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Production and Purification of Tetracyclin by *Streptomyces Aureofaciens* Using Banana Peel.

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I. INTRODUCTION

The word antibiotic comes from the Greek anti meaning 'against' and bios meaning 'life' (a bacterium is a life form).' They are also known as antibacterial, and they used to treat infections caused by bacteria. Bacteria are tiny organisms that can sometimes cause illness to humans and animals. Such illnesses as tuberculosis, salmonella, syphilis and some forms of meningitis are caused by bacteria. Some bacteria are not harmful, while others are good for us.

Antibiotics may be informally defined as the subgroup of anti-infective that are derived from bacterial sources and are used to treat bacterial infections. Other classes of drugs, most notably the sulfonamides, may be effective antibacterial. Similarly, some antibiotics may have secondary uses, such as the use of demeclocycline (Declomycin, a tetracycline derivative) to treat the syndrome of inappropriate antidiuretic hormone (SIADH) secretion. Other antibiotics may be useful in treating protozoal infections.

1.2 Tetracycline

It is a broad-spectrum polyketide antibiotic produced by the *Streptomyces* genus of Actinobacteria, indicated for use against many bacterial infections. It is a protein synthesis inhibitor. It is commonly used to treat acne today, and, more recently, rosacea, and played a historical role in reducing the incidence of mortality because of cholera. It is marketed under the brand names **Sumycin**, **Terramycin**, **Tetracyn**, and **Panmycin**, among others. **Actisite** is a thread-like fiber form, used in dental applications. It is also used to produce several semi synthetic derivatives, which together are known as the tetracycline antibiotics.

Among the enzymes participating in the biosynthesis of tetracycline's, three have been described so far:S-adenosylmethionine dedimethylamino-4-aminoanhydrotetracycline N-methyltransferase (Miller& Hash, 1975a), anhydrotetracycline (ATC) oxygenase (Behal et al., 1979) and NADP:tetracycline 5a(lla)-dehydrogenase (Miller & Hash, 1975b; Erban et al., 1985). None of them has as yet been isolated in pure state.
Dried soil, a great source of inoculum for Streptomyces

1.3 APPLICATIONS OF TETRACYCLINES

- **Administration and Pharmacokinetic Behavior in Humans :**

Tetracyclines are usually administered orally, although some are also available as parenteral products . Rolitetracycline is available only as a parenteral product. The ability to use either oral or parenteral formulations of doxycycline has been used advantageously to permit switching programs from intravenous to oral administration

The dosing regimens and pharmacokinetic properties of the tetracyclines have been extensively reviewed in previous publications .Therefore only a brief summary is provided here. Absorption following oral administration occurs largely in the stomach and proximal small intestine and is influenced by the presence of food, milk, or divalent cations, particularly, calcium, with which tetracyclines form nonabsorbable chelates. Levels achieved in serum after normal oral dosing are in the range of 2 to 5 µg/ml, and most tetracyclines have to be given four times daily to maintain therapeutic concentrations in the serum.

However, the long elimination half-lives of doxycycline and minocycline permit once- or twice-daily dosing. Tetracyclines generally penetrate moderately well into body fluids and tissues and are excreted in the urine. For

instance, levels in sputum about 20% of those in serum, can be achieved, which explains why the tetracyclines have a role in the treatment of respiratory tract infections. Tetracyclines also penetrate into the sebum and are excreted in perspiration, properties which contribute to their usefulness in the management of acne.

- **Human Therapy and Prophylactics**

Although 9-(*t*-butylglycylamido)-minocycline, a third-generation compound, is currently undergoing clinical trials, it is now nearly 30 years since the last tetracycline, minocycline, was introduced . During this period, as already discussed, there have been increases in the incidence of bacterial resistance to the tetracyclines and in availability of more active and better tolerated agents from different antimicrobial classes. Consequently, in recent years the clinical use of tetracyclines has significantly declined in most countries since they are no longer drugs of choice in many instances .

However, in other cases new applications have been identified. For instance, tetracycline has been used as part of a triple therapy for management of gastritis and peptic ulcer disease associated with *Helicobacter pylori*. Although omeprazole, clarithromycin, and amoxicillin (or metronidazole) are standard therapy, the role of tetracycline may increase as more clarithromycin- and methronidazole-resistant *H. pylori* isolates are encountered . Tetracyclines are active against malaria, and this has unexpectedly become important for prophlylaxis following the rapid increase of mefloquine-resistant *P. falciparum* strains.. These antibiotics have also been evaluated for their potential in other situations. However, such applications may not necessarily gain widespread acceptance or become components of standard therapeutic regimens. For instance, on the basis of limited clinical evaluation, Ji et al. consider that minocycline, in combination with ofloxacin, may have a role in the treatment of leprosy. Although larger clinical trials would be needed to establish benefit, it seems likely that a combination of minocycline and ofloxacin could provide the opportunity for supervised monthly administration of these antibiotics, thereby greatly improving patient compliance.

- **Veterinary Medicine**

The tetracyclines have applications for the treatment of infections in poultry, cattle, sheep, and swine. In some cases, e.g., for therapeutic treatment of large numbers of poultry reared on commercial farms, the antibiotics are added directly to feed or water or can be administered in aerosols. The use of tetracyclines in the rearing of farm animals has been reviewed in recent years and readers are referred to these earlier papers for details. Tetracyclines are also used for treatment of infections in domestic pets .

- **Animal Growth Promoters**

Antibiotics represent one of the few classes of drugs that can be used in food animals both therapeutically to treat disease and subtherapeutically, usually over long periods, to improve their rate of growth and feed conversion efficiency. The practice of adding low concentrations of antibiotics, defined in the United States as < 200 g/ton of feed, to animals to improve growth and feed efficiency is referred to as growth promotion or growth enhancement . An obvious outcome of this practice is that animals need less food to reach marketable weight. The mechanisms responsible for growth promotion have not been fully elucidated but appear to include enhancement of vitamin production by gastrointestinal microorganisms, elimination of subclinical populations of pathogenic organisms, and increased intestinal absorption of nutrients .

The growth-promoting properties of tetracyclines were discovered in 1949, when it was observed that low levels of chlortetracycline in livestock rations beneficially affected the rate of growth and feed utilization by young chickens. The initial observations in chickens were confirmed and soon extended to swine and cattle, leading to the development of both chlortetracycline and oxytetracycline as animal growth promoters. In the United States these antibiotics were approved by Food and Drug Administration as feed additives in 1951 (chlortetracycline) and 1953 (oxytetracycline).

Increasing concerns about growth promoters followed the publication in the United Kingdom of the Swann report in 1969. This report, suggesting that subtherapeutic application of tetracyclines and other antibiotics to farm animals might contribute to the development of resistant human isolates, led to a ban of the use of tetracyclines for growth promotion in Europe in the early 1970s (EC directive 70/524;. Although issues surrounding the use of growth-promoting antibiotics have been widely discussed in other countries, particularly the United States , and Australia, no such ban has been imposed on the use of tetracyclines for growth promotion in these and many other countries.

- **Other Uses**

Tetracyclines are used in aquaculture to control infections in salmon, catfish, and lobsters . They are also sprayed onto fruit trees and other plants to treat infection by *Erwinia amylovara*, injected into palm trees to treat mycoplasma infections (lethal yellow), and used to control infection of seeds by *Xanthomonas campestis* (black rot). They also have applications in the treatment of insects of commercial value; e.g., oxytetracycline is

used to treat foulbrood disease of the honeybee, which is caused by either *Bacillus larvae* or *Streptococcus pluton*

1.4 BANANA

Banana is the common name for herbaceous plants of the genus *Musa* and for the fruit they produce. Bananas come in a variety of sizes and colours when ripe, including yellow, purple, and red. Almost all modern edible parthenocarpic bananas come from the two wild species *Musa acuminata* and *Musa balbisiana*. The scientific names of bananas are *Musa acuminata*, *Musa balbisiana* or hybrids *Musa acuminata* × *balbisiana*, depending on their genomic constitution. The old scientific names *Musa sapientum* and *Musa paradisiaca* are no longer used.

Elaichi Bananas

Its Called Elakki Bale (baaley or baahlay), these bananas are very common in most parts of Karnataka and is similar in taste to a variety called njaali-poovan in Kerala. These are usually very small, 1.5 to 4 inches, and very sweet & tasty.

Common name : Mysore cardamom seeds, Malabar cardamom, ebil, kakelah seghar, capalaga, gujalatti elachi, ilachi, ailum, amomum cardamomum, A. repens, Alpina Cardamom, matonia cardamomum, cardamomum minus, Cardamomi Semina

Composition of Bananas

Bananas are rich sources of vitamins, minerals, sugar and other nutrients. Compared to an apple, a banana has four times more protein, twice as much carbohydrate, thrice as much phosphorous, five times vitamin A and iron, and twice as much of vitamins and minerals.

- Apart from vitamins A and C, there are large amounts of the vitamin B group: thiamin, riboflavin, niacin and B6. Some folic acid is also present.
- Bananas have essential minerals like sodium, potassium, calcium and iron. They are particularly rich in potassium.
- Bananas contain proteins, including three essential amino acids and Tryptophan. The latter is a protein which is converted in the body to serotonin, a neurotransmitter.
- Carbohydrates and natural fiber are also found in bananas.
- They have large amounts of easily digestible sugars.

USES

- Healing Power and Curative Properties of Cardamom

The aroma and therapeutic properties of cardamom are due to its volatile oil. Tinctures of cardamom are used chiefly in medicines to relieve flatulence and for strengthening digestion activities**.**

Digestive Disorders : Cardamom reduces the air and water elements, increases appetite and soothes the mucous membrane. It relieves gas and heart-burn caused by garlic and onion. Ground cardamom seed mixed with ginger, cloves and coriander, is an effective remedy for indigestion. A tea made from cardamom is valuable in headache caused by indigestion.

Bad Breath : The aromatic flavour in cardamom is a breath freshener. A . few seeds chewed for a brief period will remove foul smell.

Genito-Urinary Disorders. : Its powdered seeds mixed with a tablespoon of banana leaf and *amla* juice taken thrice a day, will serve as an excellent diuretic for the treatment of gonorrhoea, cystitis, nephritis, burning micturation or urination and scanty urination.

Depression : Powdered seeds of cardamom are boiled in water with tea. It gives a very pleasing aroma to the tea. This can be used as a remedy in the treatment of depression.

Impotency : The herb is useful in sexual dysfunctions like impotency and premature ejaculation. A pinch of powdered cardamom seeds boiled in milk and sweetened with honey every night would yield excellent results. Excessive use of cardamom at times may lead to impotency.

Oral Disorders : Gargling with an infusion of cardamom and cinnamon cures pharyngitis, sore-throat, relaxes uvula, or the fleshy conical portion at the back of the tongue, and hoarseness during the infective stage of influenza. Its daily gargle protects one *from* the flu.

Other Uses and benefits of Cardamom

Hiccups: An infusion made by boiling a couple of pounded whole cardamoms in a cup of water along with 5 leaves of mint is useful in relieving hiccups.

In India , cardamom is used as masticatory and often included in *pan-supari.* It is used for flavoring curries, cakes, bread and for other culinary purposes, like flavouring coffee or confectionery.

The essential oil of cardamom is used for pharmaceutical purposes, perfumery, flavouring liquers and bitters, in the preparation of tincture and as a stimulant..
Elaichi banana (local name : elakki bale) : elaichi banana is small variety of banana that is dwarf size like 3-4 inches(half the size of big banana),,and is often sweeter, more pulpier and fresh ones have distinct sweet smell.

RASA BALE HANNU
The *rasa bale hannu* a variety of banana, native to Karnataka state, India
Antibiotic tetracycline is produced using banana peels of ELAKKI BALEHANNU and RASA BALEHANNU. These are one of the types of banana mostly grown in South India.

1.5 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY - HPLC
High performance liquid chromatography is a powerful tool in analysis. This page looks at how it is carried out and shows how it uses the same principles as in thin layer chromatography and column chromatography.

Introduction
High performance liquid chromatography is basically a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster.
It also allows you to use a very much smaller particle size for the column packing material which gives a much greater surface area for interactions between the stationary phase and the molecules flowing past it. This allows a much better separation of the components of the mixture.
The other major improvement over column chromatography concerns the detection methods which can be used. These methods are highly automated and extremely sensitive.

The column and the solvent
Confusingly, there are two variants in use in HPLC depending on the relative polarity of the solvent and the stationary phase.
Normal phase HPLC
This is essentially just the same as you will already have read about in thin layer chromatography or column chromatography. Although it is described as "normal", it isn't the most commonly used form of HPLC. The column is filled with tiny silica particles, and the solvent is non-polar - hexane, for example. A typical column has an internal diameter of 4.6 mm (and may be less than that), and a length of 150 to 250 mm. Polar compounds in the mixture being passed through the column will stick longer to the polar silica than non-polar compounds will. The non-polar ones will therefore pass more quickly through the column.

Reversed phase HPLC
In this case, the column size is the same, but the silica is modified to make it non-polar by attaching long hydrocarbon chains to its surface - typically with either 8 or 18 carbon atoms in them. A polar solvent is used - for example, a mixture of water and an alcohol such as methanol.
In this case, there will be a strong attraction between the polar solvent and polar molecules in the mixture being passed through the column. There won't be as much attraction between the hydrocarbon chains attached to the silica (the stationary phase) and the polar molecules in the solution. Polar molecules in the mixture will therefore spend most of their time moving with the solvent. Non-polar compounds in the mixture will tend to form attractions with the hydrocarbon groups because of van der Waals dispersion forces. They will also be less soluble in the solvent because of the need to break hydrogen bonds as they squeeze in between the water or methanol molecules, for example. They therefore spend less time in solution in the solvent and this will slow them down on their way through the column. That means that now it is the polar molecules that will travel through the column more quickly. Reversed phase HPLC is the most commonly used form of HPLC.

The whole process
Injection of the sample
Injection of the sample is entirely automated, and you wouldn't be expected to know how this is done at this introductory level. Because of the pressures involved, it is *not* the same as in gas chromatography (if you have already studied that).
Retention time
The time taken for a particular compound to travel through the column to the detector is known as its ***retention time***. This time is measured from the time at which the sample is injected to the point at which the display shows a maximum peak height for that compound.
Different compounds have different retention times. For a particular compound, the retention time will vary

depending on:

- the pressure used (because that affects the flow rate of the solvent)
- the nature of the stationary phase (not only what material it is made of, but also particle size)
- the exact composition of the solvent
- the temperature of the column

That means that conditions have to be carefully controlled if you are using retention times as a way of identifying compounds.

The detector

There are several ways of detecting when a substance has passed through the column. A common method which is easy to explain uses ultra-violet absorption. Many organic compounds absorb UV light of various wavelengths. If you have a beam of UV light shining through the stream of liquid coming out of the column, and a UV detector on the opposite side of the stream, you can get a direct reading of how much of the light is absorbed. The amount of light absorbed will depend on the amount of a particular compound that is passing through the beam at the time. You might wonder why the solvents used don't absorb UV light. They do! But different compounds absorb most strongly in different parts of the UV spectrum. Methanol, for example, absorbs at wavelengths below 205 nm, and water below 190 nm. If you were using a methanol-water mixture as the solvent, you would therefore have to use a wavelength greater than 205 nm to avoid false readings from the solvent.

1. ***OBJECTIVES***

- Isolation and identification of streptomycin aureofacians.
- Solid state fermentation using 2 types of BANANA PEEL (*Elettaria Cardamomum* bale and rasa bale) for tetracycline antibiotic.
- Purification and analysis by HPLC

II. REVIEW LITERATURE

ANTIBIOTICS

A broad-spectrum antibiotic can be used to treat a wide range of infections. A narrow-spectrum antibiotic is only effective against a few types of bacteria. There are antibiotics that attack aerobic bacteria, while others work against anaerobic bacteria. Aerobic bacteria need oxygen, while anaerobic bacteria don't (***Lindblad WJ ,2008***)

. Although antibiotics are useful in a wide variety of infections, it is important to realize that antibiotics only treat bacterial infections and are useless against viral infections and fungal infections such as ringworm(***Forrest RD (March 1982***).

Although there are well over 100 antibiotics, the majority come from only a few types of drugs. The main classes of antibiotics are, penicillins such as penicillin and amoxicillin ,Cephalosporins such as cephalexin (Keflex) ,Macrolides such as erythromycin (E-Mycin), clarithromycin (Biaxin), and azithromycin (Zithromax), Fluoroquinolones such as ciprofloxacin (Cipro), levofloxacin (Levaquin), and ofloxacin (Floxin) ,Sulfonamides such as co-trimoxazole (Bactrim) and trimethoprim (Proloprim), Tetracyclines such as tetracycline (Sumycin, Panmycin) and doxycycline (Vibramycin) ,Aminoglycosides such as gentamicin (Garamycin) and tobramycin (Tobrex)(***Sykes R ,2001***).

Agents that kill or inhibit microorganisms may be classified as disinfectants, antiseptic or antibiotics. Antibiotics are m molecules that are produced by one microorganism that kill (bactericidal) or inhibit (bacteriostatic) other microorganisms (***Dor lands Medical Dictionary:antibacterial". Archived from the original on 2010-11-17***).

On the whole, the last 55 years have seen the discovery of more than 12,000 antibiotics.
The actinomycete yielded about 70 % of these, and the remaining 30 % are products of filamentous fungi and non-actinomycete bacteria. Most of the bioactive compounds from actinomycete sort into several major structural classes such as amino glycoside (e.g., streptomycin and kanamycin), ansamycins(e.g., rifampin, adoxorubicin), b-lactam(cephalosporins), macrolides(e.g.,erythromycin),and tetracycline(***Waksman SA. The Actinomycetes. 1st edition,Watham, MASS, USA, 185-191)***

Antibiotics are the best known products of actinomycete. Over 5,000 antibiotics have been identified from the cultures of Gram-positive and Gram-negative organisms, and filamentous fungi, but only about 100 antibiotics have been commercially used to treat human, animal and plant diseases. The genus, Streptomycete, is responsible for the formation of more than 60 % of known antibiotics while a further 15 % are made by a number of related Actinomycetes (***Basavaraj K Nanjwade et al. May-June, 2010)***

Antibiotics are among the most frequently prescribed medications in modern medicine. They were used to cure disease by killing or injuring bacteria. The first antibiotic was penicillin, discovered accidentally from a mold culture. Today, over 100 different antibiotics are available to doctors to cure minor discomfort as well as life-threatening infections (***Everett Stephens,2000***).

Although there are a number of different types of antibiotic they all work in one of two ways:
A bactericidal antibiotic kills the bacteria. Penicillin is a bactericidal. A bactericidal usually either interferes with the formation of the bacterium's cell wall or its cell contents,A bacteriostatic stops bacteria from multiplying (***von Nussbaum F. et al. 2006***).

To create a drug, nature's blueprints often have to be improved through semisynthesis or total synthesis (chemical postevolution). Selected contributions from industrial and academic groups highlight the arduous but rewarding path from natural products to drugs. Principle modification types for natural products are discussed herein, such as decoration, substitution, and degradation. The biological, chemical, and socioeconomic environments of antibacterial research are dealt with in context. Natural products, many from soil organisms, have provided the majority of lead structures for marketed anti-infectives. Surprisingly, numerous “old” classes of antibacterial natural products have never been intensively explored by medicinal chemists. Nevertheless, research on antibacterial natural products is flagging. Apparently, the “old fashioned” natural products no longer fit into modern drug discovery. The handling of natural products is cumbersome, requiring nonstandardized workflows and extended timelines. Revisiting natural products with modern chemistry and target-finding tools from biology (reversed genomics) is one option for their revival.(***Franz von Nussbaum Dr. et al 31 JUL 2006***)

Natural products have provided the majority of lead structures for marketed antibacterials. In addition, they are biological guide principles to new therapies. Nevertheless, numerous "old" classes of antibiotics such as the longicatenamycins have never been explored by chemical postevolution. Longicatenamycin A is the first defined longicatenamycin congener that has been totally synthesized and tested in pure form. This venture required the de novo syntheses of the non-proteinogenic amino acids (2S,3R)-beta-hydroxyglutamic acid (HyGlu), 5-chloro-D-tryptophan (D-ClTrp), and (S)-2-amino-6-methylheptanoic acid (hhLeu). In the key step, the sensitive HyGlu building block was coupled as a pentafluorophenyl active ester to the unprotected H-D-ClTrp-Glu-hhLeu-D-Val-D-(Cbz)Orn-OH fragment. This first total synthesis of longicatenamycin A provided new congeners of the natural product (deacetyllongicatenamycin, dechlorolongicatenamycin, and longicatenamycin-A-amide).
(***von Nussbaum F, et al 2008 Apr***)

TETRACYCLIN

The tetracyclines are a family of polyketide antibiotics produced by *Streptomyces* genus of actinobacteria. The different form of tetracyclines are tetracycline, chlortetracycline demeclocycline, oxytetracycline and other most active types. These antibiotics are broad-spectrum in nature. At present, reports on the clinical use of tetracyclines have been generally confined to respiratory tract infection, sinuses, middle ear infection, urinary tract infections, intestinal infection and also gonorrhoea (***Chopra and Howe, 1978 and speer 1992***).

The tetracyclines are a family of broad-spectrum antibiotics which inhibit protein synthesis by preventing the binding of amino acyl transfer ribonucleic acid to the bacterial ribosome.
Although a large number of tetracycline derivatives are now known ,relatively few have found extensive clinical use apart from tetracycline itself, chlortetracycline, oxytetracycline, and, more recently, doxycycline and minocycline (for the structures of these compounds, see reference. These tetracyclines provide safe, inexpensive, and effective treatment for many bacterial infections and are also used for antimicrobial prophylaxis. However, the emergence of tetracycline-resistant bacteria has considerably reduced the usefulness of these antibiotics. (***I. CHOPRA AND T. G. B. HOWE 1978***)

The production of tetracycline by fermentation was disclosed by Minieri et al. (1953).

It is capable of producing at least two antibiotic substances as pointed out by Backus et al. (1954). The simultaneous production of two or more antibiotics in a fermentation is well known, and the substances formed may be either closely related on a chemical or a biological basis or widely separated. Typical examples of closely related compounds produced simultaneously by the same organism are the penicillin's (Clarke, 1949), streptomycins (Waksman, 1949), polymyxins (Brownlee, 1949), bacitracins (Newton and Abraham, 1950), cephalosporins (Crawford et al., 1952), nisins (Berridge et al., 1952), neomycins (Waksman, 1953), rhodomycins (Brockmann et al., 1951), and candicidins (Lechevalier et al., 1953).

Tetracyclines were discovered in the 1940s and exhibited activity against a wide range of microorganisms including gram-positive and gram-negative bacteria, chlamydiae, mycoplasmas, rickettsiae, and protozoan parasites. They are inexpensive antibiotics, which have been used extensively in the prophlylaxis and therapy of human and animal infections and also at subtherapeutic levels in animal feed as growth promoters. The first tetracycline-resistant bacterium, Shigella dysenteriae, was isolated in 1953. Tetracycline resistance now occurs in an increasing number of pathogenic, opportunistic, and commensal bacteria. The presence of tetracycline-resistant pathogens limits the use of these agents in treatment of disease. (***Ian Chopra and Marilyn Roberts et al, Jun 2001***)

Biological wastes contain several reusable substances of high value such as soluble sugars and fibre. Direct disposal of such wastes to soil or landfill causes serious environmental problems. Thus, the development of potential value-added processes for these wastes is highly attractive. These biological wastes can be used as support-substrates in solid-state fermentation (SSF) to produce industrially relevant metabolites with great economical advantage. In addition, it is an environmentfriendly method of waste management. Biological wastes for the production of value-added products using the SSF technique.(***Susana Rodríguez Couto Dr. 9 JUN 2008***)

Tetracyclines are broad-spectrum antibiotics, and the hydrocarbon derivatives of octahydronaphthacene with four annelated 6-membered rings. Submerged culture was usually used for tetracycline production, resulting in much energy input and waste water production.324I n addition, culture media and culture conditions would affect the kind and the quantity of antibiotic production. (***Shang-Shyng Yang and Meei-Yueh Ling , China , 1988)***

For saving energy in antibiotic production and reducing the amount of agricultural wastes, solid state fermentation was used in this study to produce tetracycline with sweet potato residue by Streptomyces viridifaciens ATCC 11989. It was found that the optimal media for tetracycline production were sweet potato residue 100 g, organic nitrogen (rice bran, wheat bran, or peanut meal) 20 g, (NH(4))(2)SO(4) 2.4 g, KH(2)PO(4) 0.4 g, CaCO(3) 1.8 g, NaCl 0.6 g, MgCl(2) 0.8 g, soluble starch 10 g, methionine 0.2 g, histidine 0.8 g, and monosodium glutamate 1.6 g with initial moisture content 68-72%, and initial pH 5.8-6.0. Each gram of dry weight substrate was inoculated with 1.0 x 10(8) conidia and incubated at 26 degrees C for 5-7 days, producing 4720 mug of total tetracycline equivalent potency. When incubated at 26 degrees C with the initial moisture content 68%, the conidia in solid media germinated on the second day, mycelia grew abundantly on the third day and reached stationary phase on the sixth day. The antibiotic production was consistent with the morphogenesis of S. viridifaciens: activity could be detected on the third day, had the maximal potency on the sixth day, and decreased slightly on the tenth day. (***Shang-Shyng Yang Meei-Yueh Ling*** 2004)

In the present study, we found that tetracyclines can be most effective in reducing alveolar bone loss when applied locally. The combined local treatment of alendronate and tetracycline may have a synergistic effect. (***Yaffe A***, ***Herman A, Bahar H, Binderman I. et al 2003 Jun***)

Previous in vitro studies have suggested that tetracycline-HCl (TTC-HCl) is adsorbed and actively released from root dentin. The aim of the current study was to evaluate the binding to and release of TTC-HCl from human root dentin surfaces in vivo, and to evaluate the clinical utility of TTC-HCl irrigation as an adjunct to scaling and root planing. (***Lars A. Christersson, Ola M. Norderyd ,Craig S. Puchalsky et al 13 DEC 2005***)

Tetracyclines are called "broad-spectrum" antibiotics, because they can be used to treat a wide variety of infections. Physicians may prescribe these drugs to treat eye infections, pneumonia, gonorrhea, Rocky Mountain spotted fever, urinary tract infections, certain bacteria that could be used in biological weapons, and other

infections caused by bacteria. The medicine is also used to treat acne. The tetracyclines will *not* work for colds, flu, and other infections caused by viruses. Tetracyclines are generally a low-cost alternative among antibiotics. (***Roberts, Marilyn C. February 15, 2003***)

Tetracycline applied with a microbrush may be an alternative treatment for persistent periodontitis that can probably be mediated by reduction of microorganism proliferation (***Bosco JM, Lopes BM, Bosco AF, Spolidorio DM, Marcantonio RA.et al 2009 Jan***)

STREPTOMYCES AUREOFACIANS

Pairs of non-tetracycline-producing mutants of *Streptomyces rimosus* or *S. aureofaciens* were grown side by side on agar. Their ability to produce antibiotic by cosynthesis was tested by placing a strip of agar cut from the combined culture on plates containing *Bacillus subtilis.* The activity was revealed as an inhibition halo formed on *B. subtilis,* opposite one or other mutant strain. The strain surrounded by the halo was considered as a converter of an intermediate product secreted by the other strain. Two types of mutants were observed: a rare type probably affecting the main pathway of antibiotic biosynthesis, and a more frequent type probably affecting some regulatory process. (***V. DELIĆ, JASENKA PIGAC and G. SERMONTI 1969).***

The ability of Streptomyces sp. OXCI, S. rimosus NRRL B2659, S. rimosus NRRL B2234, S. alboflavus NRRL B1273 S. aureofaciens NRRL B2183 and S. vendagensis ATCC 25507 to produce tetracycline using some local agricultural wastes as solid state media, were assessed. The wastes employed include peanut (groundnut) shells, corncob, corn pomace and cassava peels. Bacillus subtilis ATCC 6633 was used to assay antimicrobial activity. All the strains produced tetracycline in a solid-state fermentation process containing peanut (groundnut) as the carbohydrate source. Streptomyces sp. OXCI had the highest ability for tetracycline production with peanut shells as the substrate in solid fermentation (13.18 mg/g), followed by S. vendagensis ATCC 25507 (11.08 mg/g), S. rimosus NRRL B1679 (8.46 mg/g), S. alboflavus NRRL B1273 (7.59 mg/g), S. rimosus NRRL B2234 (6.37 mg/ g), S. aureofaciens NRRL B2183 (4.27 mg/g). Peanut (groundnut) shells were the most effective substrate (4.36 mg/ g) followed by corncob (2.64 mg/g), cassava peels (2.16 mg/g) and corn pomace (1.99 mg/g). The composition of the peanut (groundnut) shell medium optimal for tetracycline production were peanut shells 100 g, organic nitrogen (peanut meal) 10 g, $(NH_4)_2SO_4$ 1 g, KH_2PO_4 0.5 g, $CaCO_3$ 0.5 g, NaCI 0.5 g, $MgSO_4$. $7H_2O$ 0.5 g, soluble starch 10 g, peanut oil 0.25 ml with initial moisture content of 65-68%, and initial pH 5.3-6.3. Substrate (1 g dry weight) was inoculated with 1.0 x 10^8 conidia per ml and incubated at 28-31 °C for 5-7 days, producing 13.18 mg/g of total tetracycline. Tetracycline detection started on day 3 and attained its maximum level on day 5. (***ASAGBRA Agnes E. ; SANNI Abiodun I. ; OYEWOLE Olusola B.et al , 2005***)

The self-cycling frementation (;rSCF) technique was applied to culture of *Streptomyces aureofaciens*. SCF is a method of continuous fermentation in which the metabolism of a microorganism is monitored by a measurement such as dissolved oxygen. These data are sent to a computer to allow it to control the system. Tetracycline production was observed only at exceedingly low iron concentrations in the growth medium. Repeatability of cycles was found to be dependent upon the presence of tetracycline in the fermentation broth as well as the strain of microorganism grown in the fermentor. Tetracycline was produced by an improved specific rate when compared to results in the literature for this organism grown using the batch method. (***John Wiley & Sons, Inc 1994***).

In this fermentation the composition of the medium and the strain of streptomycete are both important factors, since Streptomyces aureofaciens is capable of producing at least two antibiotic substances as pointed out by Backus et al. (1954). In the presence of chloride, which is incorporated within the chlortetracycline 2 molecule (Broschard et al., 1949) and which is therefore essential for its production as disclosed by Petty and Matrishin (1950), the antibiotic formed was predominately chlortetracycline. In media low in chloride tetracycline predominates and the chlortetracycline fraction diminishes since 1 ppm of available chloride
ion can produce at most 14 ,ug/ml chlortetracycline. The simultaneous production of two or more antibiotics in a fermentation is well known, and the substances formed may be either closely related on a chemical or a biological basis or widely separated. Typical examples of closely related compounds produced simultaneously by the same organism are the penicillins (Clarke, 1949), streptomycins (Waksman, 1949), polymyxins (Brownlee, 1949), bacitracins (Newton and Abraham, 1950), cephalosporins (Crawford et al., 1952), nisins (Berridge et al., 1952), neomycins (Waksman, 1953), rhodomycins (Brockmann et al., 1951), and candicidins (Lechevalier et al., 1953).(***marjorie a. darken, herman berenson, richard j. shirk, and newell 0. sjolander july 27, 1959***)

A shift down in temperature causes in *Streptomyces aureofaciens* a transient repression of polypeptide synthesis. During the acclimation phase 32 proteins were synthesized. The addition of tetracycline (200 μg/ml) to cells

from exponential phase of growth leads to induction of 27 novel proteins and 17 upregulated proteins migrated in 2-D gel as proteins expressed upon cold shock. Immunoblot analysis using antibodies raised against CspB, CspC, and CspD of *Bacillus subtilis* revealed five cross-reactive proteins of the Csp family. Proteins CspB and CspD are predominantly induced at low temperature or by the presence of tetracycline. Expression of Csp proteins during the acclimation phase is regulated on the transcription level. Proteins of the Csp family have been shown to be associated with ribosomes and can be removed by 1 M NH_4Cl. As expression of Csp proteins differs during development or temperature shift down, these proteins can be considered as trans-acting factors to form contacts with the coding region of specific mRNAs (***Karel Mikulík, Quoc Khanh-Hoang, Petr Halada, Silvie Bezou š ková, Old î ich Benada and Vladislav Bêhal et al*** *2 April 2002).*

By Northern blot analyses with DNA probes carrying 6-demethylchlortetracycline (6-DCT) biosynthetic genes from Streptomyces aureofaciens NRRL3203, a highly expressed gene (tcrC) was detected in a high titer producing mutant derived from the parental strain NRRL3203 by NTG mutagenesis. The analysis of the nucleotide sequence of the 2.8-kb BamHI fragment containing tcrC gene showed that the predicted tcrC gene product is a protein consisting of 512 amino acids. The deduced amino acid sequence had a high level identity with that of the self-defense gene (tet347) of Streptomyces rimosus, known to mediate oxytetracycline efflux. The tcrC gene-inactivated strains generated from strain NRRL3203 by gene replacement had a 90% decrease in the level of resistance to tetracycline and the antibiotic productivity when compared with the parental strain. (***Dairi T, Aisaka K, Katsumata R, Hasegawa M.et al 1995 Oct;.)***
A number of nutrient media have been described by ***Goodman (1954), Katagiri (1954), Niedercorn (1952), Petty et al. (1953), Van Dyke and De Somer (1952),*** and others that allow Streptomyces aureofaciens Duggar to grow in aerated, liquid culture and to accumulate substantial quantities of 7-chlorotetracycline.' The concentrations of 7-chlorotetracycline accumulated on these different media vary from about 0.1 to 2.5 g per L. These media are, in general, complex in composition, making it difficult to define the requirements of the organism for growth and to determine the origins of the carbon and the nitrogen appearing in the accumulated 7-chlorotetracycline2 This paper describes a series of simple, chemically defined nutrient media suitable for growth and 7-chlorotetracycline
production by S. aureofaciens strain BC-41, a strain which is characteristic of this species. It is a descendant, through a series of mutation treatments and selections, of the original S. aureofaciens strain A-377 soil isolate of Duggar.)

Streptomyces aureofaciens ATCC 10762 grown in rotary-shaken submerged cultures produced substantial amounts of tetracycline only when the defined medium was deprived of iron. The biosynthesis of tetracycline was inhibited either by free iron at concentrations above 1-2 mumol l-1, or by chelated iron provided by the siderophores of this bacterial strain. Late static iron-containing cultures allowed cell differentiation and sporulation and led to tetracyclines synthesis. A nitrosoguanidine-induced mutant able to synthesize tetracycline in the presence of iron in shaken submerged cultures was isolated and compared to the wild-type strain. However, no constitutive siderophore-mediated iron transport occurred in the mutant. These results suggest the involvement of a putative iron-controlled repressor in the biosynthesis of these secondary metabolites during vegetative growth and primary metabolism of the bacterium.(***Béchet M, Blondeau R, et al. 1998 May***)
The exact time course of phosphate consumption in a tetracycline production by*Streptomyces aureofaciens* has been determined. The data have been compared with model simulations according to a model proposed by Votruba et al. (1984). This led to a revision of his equation for the rate of phosphate consumption and to the proposal that phosphate is consumed proportionally to the growth rate. In contradiction to the model simulations it was found that the length of the time lag of the production is independent of the initial phosphate concentration. While the model explains the time lag through inhibition of the production by phosphate, the measured data show that there must be another or an additional reason for the lag. Simultaneously with the start of the production the organism changes from an organic substrate to ammonia as nitrogen source.(***Anton Ross , Karl Schügerl et al. 1988***)

Ribosomes from cells of *Streptomyces aureofaciens* producing tetracycline antibiotics (Tc-ribosomes) differ in electrophoretic mobility of ribosomal proteins S2, S10 and L19 from those of the same strain, where the production of tetracyclines was suppressed by changed cultivation conditions (C-ribosomes). Purified tight vacant couples C- and Tc-ribosomes are equally active in the translation of poly(U). Both types of *S. aureofaciens* ribosomes are more sensitive to tetracycline and chlortetracycline than ribosomes of *Escherichia coli* in the Phe-tRNA binding and the translation of poly(U) (***Karel Mikulík, Anna Jiráňová, Ivan Janda, Jaroslav Weiser et al. 7 February 1983)***

SOLID STATE FERMENTATIONS

Pineapple peel is the principal solid waste product of the juice processing industry. The disposal of the fresh peels is becoming a major problem to many food processing industries. Dry pineapple peels are rich in biodegradable organic material and suspended solids; therefore, this waste was used as a novel substrate in present solid substrate fermentation. The effect of medium ingredients such as carbon, inorganic and organic nitrogen sources, inorganic salts on tetracycline production by various strains of *Streptomyces* [*S. aureofaciens* NCIM (2417, 2614, 2615), *S. rimosus* NCIM 2213 and *S. viridifaciens* NCIM 2506] in solid-state fermentations (SSF) was observed. (**Basavaraj M. Vastrad1 and Shivayageeswar E. Neelagund2,et al *2011).***
The last decade has witnessed an unprecedented increase in interest in solid state fermentation (SSF) for the development of bioprocesses, such as bioremediation and biodegradation of hazardous compounds, biological detoxification of agro-industrial residues, biotransformation of crops and crop-residues for nutritional enrichment, biopulping, and production of value-added products, such as biologically active secondary metabolites, including antibiotics, alkaloids, plant growth factors, etc. enzymes, organic acids, biopesticides, including mycopesticides and bioherbicides, biosurfactants, biofuel, aroma compounds, etc. SSF systems, which during the previous two decades were termed as a 'low-technology' systems, appear to be a promising one for the production of value-added 'low volume-high cost' products such as biopharmaceuticals. (*Ashok Pandey, Carlos R Soccol and David Mitchell* ***July 2000***).

Solid state (substrate) fermentation (SSF) has been used successfully for the production of enzymes and secondary metabolites. These products are associated with the stationary phase of microbial growth and are produced on an industrial scale for use in agriculture and the treatment of disease. Many of these secondary metabolites are still produced by submerged liquid fermentations (SmF) even though production by this method has been shown to be less efficient than SSF. As large-scale production increases further, so do the costs and energy demands. SSF has been shown to produce a more stable product, requiring less energy, in smaller fermenters, with easier downstream processing measures. In this article we review an important area of biotechnology, since the recent evidence indicates that bacteria and fungi, growing under SSF conditions, are more than capable of supplying the growing global demand for secondary metabolites. (***T. Robinson, D. Singh and P. Nigam 2001***).

Streptomycetes are soil microorganisms exposed to various stresses that activate specialized responses whose coordinated action promotes growth under adverse conditions. Ribosomes having a highly cooperative structure are potential target for control mechanisms that generate signal and activate adaptive regulons or developmental programs. We examined how *Streptomyces aureofaciens* producing tetracycline responds to the presence of antibiotics and stresses induced by the changes in temperature. Tetracycline interacts with 16S RNA and decreases its thermodynamic stability. The drug also inhibits binding of ternary complex Phe-tRNA.EF-Tu.GTP to purified ribosomes. We have found that antibiotics that cause ribosome stall or pause could increase the requirement for mRNA in the process *trans*translation. Increase in tmRNA level was also demonstrated upon downshift in temperature. **(*K. Mikulík and P.Palečková 2007*)**

Enzyme production is an increasing field of Biotechnology. Most enzyme manufacturers produce enzymes by submerged fermentation (SmF) techniques. However, in the last decades there has been an increasing trend towards the use of the solid-state fermentation (SSF) technique to produce several enzymes. SSF is known from ancient times in Asian countries thus, SSF is used, for example, in the production of koji and sake. However, in western countries SSF was nearly ignored after 1940. This was due to fact that SmF had become a model technology for production of any compound by fermentation as a result of the development of penicillin. Table 1 gives a brief summary of the historical evolution of
SSF . This technique reproduces the natural microbiological processes like composting and ensiling. This natural process can be utilised in industrial applications in a controlled way to produce a desired product. In addition, it presents several advantages over the traditionally employed SmF (***J. L. Toca-Herrera1, J. F. Osma, S. Rodríguez Couto et al. 20009, Spain***)

Solid-state (substrate) fermentation (SSF) has been defined as the fermentation process occurring in the absence or near-absence of free water. SSF processes generally employ a natural raw material as carbon and energy source. SSF can also employ an inert material as solid matrix, which requires supplementing a nutrient solution containing necessary nutrients as well as a carbon source. Solid substrate (matrix), however, must contain enough moisture. Depending upon the nature of the substrate, the amount of water absorbed could be one or several times more than its dry weight, which leads relatively high water activity (a_w) on the solid/gas interface in order to allow higher rate of biochemical process. Low diffusion of nutrients and metabolites takes place in lower water activity conditions whereas compaction of substrate occurs at higher water activity. Hence, maintenance of adequate moisture level in the solid matrix along with suitable water activity are essential

elements for SSF processes. Solid substrates should have generally large surface area per unit volume (say in the range of 10^{3}-10^{6} m^{2}/cm^{3} for the ready growth on the solid/gas interface). Smaller substrate particles provide larger surface area for microbial attack but pose difficulty in aeration/respiration due to limitation in inter-particle space availability. Larger particles provide better aeration/respiration opportunities but provide lesser surface area. In bioprocess optimisation, sometimes it may be necessary to use a compromised size of particles (usually a mixed range) for the reason of cost effectiveness. For example, wheat bran, which is the most commonly used substrate in SSF, is obtained in two forms, fine and coarse. Former contains particles of smaller size (mostly smaller than 500-600 μ) and the latter mostly larger than these. Most of SSF processes use a mix of these two forms at different ratios for optimal production. (***Prof Ashok Pandey13 June 2008***)

HPLC

Compounds stick to reverse phase HPLC columns in high aqueous mobile phase and are eluted from RP HPLC columns with high organic mobile phase. In RP HPLC compounds are separated based on their hydrophobic character. Peptides can be separated by running a linear gradient of the organic solvent. I often tell my fellow researchers to run the 60/60 gradient when chromatographing an unknown. The 60/60 gradient means that the gradient starts at near 100% aqueous and ramps to 60% organic solvent in 60 minutes. The majority of peptides (10 to 30 amino acid residues in length) will elute by the time the gradient reaches 30% organic (***Andrew Guzzetta July 22nd, 2001*)**

High Pressure Liquid Chromatography (HPLC) is a popular method of analysis because it is easy to learn and use and is not limited by the volatility or stability of the sample compound. Modern HPLCs have many applications including separation, identification, purification, and quantification of various compounds. There are many players who are into the HPLC business in the world. A few such players are Alltech Associates, Baxter Healthcare Corporation, Beckman Instruments, Inc. Applied Biosystems, Millipore, Thermo Electron, Hitachi, Shimadzu, Bio-Rad Laboratories, Dupont Company , Tosohaas, Gilson, HPLC Technology, Proteinlabs and Waters Chromatography (***Narayan Kulkarni, August 16, 2005***)

Although gas chromatography is the dominant technique for fatty acid analysis, high-performance liquid chromatography has an important role to play in applications such as the handling of less usual samples, avoidance of degradation of heat-sensitive functional groups, and for micro-preparative purposes. Several approaches for development of improved methods are suggested, especially for reversed-phase applications ***(P.J. Barnes & Associates 1997).***

High performance liquid chromatography, also called high-pressure liquid chromatography (HPLC), separates compounds on the basis of their interaction with the solid particles of a tightly packed column and the solvent of the mobile phase. Modern HPLC, such as the one used in this laboratory, uses a nonpolar solid phase, like C18, and a polar liquid phase, generally a mixture of water and another solvent. High pressures of up to 400 bar are required to elute the analyte through the column before they pass through a diode array detector (DAD). A DAD measures the absorption spectra of the analytes to aid in their identification. HPLC is useful for compounds that cannot be vaporized or that decompose under high temperatures, and it provides a good compliment to gas chromatography for detection of compounds.(Kalkhoff, S.J., Kolpin, D.W., Thurman, E.M., Ferrer, I., and Barcelo, D., 1998)

BANANA

The skin of a banana has been used to great comic effects in numerous slapstick routines for many years. It's also good for the skin and is a traditional cure for warts. You can polish shoes and silver with it. You can make wine with it and it's even been known to find itself being dried, wrapped in paper and smoked. Now, research published in the journal of the American Chemical Society claims that mashed up peel can remove heavy metals from river water. (***paul-ridden* March 14, 2011**)

Banana skins may quickly find homes in the compost bin for many - but a new study from Brazilian scientists has demonstrated that purifying water is one of the banana's hidden talents. Researchers from the Department of Chemistry and Biochemistry, in Sao Paulo, have tested minced banana peel on water laced with lead and copper, and found it performs better than many other materials. That could be a real boon to water sanitation in developing countries, say the researchers in the latest Industrial & Engineering Chemistry Research. (***Martin Leggett* 10 Mar 2011***)*

The Banana as a heart friendly fruit is rich in potassium, an essential mineral for maintaining normal blood pressure and heart function. With around 460 mg of Potassium and only 1 mg of sodium, a banana a day helps to protect against high blood pressure and CVD, especially atherosclero**sis.** According to research in "The New England Journal of Medicine, 'eating bananas as part of a regular diet can cut the risk of death by strokes by as

much as 40%! ` Apart from its benefits on CVD the high potassium content also help to promote bone health. It works against the increased urinary calcium loss caused by the high-salt diets from fast and junk foods, typical of today's diets thus helping to prevent bones from thinning out at a fast rate. Banana also helps to replenish potassium salts lost during bouts of vomiting or diarrhea. While ripe bananas are known to prevent constipation and in the treatment of peptic ulcers**,** the green ones are used in the treatment of diarrhea and hemorrhoids. It is also seen to be helpful with infant diarrhea, in celiac disease and in colitis**.** In the case of psoriasis it is said that when rubbed on the red, scaly patches of skin it gives relief without the side effects. (***Vijayalakshmi Iyenga*** **May 25, 2009**)

Musa sapientum peels were analysed for minerals, nutritional and anti – nutritional contents. The result of mineral content indicate the concentrations (mg/g) of potassium, calcium, sodium, iron, manganese, bromine, rubidium, strontium, zirconium and niobium to be 78.10, 19.20, 24.30, 0.61, 76.20, 0.04, 0.21, 0.03, 0.02 and 0.02 respectively. The percentage concentrations of protein, crude lipid, carbohydrate and crude fibre were 0.90, 1.70, 59.00 and 31.70 respectively. The results indicate that if the peels are properly exploited and process, they could be a high-quality and cheap source of carbohydrates and minerals for livestock. (***Anhwange, B. A. et al, 8 (6), 2009***.*)*

Four different preparation methods of banana peel, dry milling, wet milling, wet milling and tap water washing, and wet milling and hot water washing were investigated on their effects on the chemical composition and properties of the banana peel dietary fibre concentrate (BDFC). The dry milling process gave the BDFC a significant higher fat, protein, and starch content than the wet milling process, resulting in a lower water holding capacity (WHC) and oil holding capacity (OHC). Washing after wet milling could enhance the concentration of total dietary fibre by improving the removal of protein and fat. Washing with hot water after wet milling process caused a higher loss of soluble fibre fraction, resulting in a lower WHC and OHC of the obtained BDFC when compared to washing with tap water. Wet milling and tap water washing gave the BDFC the highest concentration of total and soluble dietary fibre, WHC and OHC. (***Phatcharaporn Wachirasiri, Siripan Julakarangka, Sorada Wanlapa et al. 10 November 2008***)

Chemical and biological evaluation of both the edible and nonedible portion of banana fruit was carried out. The possibility of using the nonedible portion (peel) as animal feed was also explored. The results showed a remarkable difference concerning the chemical composition of both the edible and nonedible portions. The amino acid contents were proved to be vastly deficient with regard to the indispensable amino acids with exception of phenylalanine which was found in good amounts in the edible portion (pulp). The biological results demonstrated that neither the pulp nor the peel portions yielded good P.E.R. values when used at 6% protein level. The P.E.R values showed negative values which amounted to –1.75, –5.85 and –4.67 for the pulp and peel diets respectively. Incorporation of the stock diet to the peel diet resulted in a slight increase which amounted to 6% in both male and female rat groups (***Phatcharaporn Wachirasiri, Siripan Julakarangka , Sorada Wanlapa.et al. 10 November 2008***)

The contents of moisture, protein, ash, ascorbic acid, glucose, fructose, total sugars, and total and insoluble fiber were determined in cultivars of bananas (Gran Enana and Pequeña Enana) harvested in Tenerife and in bananas (Gran Enana) from Ecuador. The chemical compositions in the bananas from Tenerife and from Ecuador were clearly different. The cultivar did not influence the chemical composition, except for insoluble fiber content. Variations of the chemical composition were observed in the bananas from Tenerife according to cultivation method (greenhouse and outdoors), farming style (conventional and organic), and region of production (north and south). A highly significant (r = 0.995) correlation between glucose and fructose was observed. Correlations of ash and protein contents tend to separate the banana samples according to origin. A higher content of protein, ash, and ascorbic acid was observed as the length of the banana decreased. (***Markus Paul Forster, Elena Rodríguez Rodríguez, and Carlos Díaz Romero et al. November 21, 2002***)

The banana is often thought of as being a tropical fruit, but it is possibly one of the most easily available and popular fruits worldwide. Moreover, it is known as the "poor man's fruit" in many parts of the world. This is because it is relatively cheaper than other fruits, yet full of goodness and health benefits. The banana is commonly eaten raw as a fruit, and is a popular ingredient in salads and desserts. When unripe, it can be cooked, or fried to make banana chips.(***Nita Mukherjee Jul 9, 2009***)

In our country, bananas are no longer considered an exotic fruit: it can buy them year round, and they are inexpensive. Very well that these amazing, fantastic fruit can buy any citizen of Russia, regardless of income level and place of residence because Bananas are very useful. Among all the fruit grown in other countries, only

bananas can be safely given to children of the first year of life. Adults who like to buy bananas when there is no time for a complete meal, but want to eat something useful, without fear of overload the stomach. Bananas are also convenient to have in any situation, even if there is no place to wash your hands: should just gently release the fruit of a convenient "packaging", conceived by nature, and, holding the tip, easy to eat. Such food is not only nourishes, but also helps the body get energy and lots of useful substances, as well as uplifting. (***NaQonianz , October 25, 2010***)

III. MATERIALS AND METHODS

Pure culture of *streptomycin aureofacians* , Nacl, Starch ,casein, dihydrogen ortho phosphate, magnessium sulphate, ferrous sulphate,agar, calcium carbonate, ethanol, methanol, activated charcoal, elaichi banana peel, rasa banana peel....etc

Tetracycline is manufactured by fermentation process. The process comprises three major steps :

i Inoculum preparation.
ii. Solid state fermentation.
iii. Extraction,
iv. Recovery and purification

i. Inoculum preparation :

3 (a) COLLECTION OF SAMPLES

The pure culture of streptomycin aureofacians was purchased from Microbial Type Culture Collection and Gene bank (MTCC), Institute of Microbiology Technology. Sector 39-A Chandigarh-160036. Baring MTCC code for the organism ****325***.

3 (b) IDENTIFICATION OF ANTIBIOTIC PRODUCTION ORGANISM BY GRAM'S STAINING.

Gram-positive bacteria have a thick mesh-like cell wall made of peptidoglycan (50-90% of cell wall), which are stained purple by crystal violet, whereas Gram-negative bacteria have a thinner layer (10% of cell wall), which are stained pink by the counter-stain. There were four basic steps of the Gram stain followed:

- applying a primary stain (crystal violet) to a heat-fixed (death by heat) smear of a bacterial culture
- the addition of a trapping agent (Gram's iodine)
- rapid decolorization with alcohol or acetone, and
- *counterstaining* with safranin. Basic fuchsin is sometimes substituted for safranin since it will more intensely stain anaerobic bacteria but it is much less commonly employed as a counterstain.

Confirming the morphological features of streptomyces aureofacians. subculturing is followed.

3.(c). SUBCULTURING OF THE PURCHASED ANTIBIOTIC PRODUCING ORGANISM

Starch casein broth was prepared and autoclaved.

Break opening the pure culture tube sent by MTCC. Add 1mL of the broth to the culture tube to mix the dried culture given.

To two flask of 500mL (in duplicates) was taken and broth was added and autoclaved.
0.5mL of the pure culture was inoculated.

Incubate the flasks at 30°C for 5-7 days in shaker to get the growth of streptomycin auroefacians.

3 (d). PRODUCTION OF ANTIBIOTIC BY FERMENTATION

Streptomyces aureofacians is the micro-organism whose strain is used for the purpose of tetracycline production.

First step is the preparation of inoculum from the stock culture of the strain/The inoculum is transferred to a flask containing starch casein broth.

composition of starch casein broth

soluble starch	10gm
Potassium phosphate dibasic	2gm
Potassium nitrate	2gm
Sodium chloride	2gm
Casein	0.3gm
MgSO4. 7H2O	0.05gm
CaCO3	0.02gm
FeSO4. 7H20	0.01gm
Distilled water	1000ml

Incubate the flask in a rotary shaker at 37^0c for 5 to 6 days to increase the total biomass and for the production of antibiotic .

3 (e). SCREENING FOR ANTIBIOTIC PRODUCTION

Antibiotics acts against bacteria in two different ways .some are bacteriocidal and some are bacteriostatic.antibiotic assays can be performed in much the same way:
finding the minimal inhibitory concentration (the lowest concentration that stops growth of the test organism is a MIC) or by measuring the zone of inhibition produced by inhibiting growth of a lawn of test organism.

the media LB agar (Tryptone 10gm, yeast extract 5gm,sodium chloride 5gm,agar 10gm PH 7.2) was prepared and sterilized. Then pour the media into sterlized petriplate and allowed to solidify.

A culture of gram-negative bacteria *Escherichia coli* was spreaded on the appropriate agar plate using a sterile cotton swab.

Once the cultures have soaked into the agar plates, then the well was made at the center and the test sample of 100microliter was added to that well.

Incubate the plates at 18-24hours at 37ºC for growth of the microorganism after incubation the, the plates are examined for the zone of inhibition around the well.

The size of the zone of inhibition is depend upon the concentration of the test substance, its potency, and rate of diffusion in the medium.

3 (f). SOLID STATE FERMENTATION FOR ANTIBIOTIC PRODUCTION USING POWDERED BANANA PEEL.

Banana peel can be used for various purposes. It is because bananas serve as the amazing source of vitamins and nutrients. The result of mineral content indicate the concentrations (mg/g) of potassium, calcium, sodium, iron, manganese, bromine, rubidium, strontium, zirconium and niobium to be 78.10, 19.20, 24.30, 0.61, 76.20, 0.04, 0.21, 0.03, 0.02 and 0.02 respectively. The percentage concentrations of protein, crude lipid, carbohydrate and crude fibre were 0.90, 1.70, 59.00 and 31.70 respectively. This indicate that if the peels are properly exploited and process, they could be a high-quality and cheap source of carbohydrates and minerals for livestock.

Banana Peels used in solid state fermentation for the production of antibiotic are :
1. Elaichi banana.
2. Rasa banana.

composition of the media
Flask 1

Elaichi banana peel powder	20g
Potassium phosphate dibasic	0.2g
Potassium nitrate	0.2g
Sodium chloride	0.2g
Casein	0.03g
MgSO4. 7H2O	0.005g
CaCO3	0.002g
FeSO4. 7H20	0.001g
Distilled water	100ml

Flask 2

Rasa banana peel powder	20g
Potassium phosphate dibasic	0.2g
Potassium nitrate	0.2g
Sodium chloride	0.2g
Casein	0.03g
MgSO4. 7H2O	0.005g
CaCO3	0.002g
FeSO4. 7H20	0.001g
Distilled water	100ml

The above medium was taken in two 500mL conical flask 1 and 2 respectively.
Innoculating 2mL of the starter culture to each flask.
Incubating the flask in shaker for 5-7 at 37˚c days for the antibiotic growth.
After production of antibiotic screening of antibiotics was again done to check the activity of antibiotic production in solid state fermentation.

3 (g). EXTRACTION AND PURIFICATION

A method for extracting streptomycin from culture filtrates is by adsorption on charcoal at pH **6-8,** elution with **1.2%** (v/v) aqueous phosphoric acid, readsorption **of** the eluate on charcoal at pH **7,** elution with acidified methanol, followed by evaporation a t reduced pressure and precipitation of streptomycin by dilution of the concentrated methanol eluate with ***5*** volumes acetone or amyl acetate. An indication is given of the order of recovery, and the potency of the product obtained. The stability of streptomycin under the conditions of pH and temperature to which it may be subjected during the extraction is outlined.
After incubation the flask are removed from the shaker and 1g of activated charcoal was added to all the flask,shake the flask for 20min and then filter the contents using filter paper .Elute the sample with solution containing 0.3g of benzoic acid,10ml of sulphuric acid and 150ml methanol and precipitate the streptomycin by adding double the volume of cold methanol .

Dry the contents by placing the flask in hot air oven for 2 to 3 days.

After drying dissolve the crystal in buffer and analyse by HPLC.

IV. RESULTS

View of *streptomycin aureofacians***.**

Streptomyces can be identified as powdery substances formed as colonies. they can be isolated by carefully taking the powdery form and subculturing.

The sample brought from MTCC was directly subcultured in conical flask containing casein broth.

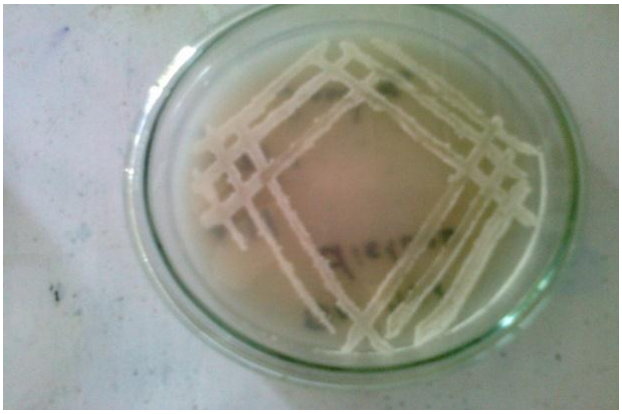
COLLECTION OF SAMPLES

The sample taken from MTCC was broke open and subcultured in two 500 mL conical flask and also on petriplate in

1 continuous
2 zig zag , fashion

.

Continuous



zig zag

IDENTIFICATION OF ANTIBIOTIC PRODUCTION ORGANISM BY GRAM'S STAINING.

To confirm the organism to be streptomycin it is carried out with gram's staining and they are observed as a thin fragments lik structure. After confirming they are carried out for production of antibiotic. Later the same checked for the concentration of antibiotic production.

SCREENING FOR ANTIBIOTIC PRODUCTION

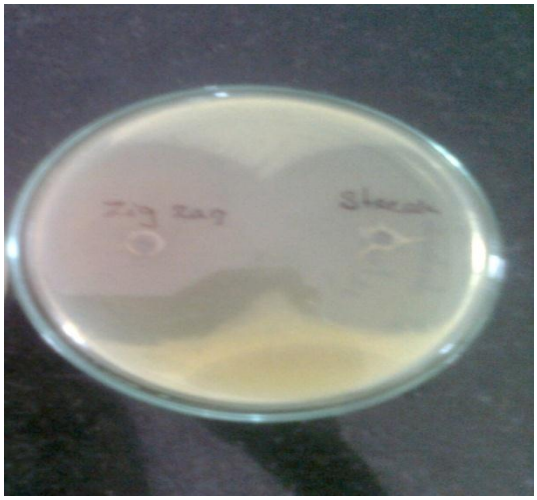
Finding the minimal inhibitory concentration (the lowest concentration that stops growth of the test organism is a MIC) or by measuring the zone of inhibition produced by inhibiting growth of a lawn of test organism.

By preparing Lb agar medium and pouring it on sterile petriplate allow to solidify, swab on the with E.coli bacteria, make 1mL of gel punch in the centre of the plate.
Add the centrifuged supernatant of the culture (1 and 2 resp) in separate plates.
Keeping in incubation for 2 – 3 days at 37˚c zone of inhibition is observed.
The antimicrobial activity inhibits the growth of E.coli forming a zone of inhibition.

Plate kept as control, without antibiotic. Were petriplate contains of the solidified agar media , with swabbed strain of E.coli, but absent of the antibiotic

Few hours after incubation.
For diameter of 1cm of the gel punch, has 1.8 cm in diameter zone of inhibition.
This indicates the rate of activity of antibiotic. It shows 0.44 % of activity.

DAY 1 of incubation.
Gives 2.2 cm of zone of inhibition. .
This shows the rate of activity of antibiotic has 0.54%.



DAY 2 of incubation.
Gives 4.2cm of zone of inhibition.
This indicates the rate of activity of antibiotic has 0.761%.

DAY 3 of incubation
Whole of petriplate is covered.
This indicated that the percentage of the production of antibiotic is increasing day by day.

$$\text{FORMULA :-} \quad \frac{\text{diameter of zone Of inhibition} - \text{diameter of gel punch}}{\text{Total zone of activity}}$$

SOLID STATE FERMENTATION FOR ANTIBIOTIC PRODUCTION USING POWDERED BANANA PEEL.

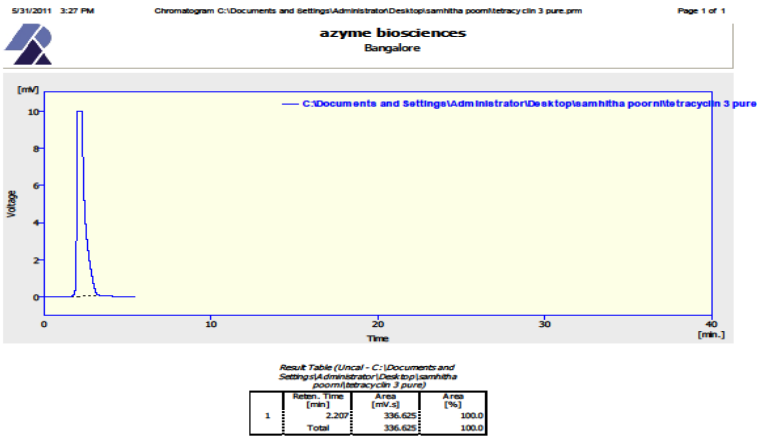
Adding powdered banana peel (both) in bottles containing casein broth media.
For the fermentation to take place on solid media 1 – 2 ml of the antibiotic was added.
Allow to ferment for 5-7 days at 37˚c for antibiotic growth.
Every alternative day was screened for the antibiotic production. The increasing zone of inhibition was determing the increase in the production of antibiotic.

EXTRACTION AND PURIFICATION

After incubation the flask are removed from the shaker and 1g of activated charcoal was added to all the flask, shake the flask for 20min and then filter the contents using filter paper. Elute the sample with solution containing 0.3g of benzoic acid,10ml of sulphuric acid and 150ml methanol and precipitate the streptomycin by adding double the volume of cold methanol .
Dry the contents by placing the flask in hot air oven for 2 to 3 days.
After drying dissolve the crystal in buffer and analyzed by HPLC.

HPLC

HPLC was run to determine the rate of production of antibiotic in both the samples (banana peel)

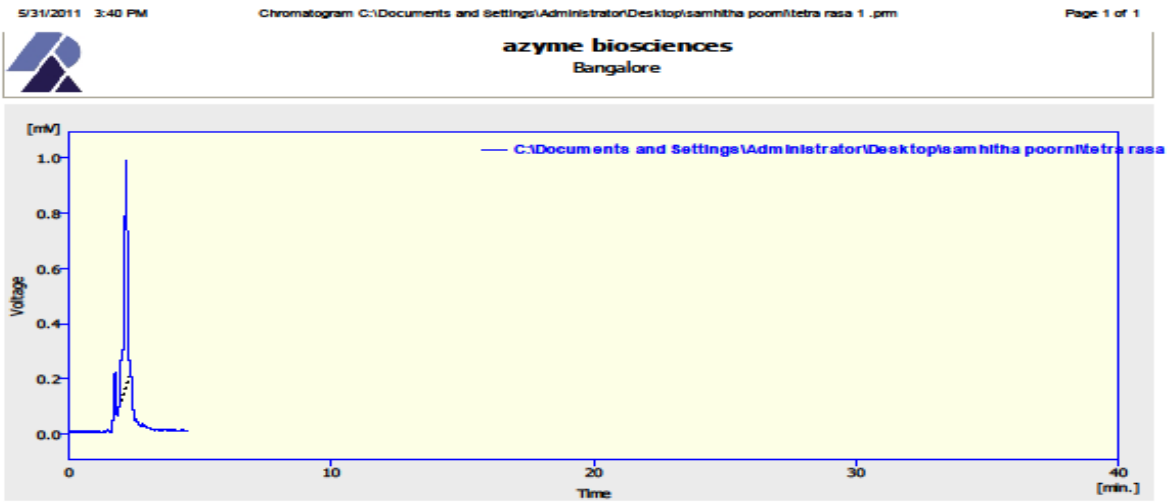


Result Table (Uncal - C:\Documents and Settings\Administrator\Desktop\samhitha poorn\tetracyclin 3 pure)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	2.207	336.625	100.0
	Total	336.625	100.0

This peak shows the value of HPLC result for std tetracyclin which was taken at 10mg in 25 mL. it gives an area of 336.625.

$$\text{FORMULA :-} \quad \frac{\text{sample}}{\text{Std}} \times \frac{\text{std amt}}{\text{dilution}} \times \frac{\text{dilution}}{\text{sample amt}}$$



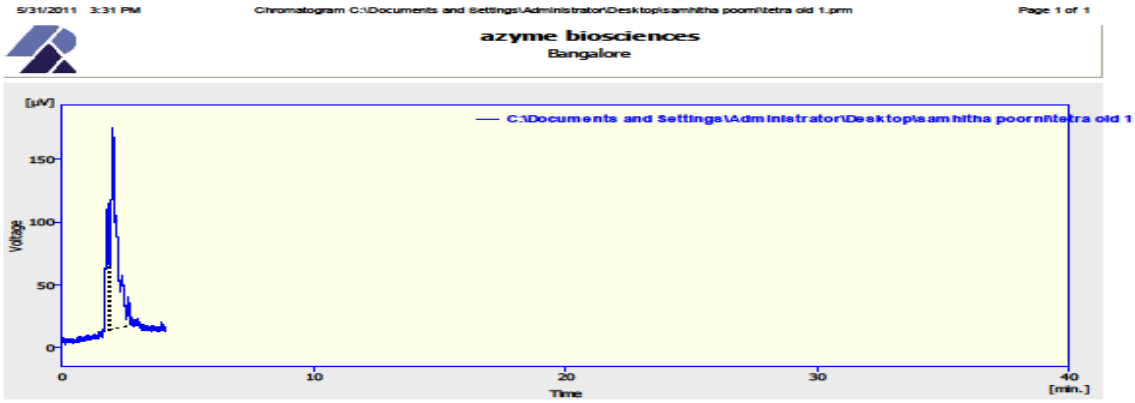
Result Table (Uncal - C:\Documents and Settings\Administrator\Desktop\samhitha poorn\tetra rasa 1)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	2.183	7.167	100.0
	Total	7.167	100.0

This 2nd peak shows the value of HPLC result for Tetracycline by Rasa banana. which was taken at 25mg in 0.5 mL. it gives an area of 7.167.

Calculation :- $\frac{7.167}{336.625} \times 0.4 \times 50 \times 100$

= 42.4 %



Result Table (Uncal - C:\Documents and Settings\Administrator\Desktop\samhitha poorni\tetra old 1)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	1.802	0.817	22.7
2	2.007	2.784	77.3
	Total	3.601	100.0

This 3rd peak shows the value of HPLC result for Tetracycline by Elaichi banana. which was taken at 25mg in 0.5 mL. It gives an area of 2.784 %.

Calculation :- $\frac{2.784}{336.625} \times 0.4 \times 50 \times 100$

= 16.6 %

They are then calculated for the percentage of result. Its as follows :
Rasa banana shows 42.4% of tetracycline production.
Elaichi banana shows 16.6% of tetracycline production.
This shows that Rasa banana shows increasing rate of tetracycline production than Elaichi banana.

FORMULA :- $\frac{\text{sample}}{\text{Std}} \times \frac{\text{std amt}}{\text{dilution}} \times \frac{\text{dilution}}{\text{sample amt}}$

V. DISCUSSION

Antibiotics are traditionally produced by submerged fermentation, and their yields tend to be low due to the energy input (Tomasini, et al 1997). The advantages of solid-state fermentation include
(i) it is more competitive process, and it may be a viable option for the industrial production of secondary metabolites (Robinson, et al 2001),
(ii) It requires lower manufacturing cost by utilizing unprocessed and moderately processed raw materials

(Adinarayana, et al 2003),
(iii) it is less sensitive to contamination when compared to submerged fermentation (Grohmann, 1993). SSF has successfully achieved higher titers of antibiotics (Farzana, et al 2005, Robinson, et al 2001), so this technique has encouraged us for tetracycline production. Moreover, submerged fermentation is usually employed for commercial production of tetracycline (Mamoru, et al 1986).

Initial pH : The *Streptomyces* strains could adapt initial pH range from 5 to 7 and optimum level at pH 6.5. As the metabolic activities of the *Streptomyces* are very much sensitive to the initial pH change (Agenes, et al 2005, Yang and Swei, 1996, Yang and Yuan, 1990, Yang and Ling, 1989). Evaluation of the optimum levels of initial pH is very important for overall economic feasibility of the production process.

Particles Sizes : The particles sizes ''C'' (6 x 4 mm) of substrate was found to be optimal size of the substrate for maximum tetracycline production **(**Basavaraj M. Vastrad and Shivayageeswar E. Neelagund *2011)* Variation of substrate particle size resulted in reduction of antibiotic production. So the banana peel was dried and growned to get the size approximately (6×4 mm).

Incubation Time : Tetracycline was detected at the third day incubation, had maximal activity at *5-6* days, and decreased gradually in 1-month period. In solid state fermentation, the mechanism of tetracycline secretion was same as that proposed in submerged fermentation. However, in submerged fermentation, antibiotic activity decreased sharply after prolonged incubation due to cell autoiysis. (Y. Okami and G. Oomura, Tokyo, 1979), Therefore, antibiotic production by solid state fermentation was more stable than that in submerged fermentation and the product could be temporarily stored without losing activity significantly.' (C. W. Hesseltine, **14** 1972).

Temperature : Tetracycline production had the maximal value at 26˚C, and decreased sharply when incubation temperature was higher than 37°C or less than 20°C. Each gram of dry substrate produced 1380 pg of total tetracycline equivalent potency at 26°C.

Organic Salts : However, previously it has been reported (Agenes, et al 2005, Yang and Swei, 1996, Yang and Yuan, 1990, Yang and Ling, 1989) that maximum tetracycline production was shown in the presence of 1% (w/w) CaCO3. In the present experiments, apparently 1% (w/w) CaCO3 enhances maximum tetracycline production.

Inorganic salts : Tetracycline production by different *Streptomyces* strains in SSF was supported by the presence of various inorganic nitrogen sources (1% w/w) such as ammonium chloride, ammonium sulphate, ammonium nitrate (Agenes, et al 2005, Yang and Swei, 1996, Yang and Yuan, 1990, Yang and Ling, 1989). Ammonium sulphate (1% w/w) enhances maximum tetracycline production even ammonium nitrate and potassium nitrate also shown significant effect on tetracycline production as it was all added to the casein broth. All the supplemented organic nitrogen sources were found to enhance the tetracycline production. Available reports on the role of organic nitrogen sources on tetracycline secretion was found to be similar (Agenes, et al 2005, Yang and Swei, 1996, Yang and Yuan, 1990, Yang and Ling, 1989). It has been reported that additional carbon source slightly stimulated tetracycline production (Agenes, et al 2005, Yang and Yuan, 1990).

VI. Conclusion

Banana peel waste was found to be the best solid substrate for the production of tetracycline by the *Streptomyces* strains in SSF. The initial pH, substrate particle size, incubation period and incubation (*Basavaraj and Shivayageeswar/Rec Res Sci Tech 3 (2011) 01-08)* temperature factors were found have significant effect on the growth of *Streptomyces* strains and tetracycline production. Supplementation of banana peels waste with additional inorganic salts; organic and inorganic nitrogen sources proved to be beneficial and increased the yield of tetracycline after optimization of the nutritional parameters. But carbon source doses not shows significant effect on tetracycline production. Therefore, the Banana peel waste could be successfully used as novel solid substrate to produce tetracycline antibiotic under optimized SSF parameters to achieve a very good yield.

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