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Strategies for Enhancement of Microbial Secondary Metabolites

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ABSTRACT

Formation and production of microbial secondary metabolites are regulated by growth rate, nutrients, induction and inactivation of enzymes. Regulation of SM influenced by unique low molecular compound, sigma factors and gene product produced in stationary phase. These products are often coded by clustered genes present on chromosomal DNA and rarely on Plasmid DNA. Techniques for enhancement of secondary metabolite can be based on use of Elicitors, quorum sensing, genetic engineering, improvement methods such as amplification of secondary metabolite biosynthetic genes, amplification of regulatory genes, inactivation of competing pathways and Co-cultivation with microbe of same species and different species.

Introduction

Various strains of microbial species produce secondary metabolites; these are substances with variable chemical structures. There are numerous types of biologically active secondary metabolites apart from antibiotics which make them economically important for industries. These molecules are produced by microbe for their protection against other biological stimulus or harms; including insects, plants, and other microorganisms, or regulating biochemical pathway for higher organism (Sharma, 2014). Microorganisms often regulate the gene expression by use of secondary metabolites or small chemicals such as Homoserine lactones (HL) (Rao, 2010). These molecules are not involved directly in growth, development and replication of producer microbes and mostly they are needless in their life cycle. Secondary metabolites produced by microbes are extremely important for human health and nutrition (Sharma, 2014).

SM isolated from microbes includes antibiotics: which includes antibacterial, antiprotozoal and antifungal action; Antibiotics are chemically complex substances that inhibit bacterial growth or kills bacteria (Manivasagan. P, 2014) Antibiotic is a Greek word made from (anti) against and (bios) life. Antibiotics are produced by microbes as secondary metabolites to compete other microorganism.

Studies show that biosynthetic potential of many bacterial and fungal strains are much higher than that observed by fermentation, Production level of secondary metabolite is influenced by unique nutrient components, inducer, repressor, enzyme inactivation and enzyme induction. Overproduction of secondary metabolites can be on the base of microbial response (quorum sensing, elicitors), metabolic engineering, genetic engineering and ribosome engineering (Davati, 2013).

In co culture, when microorganisms interact to gather it may turn on cryptic gene clusters which trigger production of novel metabolites. Co-cultivation based elicitation of microorganisms may lead production of compounds which monoculture may not be able to produce it. There are many possible scenarios for example, physical cell – cell interaction, production of enzymes which activate production of precursor and production of small molecule such as quorum sensing molecules/ auto regulator, siderophores, etc (Marmann, 2014).

1. Bioactive Secondary metabolite

SM isolated from microbes includes antibiotics: which includes antibacterial, antiprotozoal and antifungal action, antiviral and antitumor agents, other medicines like antihyperlipidemia, enzyme inhibitor, pigments, biopestisides, herbicides, food additive, preservative, growth factor, immune modulating agents and toxins (Sharma, April-2014).

1.1 Antimicrobial agent

Antibiotics are chemically complex substances that inhibit bacterial growth or kills bacteria (Manivasagan, 2014) Antibiotic is a Greek word made from (anti) against and (bios) life. Antibiotics are produced by microbes as secondary metabolites to compete other microorganism. Production of antibiotic was first noticed by Fleming in 1929 when he observed lyses of *Staphylococcus aureus* by contaminant mold called *Penicillium notatum,* and he named it penicillin. At start of discovery of this antibiotic it was mostly used for treatment of wound infection during world war two but today antibiotics are widely used in chemotherapy, veterinary medicine, plant pathology and food preservation (Hassan, 2012). Antibiotics are well known example of natural product, 1940s to 1970s was the golden age of antibiotics, due to serendipitous discovery of penicillin and its development in 1940 by Chain and Florey (Zhang, 2003).

1.2 Anti-fungal activity

Amphotericin B is a macrolide polyene antifungal antibiotic having broad spectrum activity which was isolated in 1955 from *S. nodosus* broth (Solecha. J, june 2012).However many antifungal have been isolated from variety of microorganisms but still studies are conducted for identification of novel antifungal against pathogenic fungi (Manivasagan. P, 2014), (Paolo Monciardini, 2014).According to recent medical reports, fungal infection of different fungi species reached at level of crisis in immunocopromised individuals, the most prevalent opportunistic fungi, Aspergillus and Candida species reported to be multi resistant against antifungal compounds (Prakash S, 2012).

1.3 Anti-parasitic activity

Anti-parasites are a group of compound which is used in treatment of disease caused by parasites. Milbemycins isolated from *Streptomyces hygroscopicus subsp. aureolacrimosus* are macrolides lactone having 16 members and they are having broad spectrum antihelminthic and insecticidal properties (Hiroshi Mishima, 1983). Avermectins are macrocyclic lactones produced by *Streptomyces avermitilis* derived from soil which are active against arthropod of domestic animals (Solecha. J, june 2012). Cyclic depsipeptide valinomycin, butenolide and indolocarbazole alkaloid staurosporine which are having anti-parasitic activity against *Leishmania major* and *Trypanosoma brucei,* are isolated and purified by bioassay. Valinomycine was isolated for the first time from marine source (Sheila Marie Pimentel-Elardo, 2010). Trioxacarcins (A-D) which are isolated from *Streptomyces bottropensis* and *Streptomyces ochraceus* are complex compound with anti-malarial activity comparable to artemisinin; in addition some of them have antitumor and antibacterial activities (Rajendra P. Masker, 2004)

1.4 Bacteria as source of bacteriocins (antimicrobial proteins)

Bacteriocins are ribosomal peptide with antimicrobial property having narrow or broad bactericidal spectrum which produced by bacteria, these peptides are toxic to bacteria which are closely related to producing bacterial strain. There are many bacteria which are able to produce bacteriocins, few of them used for food preservation and development of desirable microbial flora in food, food grade lactic acid bacteria produce many metabolite with antimicrobial potential which control growth of pathogenic and spoilage bacteria in food. Nicin which produced by *Lactococcus lacti s*is the only bacteriocin that used practically in some food processing industries (Gupta C, 2014)

1.5 Antiviral activity

Antiviral agent isolated from marine source represents a significant natural resource with multi potential uses having the following application:

- Control of human enteropathogenic virus disease and contamination transmission in water polluted by sewage which is important for communities which use coastal water for recreational activities and food industries.
- Chemotherapy of human and lower animal viral disease (Manivasagan, 2014).

Tunicamycin is fatty acyl nucleoside isolated from *S. lyososuperficusnov* sp In 1971 in broth fermentation by Takatsuki and Tamura et al, later on coryne toxin and streptovirudin were isolated from actinomycetes strains, both compounds have activities against viral envelope, streptovirudin isolated from *Streptomyces griseoflavus* subsp *thuringiensis* (Solecha, 2012).

Currently, only few compounds with antiviral activity derived from marine actinobacteria. Benzastatin C obtained from *Streptomyces nitrosporeus*shown antiviral activity against herpes simplex virus type I, herpes simplex virus type II and vesicular stomatitis virus.

1.6 Anticancer activity

Cancer is a disease in which abnormal cells are growing uncontrollably. Normal cell are dictate by signals for division (Hejmadi, 2010), cancer is still one of the most serious health problem for human, in women, breast cancer is the second most universal cause of death (Manivasagan. P, 2014). Cancer is treated by surgery, radiotherapy, immunotherapy and chemotherapy and selection of technique for individual is based on situation. Many antitumor drugs such as Salinosporamide A are derived from marine actinobacteria, *Streptomyces tropic.* Actinomycin D isolated from *S. antibioticus* was one of the first natural component used in the treatment of tumour, this metabolite prevent the transcript's elongation by RNA polymerase by binding at the transcription initiating complex, this medicine is still used for treatment of wilms tumors in children (Sanchez, 2009). Other groups of anticancer metabolites which obtained in 1960 during process of *S. peuceticus* include doxorubicin, daunorubicin and anthracyclines. Epirubicin is recently identified anthracyclines group member which approved by FDA in 1999 and it has less side effects and good therapeutic result than doxorubicin. This anticancer antibiotic is used for treatment of leukemia, lungs cancer, breast cancer and ovarian cancer (Solecha, 2012).

1.7 Other secondary metabolites

Streptomyces sp produce secondary metabolite such as clavulanic acid or acarbose (α-glycosidase inhibitor) which can inhibit enzymes activity. Clavulanic acid produced by *S. clavuligerus,* inhibit β-lactamases, enzyme which hydrolysis β-lactam ring of antibiotics(Neto A. B, 2005) and acarbose isolated from *Actinoplanes* sp. Lipstatins include unusual β-lactone structure produced by *S. toxytricini,* This component irreversibly inhibit pancreatic lipase enzyme that limit intestinal fat absorption. Lipstatins are available as a member of a drug termed orlistat which is used in treatment of obesity and diabetes (Weibel, 1987).

Some *Streptomyces* species produce immunosuppressive secondary metabolites such as cyclosporine A, Tacrolimus and sirolimus (rapamycin). Tacrolimus is produced by *S. tsukubaensis*which was approved by FDA in 1994 to be used after liver and other organs transplantation, topically this component is used in atopic dermatitis. Sirolimus (rapamycin) which belongs to macrolides is the only non-nephrotoxic immunosuppressant produced by *S. hygroscopicus*; thus this component often used after kidney transplantations and it also used widely as antiproliferative component (Solecha, 2012).

Spinosyns (A, D) produced by *Saccharopolyspora spinosa* are insecticides which interact with neurotransmitters and brain receptors (nicotinic acetylcholine receptors). During fermentation, this organism produce mixture of spinosyn A as major component and spinosyn D, 6- methyl spinosyn as minor component. These components selectively target insects and they are less toxic to others (Kirst, 2010).

2. Strategies for enhancement of SM production

Studies show that biosynthetic potential of many bacterial and fungal strains are much higher than that observed by fermentation, by expression of silent pathways and genes chemical diversity available in the microorganism will be maximized. Activation method for silent (cryptic) metabolic pathways is either molecular techniques or cultivation dependent techniques (Robin K, 2011). For enhancement and production of large scale antibiotics, it requires knowledge of microbial producers, physiology, regulation of pathway involved

in biosynthesis of antibiotics and control methods (Hassan, 2012). L-form of *Streptomyces viridifaciens* which is induced by Lysozyme and penicillin producing antibiotic in less amount as compare to walled form (Innes, 2001), Very less changes in cultivation and incubation conditions such as temperature, pH, aeration or media composition, will completely change the metabolic property of microorganism. From cultivation of six microbes in different cultural conditions more than 100 chemical compounds of greater than 25 structural classes isolated (Robin K, 2011).

2.1 Microbial response

It has been proved that elicitors such as stress factors enhance secondary metabolites biosynthesis. These factors are classified based on their nature and origin; physical or chemical. Abiotic stress improves antibiotic production by *Streptomyces* spp. As a source of Carbone traditional carbohydrates have been used in fermentation processes. On the other hand they have also used as bacterial and fungal elicitor in small amounts (mg L^{-1}). In one approach, carbohydrate elicitors (oligoguluronate, oligosaccharides, oligomannuronate and mannan oligosaccharides) on fungal systems: *Ganoderma* spp, *Penicillium* spp and *Corylopsis* spp, improve production (Davati, 2013).

2.2 Quorum sensing

Communicating of cells to each other when their density arrives to threshold concentration, through the release of chemical signals is called Quorum sensing. This process for the first time discovered in bacteria (Gram negative, then in gram positive) and then in dimorphic fungi. This signals are not same for all microbial systems; examples are modifies or unmodified peptides, γ -butyrolactones and it derivatives and acyl homoserine. Many microbial physiological activities such as competence, conjugation, biofilm formation, motility, virulence, sporulation, symbiosis and different secondary metabolites are regulated by quorum sensing. This communication process is a great potential for enhancement of secondary metabolite production for industrial purpose(Davati, 2013).

Production of Secondary metabolites often stimulated by precursors either by induction of biosynthetic enzymes, increasing amount of limiting precursor or both. Small molecules other than precursor (usually amino acid) also act as inducers. Auto inducers are well known which includes oligopeptides of Gram-positive bacteria, N-acylhomoserine lactones of Gram-negative bacteria, γ-butyrolactones (butanolides) of Actinomycestes and B-factor of *Amycolatopsis mediterranei* (Demain, 1998)

Primary metabolites increase production of secondary metabolite either by supply of precursor and/or induction of one or more biosynthetic pathway. Inducer precursor include tryptophan in ergot alkaloid biosynthesis for dimethyl allyl tryptophan synthetase, methionine for ACV synthetase, expandase and cyclase in the pathway of cephalosporin in *Cephalosporiu macremonum*, leucine for bacitracin synthetase, valine for valine dehydrogenase of tylosin process in *Streptomyces fradiae*, lysine for lysine aminotransferase in cephamycin pathway in *Streptomyces clavuligerus*, and phenylacetate for its uptake system for penicillin G synthesis by *Penicillium chrysogenum*. Even production of SM production is induced by other than precursor molecules as production of polyketide, jadomycin B by *Streptomycin venezuelae*is induced by one hour heat shock or ethanol, both of them known to induce heat shock protein.

2.3 Chemical elicitation

Chemical elicitation includes chemical or compound of non-biological origin such as earth elements, heavy metals etc. chemical elicitation cause change metabolic profile and the response depend on how/which pathways are triggered, and how strong or weak the pathways are activated (Usama Ramadan abdelmohsen, 2015).

Table-1
Chemical elicitation of secondary metabolite

Elicitor	Strain	Induced Secondary metabolite	Mechanism of elicitation
Ethanol	*S. venezuela* eISP5230	Jadomycin B	Change In membrane permeability or serve as precursor or heat shock (Robin K, 2011)
DMSO	*S. venezuela e* ATCC 10712	Chloramphnicol	Effect on translational level(Robin K, 2011)
Lanthanum and Scandium	*S. coelicolor* A3	Actinorhodin	Upregulation of nine genes (Kozo Ochi, 2004)
ARC2	*S. coelicolor*	Germicidins A-C Actinorhodin	Inhibition of fatty acid biosynthesis
Sodium butyrate	*S. coelicolor*	Actinorhodin	HDAC inhibitor (Hua Zhu, 2014)

2.4 Genetic engineering

Strain improvement is an important field of microbiology for increasing the productivity of microbial strain, since wild strains which are isolated from nature are producing less amount of antibiotics, therefore strains improvement is a great deal to meet commercial requirements.. Ribosome engineering is an example for strain improvement in which the production of antibiotic is enhancing by modification of ribosome components (either rRAN or ribosomal protein). However high product can also be obtained by classical genetic methods without use of genetic tools or genomic information, but these methods are always resource and time consuming. In genetic engineering, overproduction of SM is regulated by structural genes which are directly involved in biosynthesis, regulatory genes, genes responsible for primary metabolites (affects biosynthesis of SM) and antibiotic resistance gene (Kozo Ochi, 2004)

Classical genetic methods are: Random selection of mutant, rational selection of Mutation and Recombination. The most practiced strategy to induce the yield of SM is Mutation generation. This strategy enhance yield of certain antibiotics 15 to 400 times as compare to wild strains.

2.4.1 Mutation and Random selection

This technique is relied on mutation by chemical mutagens such as ethane sulphonate and N-methyl -N-nitro –N-nitrosoguanidine or physical mutagens such as UV light, followed by random screening for selection of new improved mutants.

2.4.2 Mutation and rational selection

This technique is also called directed selection techniques, in which selection is done for particular characteristic of desired genotype which is different from final interest, but detection is easier. These methods need basic understanding of pathway regulation and metabolism of product. Like addition of toxic precursor to the medium of penicillin producing microorganism inhibits sensitive strains and resistant mutants which have capability to produce high amount of penicillin are propagated (Barrios-Gonzalez, 2003).

2.4.3 Recombination methods

Related species of fungi with high productivity of SM are recombined by protoplast fusion and new isolatewith new SM from fused strains is identified (Davati, 2013).

2.4.4 Molecular genetics improvement

Require understanding and tool for molecular genetics improvement including biosynthetic pathway, vectors and effective transformation protocols. Following strategies are used in this technique for secondary metabolite producing strains:

2.4.4.1 Secondary metabolite biosynthetic gene amplification

After identification of neutral site of chromosome where gene can be inserted without altering strain fermentation properties, then the neutral site is cloned in vector containing gene for antibiotic. After transformation neutral site is displaced by gene by homologous recombination. By duplication of *tyl*F of *Streptomyces fradiae* produce 60% more tylosin than parental strains (Barrios-Gonzalez, 2003).

2.4.4.2 Competitive pathways inactivation

Blocking pathway which competes key precursors such as cofactors, Could switch precursors to secondary metabolite biosynthesis. Gene disruption, transposon mutagenesis, or insertion of antisense gene, are used in such strategy. For example α-amino adipic acid is precursor for biosynthesis of penicillin, it also involve in lysine synthesis. By disruption of lys2 gene generate auxotroph fungi (to lysine amino acid) with 100% yields induction in penicillin production (Davati, 2013).

2.4.4.3 Amplification or disruption of regulatory genes

Amplification of ccaR[1] which is positive regulatory gene has led over production of β-lactam to three fold in *Streptomyces clavuligerus*. On the other hand disruption of mmy which is negative regulatory gene for methylenoomycin biosynthesis has led overproduction of actinorhodine up to 17 fold (Barrios-Gonzalez, 2003).

2.5 metabolic engineering

Modification of cells metabolism using molecular techniques such as recombinant DNA which allows manipulation of system with consideration of overall bioprocess efficiency. This technique is beneficial as compare to random mutagenesis and genetic engineering in several aspects, since it allows necessary engineering and avoiding unimportant changes of the cell. In some cases drug and precursors are found in natural microorganism but it is difficult to synthesize the drug or to extract in large scale. By help of metabolic engineering production of precursors and drug would be increased. Latest advances in systemic and synthetic biology (synthetic biology is strategy to activate the silent gene clusters (Usama Ramadan abdelmohsen, 2015) are allowing the engineering of the whole cell metabolism, which enabling desirable design of a cell for over production of drug precursors and drugs (Barrios-Gonzalez. J, 2003).

2.6 Biological elicitation

2.6.1 Elicitation by microbial co-culture

Actinomycestes exist with other microbes in various habitats, when microorganisms interact to gather it may turn on cryptic gene clusters which trigger production of novel metabolites. Co-cultivation based elicitation of microorganisms may lead production of compounds which monoculture may not be able to produce it. There are many possible scenarios for example, physical cell – cell interaction, production of enzymes which activate production of precursor and production of small molecule such as quorum sensing molecules/ auto regulator, siderophores, etc (Marmann, 2014). Co-culture could be done within members of same or different family.

2.4.1.1. Co-culture ofdifferent bacteriaof same family

By co-cultivation of two marine actinomycetes *Actinokineospora* sp EG49 and *Nocardia* sp. RV163 three new metabolites produced, which were not produced by any of these two individually, these metabolites are (1) N-(2-hydroxyphenyl)-acetamide, (2) tetrahydro dimethyl benzoxazinobenzoxazine and (3) 1,6-dihydroxyphenazine (Dashti Yousef, 2014). Co-Culture of multi drug resistant mutant of *Streptomyces padanus* with *Rhodococcus fascians* led to emergence of *Rhodococcus*307CO, fermentation of this emerge bacteria led to synthesis of new class of aminoglycosides, rhodostreptomycins A and B (Kurosawa Kazuhiko, 2008).

2.4.1.2 Co-Culture of bacterial species of different families

Microbial predator which kill and feed on other live bacteria by utilizing their nutrients can also activate the expression of secondary metabolite (antibiotic) producing microbial cell. It has shown that *Myxococcu sxanthus,* a predator of *S. coelicolo* induce the production of actinorhodin. On the other hand actinorhodin producing microorganism use this component as repellant signals which inhibit growth of *M. xanthus*in its surrounding aria (Juana Perez, 2011).

By co-culture of *S. griseorubiginosus 43703* as producer strain and *Pseudomonas maltophilia 1928* cause 60 fold enhance in production of antibiotic biphenomycin A as compare to production in pure culture. Even treating the above organism with lysate extract of *Pseudomonas maltophilia*1928 led to production of biphenomycin A, which means that enhancement is not due to cell- cell instruction but specific enzyme of *Pseudomonas maltophilia*1928 is responsible, which convert the precursor of above antibiotic to its active form (Masami Ezaki, 1992). In co-cultivation of *Streptomyces* and *T. pulmonis* new SM produced, which are alchivemycin and TPU-0043.This

metabolites were absent in pure culture of *Streptomyces* (Igarashi, 2005).

2.4.1.3 Co-culture of bacteria with fungi

By co-culture of *S. peuceticus* ATCC 29050 and *Aspergillus fumigatus*, two new metabolites were produced, they are sulfated formyl xanthocillin analoque fumiformamide and diformamide. *Co-cultivation of S.rapamycinicus and Aspergillus fumigates* led to activation of novel fumicyclines A and B in *Aspergillus fumigates* (Usama Ramadan abdelmohsen, 2015). By co-culture of *S. leeuwenhoekii*C34 and *A.fumigatus*MR2012 on ISP2 medium (yeast extract, malt extract, dextrose and agar) led to bacterial metabolite chaxapeptin7 production, this product was not obtained in pure fermentation using sex medium (Jennifer Wakefield, jully 2017).

Conclusion

Genetic engineering technologies such as gene recombination and mutation and Co-cultivation have led to notable improvement in the production ability of many primary and secondary metabolites. Latest genetic approaches for improvement of strain for overproduction are emerging. Among them metabolic engineering, ribosome engineering combinatorial biosynthesis and molecular breeding have been proven to be successful.

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References

[1]. Barrios-Gonzalez. J, F. F. (2003). Microbial secondary metabolites production and strain improvement. *Indian Journal of Biotechnology*.
[2]. Buttner, F. K. (2009). Streptomyces morphogenetics dissecting differentiation in a filamentous bacteriumd. *Nature review microbiology, 7*.
[3]. Dashti Yousef, T. G. (2014). Production of induced secondary metabolites by a Co-Culture of sponge-associated Actinomycetes, Actinokineospora sp. EG49 and Nocardioopsis sp. RV163. *Marine drugs*, 3046-3059.
[4]. Davati. N, a. H. (2013). Overproduction strategies for microbial secondary metabolites. *International Journal of Life science & pharma Research*.
[5]. de lima Procopio. R. E, d. S. (2012). Antibiotics Produced by Streptomyces. *Infectious Diseases, 16*(5), 466-471.
[6]. Demain, A. L. (1998). Induction of Microbial Secondary Metabolites. *Internatl Microbiol*(1), 259-264.
[7]. Denyer, S. P. (2008). *Hugo and Russell's pharmaceutical microbiology.* John Wiley & sons.
[8]. Elizaveth jane ashforth, C. F. (2010). Bioprospecting for antituberculosis leads from microbial metabolites. *Natural product reports*.
[9]. Etebu. E, A. L. (2016). Antibiotics: Classification and mechanism of aciton with emphasis on molecular perspectives. *International Journal of Applied Microbiology and Biotechnology Research*, 90-101.
[10]. Gavin J. Clark, D. L. (1995). Oxygen limitaiton can induce microbial secondary metabolite formation: investigations with minature electrodes in shaker and bioreactore culture. *Microbiolgy*, 663-669.
[11]. Gupta C, P. D. (2014). Natural Usful Therapeutic Products from Microbes. *Microbiology & Experimentation, 1*(1), 1-8.
[12]. hasani. A, K. A. (2014). Streptomycetes: Characteristis and their Antimicrobial Activities. *International Journal of Advanced Biological and Biomedical Research, 2*(1), 63-75.
[13]. Hassan, M. A. (2012). Antibiotics as Microbial Secondary Metabolites: Production and Application. *Teknologi*.
[14]. Hejmadi, M. (2010). *Introduction to Cancer Biology* (Vol. 2).
[15]. Hiroshi Mishima, J. I. (1983). Milbemycins, and new family of macrolide antibiotics structure determination of milbemycins D, E, F, G, J AND K. *Antibioitics*.
[16]. Hua Zhu, S. K. (2014). Triggers and cues that activate antiviotic produciton by actinomcetes. *Microbial biotechnology*, 371-386.
[17]. Igarashi, Y. (2005). Antibiotic production in Streptomyces induced in co-culturing system. *NISR Research GRANT*.
[18]. Innes. C.M.J, A. E. (2001). Induction. growth and antibiotic production of Streptomyces viridifaciens L-form bacteria. *Applied microbiology*, 301-308.
[19]. Jennifer Wakefield, H. M. (jully 2017). Dual induction of new microbial secondary metabolites by fungal bacteial co-cultivation. *Frontiers in microbiology*.
[20]. Juana Perez, J. M.-D. (2011). Myxococcus xanthus induces actinorhodin overproduction and aerial mycelim formatio by Stretomyces coelicolor. *microbial biotechnology*, 175-183.
[21]. Kirst, H. A. (2010). Ther spinosyn family of insecticides: realizing the potential of natural products research. *Antibiotics*, 101-111.
[22]. Kozo Ochi, S. O. (2004). Ribosome engineering and Secondary metabolite Production. *Advanced in applied microbiology, 56*.
[23]. Kurosawa Kazuhiko, I. G. (2008). Rhodostreptomycins, Antibiotics Biosynthesized following Horzontal gene transfer from streptomyces padanus to rhodococcus fascians. *JACS Communications*.
[24]. Mamatha J, S. B. (2014). Production of streptomycin from Streptomyces griseus under solid state fermentation & its production enhancemet by mutation and analysis by HPLC. *world journal of pharmacy and pharmaceutical sciences* .
[25]. Manivasagan. P, V. J. (2014). Pharmaceutically Active Secondary Metabolites of Marines Actinobacteria. *Microbiological research*, 262-278.
[26]. Marmann. A, A. A. (2014). Co-Cultivation-A Powerful emerging Tool for Enhancing the chemical diversity of Microorganism. *Marine drugs, 12*(2), 1043-1065.
[27]. Martinko, M. a. (1997). *Brock biology of microorganisms* (11 ed.).
[28]. Masami Ezaki, N. S. (1992). Biophenomycin C, A precursor of Biphenomycin A in mixed culture. *Antibiotic, 46*(1).
[29]. Micah D. shepherd, M. K. (2010). Laboratory Maintenance of Streptomyces Species. *curr proto microbiol* .
[30]. Neto A. B, H. D. (2005). A study on clavulanic acid production by streptomyces clavuligerus in batch, fed-batch and continuous processes. *Brazilian Journal of Chemical Engineering, 22*(4), 557-563.
[31]. Paolo Monciardini, M. l. (2014). Discovering new bioactive molecules from microbial source. *Microbial biotechnology*, 209-220.
[32]. Prakash S, R. R. (2012). Screening and partial purification of antifungal metabolite from Streptomyces rochei MSA14: AN isolate from marine mining soil of southwest coast of india. *Indian Journal of Geo- Marine Sciences, 42 (7)*, 888-897.

[33]. Rajendra P. Masker, E. H. (2004). Anti-cancer and antibacterial Trioxacarcins with high anti-malaria activity from marine Streptomycete and their absolute stereochemistry. *Marine bacteia* .

[34]. Ram, L. (2014). Optimization of medium for the production of Streptomycin by Streptomyces griseus. *International journal of pharmaceutical science invention*, 1-8.

[35]. Rao, N. D. (2010). Secondary metabolites and other small molecules as intercellular pathogenic signals. *FEMS Microbial lett*.

[36]. Robin K, P. (2011). Small-molecule elicitation of microbial secondary metabolites . *microbial biotechnology*, 471-478.

[37]. Sanchez, A. L. (2009). Microbial drug Discovery: 80 years of progress. *Antibiotics*, 5-16.

[38]. Sharma. A, K. N. (April-2014). Bioactive secondary Metabolites: an Overview. *International Journal of Scientific & Engineering research, 5*.

[39]. Sheila Marie Pimentel-Elardo, e. a. (2010). Anti-Parasitic Compounds from Streotinyces sp. Strains Isolated from Mediterranean Sponges. *Marine drugs*.

[40]. Solecha. J, Z. J. (june 2012). Biologically active secondary metabolites from Actinomycetes. *Central European Journal of Biology*.

[41]. Usama Ramadan abdelmohsen, T. G. (2015). Elicitation of secondary metabolism in actinomycetes. *Elsevier*.

[42]. Weibel. E. K, H. P. (1987). Lipstatin, an inhibitor of pancreatic lipase produced by streptomyces toxytricini producing organism, fermentation, isolateion and biological activity. *Antibiotics*.

[43]. Weiling Hong, J. Z. (2014). Antibiotic drugs targeting bacterial RNAs. *Acta Pharmaceutica Sinica B*.

[44]. Zhang, L. (2003). Integrated Approaches for Discovering Novel Drugs form Microbial Natural products. *Appl Microbial Biotechnol*, 226-458.

