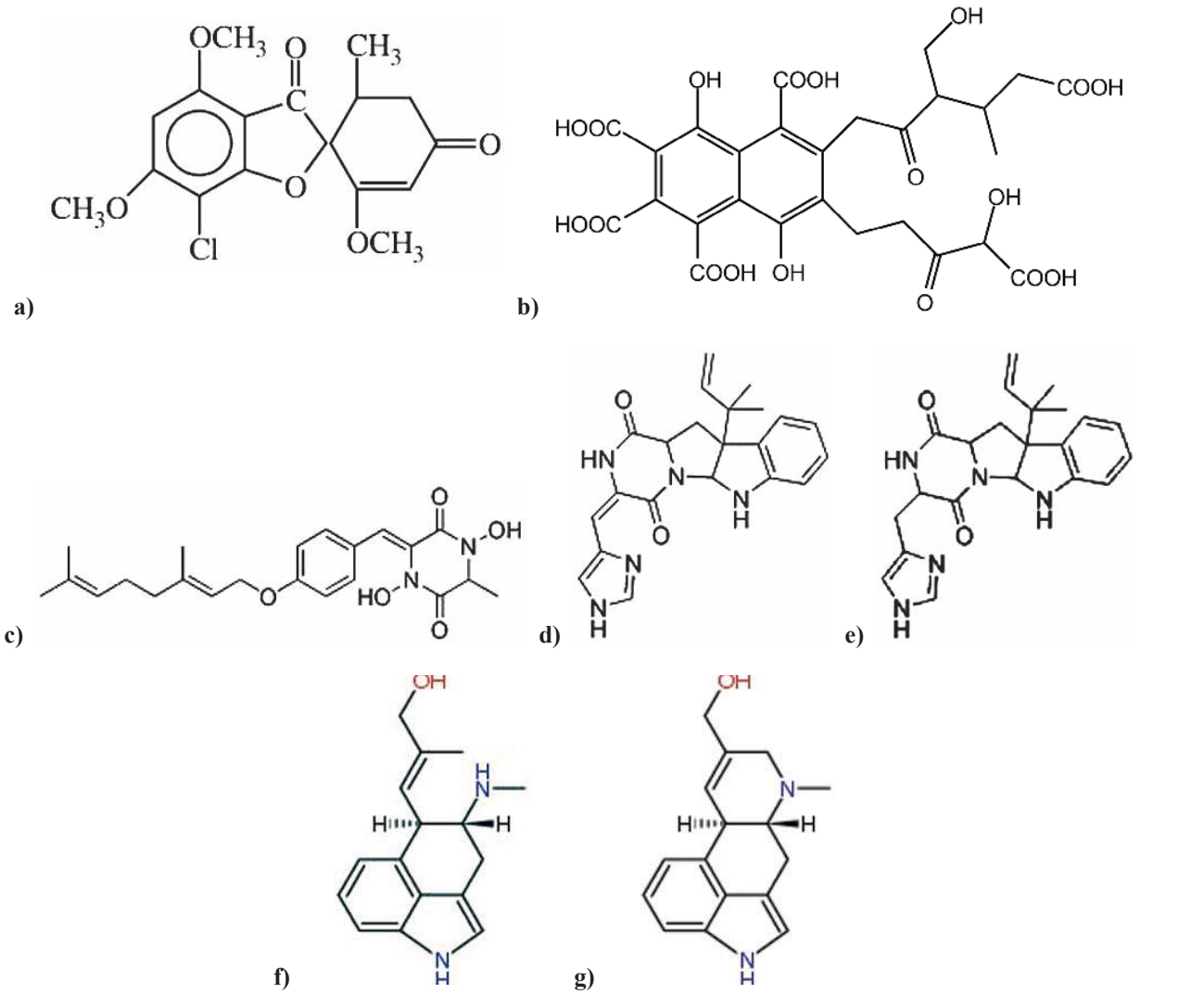

Fig. 6. Structure of bioactive substances isolated from *Penicillium griseofulvum*: griseofulvin (**6a**), fulvic acid (**6b**), mycelianamide (**6c**), roquefortine C (**6d**), roquefortine D (**6e**), chanoclavine I (**6f**), elymoclavine (**6g**).

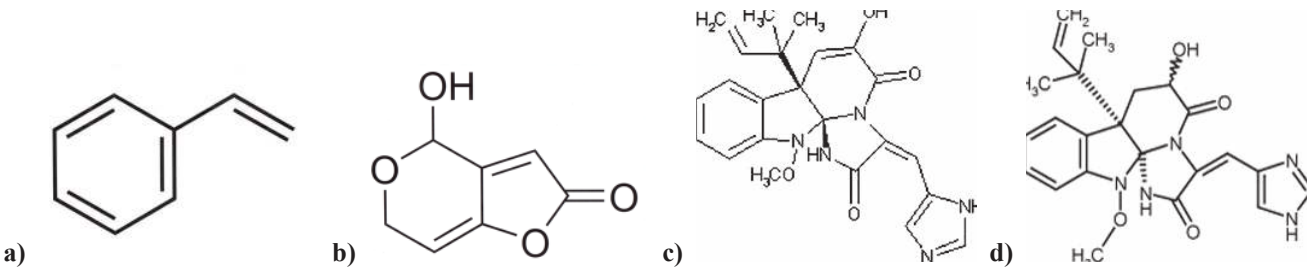

Fig. 7. Structure of bioactive substances isolated from *Penicillium coprobium*: styrene (**7a**), patulin (**7b**), meleagrin (**7c**), neoxaline (**7d**).

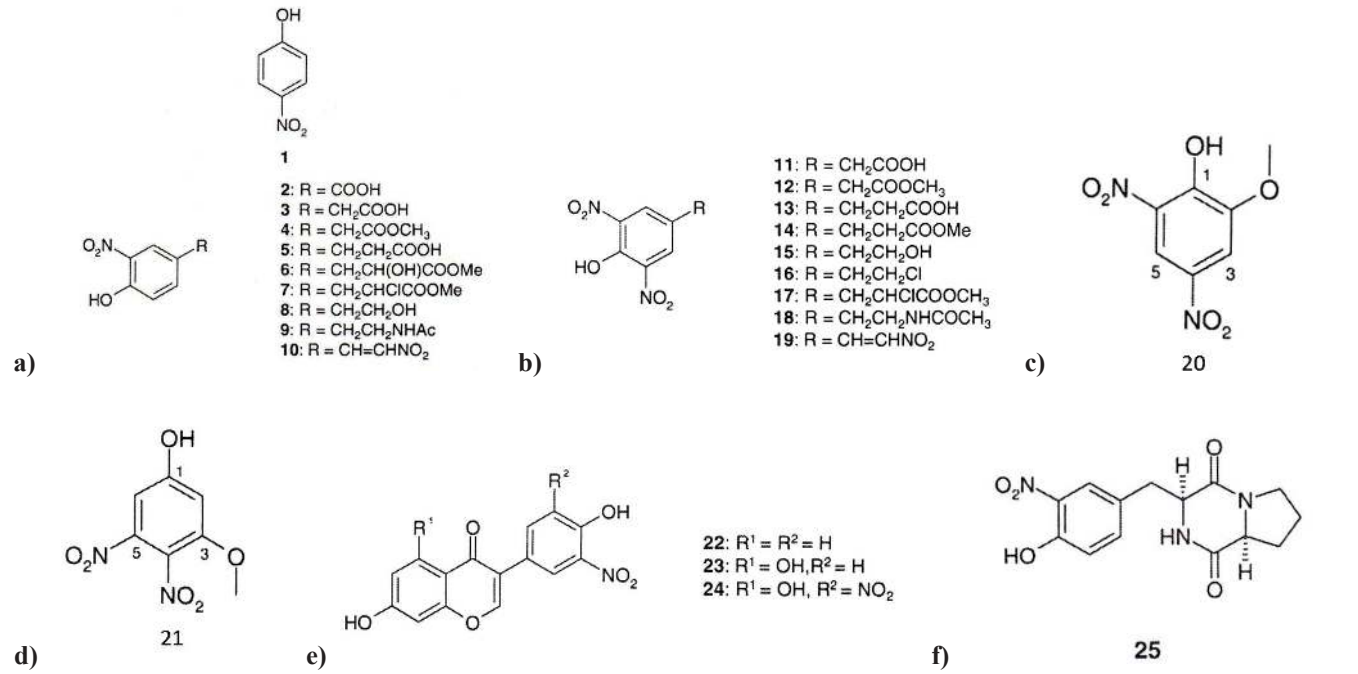

Fig. 8. Structure of nitro derivates isolated from the arctic ice bacterium *Salegentibacter* sp. strain T436: nitrophenol and its derivatives (**8a**), dinitro and trinitro derivatives of phenol (**8b**), 4,6-dinitroguaiacol (**8c**), dinitroresorcinol ether (**8d**); additional fermentation products: 3'-nitro-daidzein (**22**), 3'-nitro-genistein (**23**), 3',5'-dinitro-genistein (**24**) (**8e**) and nitro-diketopiperazine pyriculamide (**25**) (**8f**).

2-chloro-3- (4'-hydroxy-3'-nitrophenyl) propionic acid methyl ester, 3-(4'-hydroxy-3',5'-dinitrophenyl)-propionic acid methyl ester, 4'-hydroxy-3',5'-dinitrophenylethylchloride, (4'-hydroxy-3',5'-dinitrophenyl)-2-chloropropionic acid methyl ester, N-acetyl-3',5'-dinitrotyramine and 2,6-dinitro-4-(2'-nitroethenyl)phenol are new (24).

Conclusions

The search for Antarctic and Arctic microorganisms that could find industrial applications, started only in the recent years of the 20th century. The future researches will be focused on the identification (taxonomy) of new strains, isolation and elucidation of chemical structures of new antibiotics and other pharmacologically active substances, mechanism of action and search for new biological properties. These Antarctic and Arctic strains represent a potential for biotechnology (21) and also give a better understanding of the polar ecosystems. Our studies are motivated by an interest in the functional role played by natural products in the ecological interactions of the polar strains with the other members of the microbial community.

Acknowledgements

This work was supported by grant BG051PO001-3.3.04/32 financed by Operational Programme Human Resources Development (2007 – 2013) and co-financed by the European Social Fund of the European Union.

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