X. Functions of collateral metabolites produced by some actinomycetes

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Executive summary: Bacteria biosynthesize secondary metabolites (collaterals) or in other words a large class of biologically active bio compounds, which have applications in agriculture and the pharmaceutical industry. If knowledge's about bacilli are more advanced (focused on practical applications has enzymes) in the case of actinomycetes the most information has been obtained from studies on the production of antibiotics. The purpose of this paper is to summarize the information on the biosynthesis of metabolites (collateral bio compounds) and Inhibit bio compounds relevant to agriculture and the pharmaceutical industry. The paper brings information on the genes encoding the biosynthesis of important bio substances in terms of agriculture, pharmaceutical and antibiotic activity of metabolites (collateral bio compounds).

Keywords collateral metabolites; bio compounds; bacteria; actinomycetes; antibiotic activity.

General remarks

Actinomyces produce a number of enzymes that help degrade organic plant material, lignin, and chitin. Notwithstanding of diversity of their structures, bioactive collateral metabolites are synthesized from simple units used in living organisms for the biosynthesis of cellular structures. These units comprise amino acids, acetate, propionate, sugars, nucleotides *etc*. Normally to their structure and type of biosynthesis, collaterals metabolites are divided to form different groups. The production of bioactive collaterals metabolites in actinomycetes isolated from nature is before low in most cases. Actinomyces that are isolated from nature (wild type strains) produce small amounts of collateral metabolites. Actinomyces are typical bacteria ("ray fungus"); are Gram–positive filamentous rods that are not acid fast and are nonmotile. The cell wall peptidoglycan contains muramic acid, N–acetylglucosamine, glutamic acid, and one or two supplementary amino acids. Actinomyces species have lysine or lysine plus ornithine in the peptidoglycan. Most strains of *A. viscosus* and *A. naeslundii* bear well-developed long, thin surface fibrils.

1. The antibiotic activity of collaterals metabolites

The chemical composition and cellular location of some Actinomyces antigens are known. One group of saccharide antigens is cell-wall associated, protease resistant, and heat stable. *A. viscosus* also has an amphipathic antigen that is a fatty acid-substituted heteropolysaccharide and different from the teichoic and lipoteichoic acids found in most Gram–positive bacteria. The actinomycetes, especially the group of *Streptomyces* are known as specialized bio compounds potential antibiotic biosynthesis; summarizing three quarters of natural substances with antibiotic potential.

Natural substances with potential antibiotic synthesized by streptomycetes (of acetylCoA and phosphoenolpyruvate, and subsequently methylated through methionine as the methyl donor), have different chemical structure and can be classified into different types, while bio compounds with a similar function by bacteria of the genus *Bacillus* biosynthesized or other groups (lactic bacteria, *Enterobacteriaceae, Pseudomonas sp.*) are mainly peptides or peptides modified. Constituents of genus *Bacillus* are able to synthesize natural substances with antibiotic potential, as collateral metabolites in late logarithmic growth phase or early stationary phase. It was identified over 169 natural substances with antibiotic potential. *B subtilis* can synthesize 68 kinds of natural substances with antibiotic potential, while only 23 *B. brevis* synthesize natural substances with potential antibiotic (1).

Many natural substances with antibiotic potential synthesized by bacilli are active against Gram–positive, but there are exceptions. Most natural substances with antibiotic potential synthesized by bacilli are peptides, but may belong to other classes of bio compounds (amyl glycoside and phosphoric triennial). There is a controversy regarding the function of natural substances with antibiotic potential from bacteria of the *Bacillus* genus.

They are synthesized during sporulation, so it is believed that natural substances with antibiotic potential is a link to of a biochemical processes that occur in the transition from vegetative state to enhance (2).

Many strains of bacilli synthesizers of bio compounds with antimicrobial activity were subjected to special analysis of molecular mapping, identification and cloning of genes encoded (3).

Collateral bio compounds (metabolites) have antibiotic activity due to its ability to inhibit primary metabolic processes. Most act as anti–metabolites, as their functional similarity with normal metabolites allows binding to target sites and interfere with normal activity. By products generated from a given metabolic pathway inhibits another

metabolic pathway, their activity depends entirely of the configuration of natural substance with potential antibiotic configuration and distribution of functional groups at their level (4).

Since there is no strict correlation between natural substance with antibiotic potential and metabolic pathways the substance is synthesized, it is questionable assertion that all collateral bio compounds show some biological activity, given that they often have significance only for human and less for the synthesizer body (5).

Although the research on potential pharmacodynamics of natural substances were related mainly to the antibacterial activity, are now studied the action of the substance on some types of eukaryotic cells. An example is monensin, originally identified in cultures of *S. cinnamonensis*, that presently is use as bacteriostatic/coccidiostatic (used in poultry farming, antiparasitic compounds with a broad spectrum of activity against nematodes and arthropods, substances with antitumor activity, immunosuppresses, thrombolytic *etc*.) or as growth stimulator at animals (6).

A serious problem in case of producing substances with antibiotic potential is their means of synthesizing bacteria resistant to these agents that inhibit the growth or other essential biochemical processes.

To eliminate the effect of growth inhibition, synthesizers bacteria may experience more resistance mechanisms: modification of membrane permeability or inactivate toxic metabolite (7).

Because the natural substance with antibiotic potential are removed outside of the cell as they are synthesized, cell inactivation does not affect extracellular product efficiency. A study of antibiotic resistance in bacterial strains sensitive ("target") showed that natural substances with antibiotic potential excreted in the environment and acting in the vicinity of the body synthesizer has the significance of factors involved in the competition for resources.

Resistance to natural substances with potential antibiotic production has been observed in many bacterial collection strains isolated before their introduction into practice what suggests that bacterial populations come into contact with bio compounds in their natural life (8).

Some metabolites (collateral bio compounds) are toxic and even they don't have big antibiotic value, they can increase the activity of other natural substances with antibiotic potential , without that was installed the resistance of many bacterial strains. An example is the clavulanic acid synthetized by strains of *S. clavuligerus* that has small antibiotic activity, but determine an increment of action of some substances with antibiotic potential β–lactams by inhibition of β–lactamase synthetized by target bacterial strain. Natural substances with antibiotic potential known today used in practice can by classified after: mode of action, chemical nature, action spectrum *etc*.

An approach to the structure of natural substances with antibiotic potential consider them, along with a large number of metabolites synthesized by both bacteria and fungi or plants can be grouped into a family of bio compounds called poliketide (9). Polyketides are a comprehensive group of collaterals metabolites synthesized by decarboxylative condensation malonyl units often with subsequent cyclization of the polyketo chain. Al, that bio compounds have keto groups at carbon atomic level in different positions into the molecule with antibiotic potential. Bio compounds from this class are: tetracyclines, antracyclins, erythromycin, rifampicin *or rifapentine,* granaticin, avermectin (synthetized by de *s*treptomyces), aurantinin (synthetized by *Bacillus sp.*) mupirocin (synthetized by *Pseudomonas sp.*) (10) and many other substances synthetized by eukaryotic cells: micotoxins, indoles, flavonoids, *etc*.

1.1. Antibiotics that inhibits the synthesis of cellular wall

After the discovery of penicillin by Fleming in 1929, a large number of natural substances potentially related to this antibiotic were isolated, they having common chemical structure (are β–lactams) and mode of action (inhibition of bacterial cell wall synthesis). Although penicillin was found to be synthesized by fungi, was proved that natural substances with potential antibiotic similar and can be summarized by some species of Streptomyces as: *S. clavuligerus, S. lipmanii, S. lactamgens, S. cattleya* (11).

There were also synthetized β–lactams and other bio compounds that affect the functions of plasmatic membrane. Such an example is nistratin, antifungal synthetized by some species of *Streptomyce*. Another group is represented by polymyxins, bacitracins and gramicidin, substances with antibiotic potential polypeptides synthesized by bacteria called *Bacillus* (*B. polymixa, B. licheniformis, B. brevis*). That bio compounds increases plasma membrane permeability which results in massive output of amino acids or of derivatives of purine and pyrimidine bacteria, ultimately leading to cell death. Bacitracin acts on cell wall synthesis, resulting in inhibition of peptidoglycan biosynthesis of Gram–negative bacteria.

1.2. Antibiotics that inhibit protein synthesis

Of natural substances with antibiotic potential affecting protein synthesis, an important place is occupied by aminoglycosides, tetracycline and chloramphenicol; all three groups were synthesized by species of the genus *Streptomyces.* Aminoglycosides are an important group of natural substances with antibiotic potential that have the main component some saccharidess. The first antibiotic in this group was discovered in 1944 is streptomycin synthetized by *S. griseus*, after which other aminoglycosides were isolated from bacteria belonging to the genera mainly *Streptomyces* and *Micromonospora*: gentamicin synthetized by species of *Micromonospora*, neomycin synthesised by *S. fradiae*, ribostamycin synthesised by *S. ribosidificus*, kanamycin biosynthesised by de strains of *S. kanamyceticus*, butirosin

synthetized by *B. circulans etc*. (12). Aminoglycosides are substances with antibiotic potential with a large spectrum of action used for treatment of some infections produced by strains of *E. coli, Klebsiella sp., Proteus sp., Enterobacter sp.*

There are experimental dates that shows the importance of ribosomes as most important that determine the protein synthesis. The effects of aminoglycoside on bacteria are considered the consequence of their interaction with ribosomes.

Together with the interference with protein synthesis, aminoglycoside are inducing damage to plasmatic membrane, affects cellular respiration RNA accumulation and leads ultimately cell death. Regarding the mechanism of bacterial resistance to the action of these substances synthesizers potential antibiotic has shown that they have different strategies such as the production of enzymes that inactivate the antibiotic or change situs of action (13).

Bacteria synthesizers of aminoglycosides are synthesised also and enzymes that modify antibiotic substances potential: aminoglycoside phosphotransferase enzyme (APH) and acetyltransferases (AAC) (14).

Those bacteria (*S. kanamyceticus, S. tenebrarius*) can synthetize all enzymes that change situs of substances with antibiotic potential (eg ribosomal situs) by methylation action of RNAr.

Tetracycline represent an heterogenic group of substances with antibiotic potential synthesised especially by bacteria from type *Streptomyces* that they have in common the same base chemical structure (15), but are different according to the chemical groups (16) that bound to this structure: oxitetracycline (synthetized by *S. rimosus*), tetracycline (synthetized by *S. viridofaciens*), chlortetracycline (bio synthetized by *S. aureofaciens*). Tetracycline present a large spectrum of action being bacteriostatic for different Gram positive and Gram negative bacteria (exception strains of *Salmonella, Proteus* and *Pseudomonas*) and bactericides in big concentrations (17).

The main target of action of that substances with antibiotic potential protein synthesis, because don't have obvious effects on the DNA, RNA or cellular wall. It seems that there is a strong bound of the de subunit 70S of ribosomes that determine the inhibitor effect of the natural substance antibiotic potential (18).

Tetracycline has a weak link with the subunit30S that affects the blocking of connection aminoacil–RNAt to this subunit and longer polypeptidic chains. Self-resistance of synthesizer bacteria can include also the changing of normal target situs, intracellular inactivation of the antibiotic or quick elimination. Chloramphenicol is a compound with antibiotic potential, with a large action spectrum, that was isolated in 1947 from filtrate of culture of *S. venezuelae*.

The action of chloramphenicol is that it blocks RNA_m connected at ribosomes that has as result inhibition of protein synthesis (forming peptide bound) (19).

1.3. Antibiotics that inhibits replication and transcription the genetic information

From all natural substances with antibiotic potential that prevent DNA replication and synthesis of RNAm we can mention the novobiocins, coumermicin and rifamycins.

Novobiocin is a natural substance with antibiotic potential and a complex structure syntetised by *S. sphaeroides* with bacteriostatic effect over the bacterias, especially from acid environment.

Similar as action is coumermicin A, a natural substance with antibiotic potential isolated from cultures of *S. rishinensis* and *S. hazeliensis* (20). At smaller concentrations of novobiocin and cumermicin A the only affected is replication of DNA from sensitive cells, not the transcription process. The two natural substances with antibiotic potential can affect the action of ADN girase, enzyme that determine over twisting of DNA molecule, that explain the replication inhibition of transcription genetic information (21).

The resistance at novobiocin of the synthesis bacteria *S. sphaeroides* is determined of the gene *gyr B* with its product, DNA girase B resistant to the action of the antibiotic (22). Rifamycins, with the represented typically rifampicin is representing a group of natural substances with l antibiotic potential macrocyclic that acts, over mycobacteria and Gram positive bacteria. There mechanism of action is about inhibition of transcription of genetic information by blocking the action of RNA–polymerase enzymes. Another antibiotic with inhibitory–restrictive of action over the nucleic acids is granaticin, quionic antibiotic, isolated from cultures of *S. olivaceus* and *S. violaceoruber*.

His „in vitro" action is manifested, at prokaryotes and eukaryotes, over the synthesis of DNA, RNA and proteins.

Granaticin has antitumoral effects in leukemia L1210 and P388 and cytotoxic effect for cells KB (23).

2. Actions on living cells

Many natural substances with potential antibiotic not used in clinical practice due to their toxic action on animal cells.

The toxic effects resulting from direct action on one of the primary metabolism pathways, pathways that are universal on the energy or gene expression. Toxicity to animal cells do not always result in the same reaction inhibition is target organisms. Amphomycin that interfere with membrane transport intermediaries murein from bacteria to eukaryotic cells causes a block glycosylation. Some natural substances with potential antibiotic activity showed a growth inhibition of several types of organisms began to be used as antitumor substances due to their toxicity on the cells that proliferate rapidly. Among these substances occupy an important place anthracyclines and bleomycin, substances synthesized by species of the genus *Streptomyces* (24).

Originally discovered as antibacterial agents, it turned out that the two families of substance binds specifically by intercalation, the DNA molecules and bleomycin and cause cleavage of DNA chains. Selective cytotoxicity of

bleomycin resulting in decreased activity of an enzyme inactivation of tumor cells. Another type of enzyme inhibitors produced antimicrobial or antitumor activity is represented by small protein, containing a chromophore prosthetic group that intercalate in DNA macromolecules, changing their structure (25). Some collateral metabolites show specific pharmacological animal, in which case they do not have a general cytotoxicity, but their pharmacological action may sometimes be extremely toxic. An example is a vasodilator action bio compounds isolated from *S. aureofaciens* but this is not accompanied by toxicity and antibiotic activity.

Another bio compounds MY–336A, isolated from cultures of *S. gabonae* exerts antagonistic action of β–adrenergic receptors with a pharmacological action so selective, nontoxic (26).

In case of amicourmamycin A isolated from *B. pumilus* was initially stated an antibiotic action and after was proved to have also anti-inflammatory action on tested animals (27). Among bio substances initially isolated by screening for antibiotic activity experiments were found some who act to modulate the immune response.

For example, IC201 substance isolated from *S. fimbriatus and S cirratus* as antitumor agent has been shown to have activating and action on macrophages. Another bio compounds, macrolides FK 506 isolated from *S. tsukubaensis* during selection experiments of substances that inhibit the production of interleukin–2 has been shown to have immunosuppressive action (28).

2.1. Insecticidal Activity of Actinomycetes

It was showed that many collateral metabolites produced by streptomyces in particular have insecticidal action.

Some of them, like some natural collateral substances with potential macrolides antibiotic polienic, blocks cellular respiration or inhibit protein synthesis in eukaryotes, thus nonspecific agents. Other collateral metabolites, such nikkomycin (group of peptidyl nucleoside antibiotics with potent fungicidal, insecticidal and acaricidal activities) have specific action inhibiting chitin syntheses and thus act on the chitinous cell walls at insects (29).

Mibelmycin (another antibiotic), synthetized by Streptomyces hygroscopicus subsp. *Aureolacrimosus* does not have antibacterial effect but acts selective on insects with also anthelmintic activity and acaricide effect. In one study, were isolated after a complex process of selection over actinomycete strains cultures, a new substance with an insecticide and acaricide mode of action (30).

An insecticide action is made possible by the biosynthesis of one bacterial strain (with chitin hydrolysis activity) of chitinase that acts directly over the pathogen insect coatings (produce antimicrobial peptides known as defensins and cecropins). Microbial collateral metabolites also exhibit all right herbicide and pesticide activities and are biodegradable.

Though, microbial herbicides and pesticides only rarely used (e.g. bialaphos) due to their high price. Biological control is slow but can be long-drawn, low-priced, and inoffensive to living organisms and the ecosystem; it neither eliminates the pathogen nor the disease, but brings them into normal balance. On the other part stereptomyces collateral metabolites not only effective against insect but may also boost the insect yourselves from other microbial pathogen and other insect as in wasps which cultures a strain of antibiotic–producing *S. philanthi* within experienced glands on her antenna. *S. philanthi* then excrete antibiotics in to the cocoons, protecting the beewalf larvae from harmful pathogen.

3. Activity on vegetal cells

Some collateral bio compounds products from bacteria of type *Streptomyces* have phytotoxic and antifungal action. Herbicides synthetized by *S. Sagonensis* are nucleosides with phytotoxic action for dicotyledonous plants (31).

They inhibit the fungal development. Another substances as herbimycin synthetized by *S. hygroscopicus* have small antibiotic action, instead are toxic for vegetal cells. Herbimycin A inhibition development of tabacco mosaic virus and has antitumoral action for animal cells (32).

Another substance, homoalanosin, synthetized by *S. galileus* is an antimetabolite aspartic acids and glutamic acid, being applied insecticide and herbicides (33; 34). Many researchers have published data on species belonging to the genus *Bacillus* ability to inhibit the growth of phytopathogenic fungi (35).

In most cases biological combat of fungi involves using biotic facts from environment. Studies have shown the capacity of some strains of *Bacillus subtilis* to combat different phytopathogenic fungi, like: *Macrophomina phaseolina*–pathogen at peas (36), *Rhizoctomia solani*–pathogen at peas (37), beans and wheat seeds (38), *Fusarium oxysporum* and *Phytyum ultinacere*–pathogens at tomato (39).

The most common species of the genus Bacillus have shown the ability to prevent and combat the development of diseases caused by phytopathogenic fungi is: *Bacillus subtilis, Bacillus licheniformis, Bacillus polymyxa, Bacillus cereus*. Studies on the suitability of these bacterial species in biological control were based on the property to synthesize high boost resistance and longevity in natural environmental conditions, the resulting possibility of obtaining and marketing in a manner similar to chemical fungicides.

In the fight for micro nutrients available to an arsenal of chemicals that inhibit bio compounds competitive organizations. Many of these bio compounds in origin and can be synthesized ribosomal peptide or nonribozomal.

Many of *Bacillus subtilis* strains produce a number of substances such lipo oligopeptidic, iturin range, with antifungal activity, hemolytic and natural substances with potential antibiotic properties.

Chemical structure of these substances was determined by various methods {mass spectrometry (MS), gel chromatography, thin layer chromatography (TLC), liquid chromatography (HPLC).

Protein component of these substances is the oligopeptides composed of seven amino acids and lipid component containing long carbon chain fatty acids (C_{14}–C_{16}) (40). Thus, iturin AL content the heptapeptide (Asn–Tyr–Asn–Gln–Pro–Ser–Asn) and mixture of β–amino *fatty acid* chain can vary from C_{14}–C_{16}. Iturin D and iturin E content the heptapeptide (3 Asp, 1 Glu, 1 Pro, 1 Ser, 1 Tyr) and mixture of β–amino fatty acid chain can vary from C_{14}–$C_{16,}$ differing from iturin A through the presence of carboxyl groups at iturin D and a carboxymethyl group at iturin E. Mycosubtilin (and/or bacillomysin), is a substance from iturin family, contain a cyclic heptapeptide (Asn, Tyr, Asn, Gln, Pro, Ser, Asn) linked to a β–amino fatty acid.

Also surfactin and fengycin, for the iturin family, contain a β–hydroxy fatty acid (lipopeptides) and bacillomycin contain a β–amino fatty acid. More intensive studies were performed with a substance synthesized by several species of *Streptomyces (S. hygroscopicus, S. viridochromogenes)* and were named phosphinothricyl–alanyl-alanine (PTT), also known as bialaphos. This substance was initially selected for its antibiotic activity after which been observed she has in fact a far more complex action. The active component of phosphinothricyl–alanyl-alanine is phosphinothricin, which inhibitor of glutamine synthetase activity of vegetal plants, giving it a strong herbicidal action (41).

Many of metabolites (biocompounds collateral) produced by various microorganisms were tested for their possible action on enzymes. When the system assay involving key enzymes in pharmacological processes from animals, many secondary metabolites have been detected as with physiological activity "in vivo".

Many of those biocompounds have been found to be protease inhibitors, variously active against pepsin, papain, trypsin, chymotrypsin, catepsin, elastase, renin, aminopeptidase B, leucine aminopeptidase *etc*. (42). Among the protease inhibitors isolated from actinomycetes some of acting on the renin or on a zinc exopeptidase converting angiotensin to angiotensin II. The research carried out on microbial cultures has allowed isolation of inhibitors and other enzymes. This applies for glucosidase inhibitors (43), for cAMP, kinase *etc*.

4. Final remarks

The information in this paper reflect the particularities of bacteria and the importance they have both theoretical and practical, is to obtain compounds with applications in medicine, food, detergents *etc*. or in kind, as important links of the mechanisms of degradation and recycling of organic waste typically existing in soil.

Knowing their equipment enzyme biosynthesis of their potential as its genetic determinism and modern techniques related to those of bioassay and cell provides a good study of the characteristics of these bacteria, including taxonomy of the group and lead to obtaining recombinant strains genetic able to catabolize or anabolic with increased efficiency bio compounds with some practical importance.

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