Marine actinomycetes sediment samples were collected from Gulf of Mannar coastal region, Koyalpatnam, located at Tuticorin district, Tamilnadu, India. Marine actinomycetes were isolated and evaluated for activity of L-asparaginase production. A total of 10 marine actinomycetes strains were isolated. Among 10 isolates, six were belongs to Streptomyces sp, three were belongs to Micromonospora sp and one was to Micropolyspora sp. Based on phenotypic characteristics, actinomycetes strains were screened for L-asparagine production. Streptomyces sp KPMS5 and Micromonospora sp KPMS10 were showed large pink coloration on L-asparaginase production medium. The strains were further studied for maximum production and characterizations of culture condition of L-asparaginase enzyme were evaluated. Effect of substrate on L-asparaginase production was evaluated by enzyme assay. Maximum enzyme assay (1.4 mM) was observed on glucose followed by starch (1.12Mm) and then by lactose (1.17mM). Yeast extract was effectively used as substrate for maximum production of L-asparaginase by submerged fermentation. Further studies on purification and characterization are required to support the application of enzyme. The finding concludes isolates belongs to non-Streptomyces sp like Micromonospora sp is a potential novel source for L-asparaginase production.

Keywords: Leukemia; enzymes; L-asparagines; drugs; anticancer

Introduction

Many enzymes especially L-asparaginase has been attracted towards the use effective therapeutic agent against lymphocytic leukemia and many other types of cancer caused in human. Tumor cells is required to synthesis high amount of amino acid asparagines but it unable to synthesize their own, which is very closed for the better growth tumor cell, whereas non-tumor cell can synthesis their own asparagines and the cell growth is independent of its requirement (Pieters et al., 2011; Jha et al., 2012). Asparaginase is a major component required for the synthesis of protein molecules. These can be synthesized by body itself, with in the cell by an enzyme called asparagines synthetase or can be taken from outside of the cell. L-asparaginase (L– asparagines amidohydrolase E.C.3.5.1.1) is an enzyme which converts L – aspartic acid and ammonia has been used as a chemotherapeutic agent in the treatment of acute lymphoblastic leukemia there by prevents tumor cells from rapid malignant growth for over 30 years. These enzymes are selectively killed by L-asparagene deprivation. The clinical action of this enzyme is attributed to the reduction of L-asparagine (Amena et al., 2010). L-asparaginase production by microorganisms are basically used in clinical practices because the diversity of microbial population is being explored for gathering novel sources of L-asparaginase production with less side effects and better treatment(Geckil and Gencer, 2004).

Screening of microbial sources for L-asparaginase activity has been greatly intensified and well documented in filamentous fungi, yeast and bacteria like E.coli, Vibrio succinogenes, Erwinia carotovora (Deokar et al., 2010) and Bacillus sp. Among the fungi, Mucor sp., Penicillium sp., and yeast like Candida utilis have been proved to be potential producers of this enzyme (Joseph and Rajan, 2011).

Actinomycetes have gained special importance as the most potent source of bioactive secondary metabolites. Actinomycetes are group of thread like bacterial and look like fungi with high G +C content which form branching filaments or hyphae and asexual reproduction (Peela et al., 2005). Some actinomycetes, Streptomyces karnatakensis, Streptomyces griseus (Dejong,1972), Streptomyces venezuelae , Streptomyces longisporusflavus, Streptomyces
gulbargensis, marine Streptomyces sp PDK2 and Nocardia asteroids (Gunasekaran et al.,1995) have been reported to be a potential L-asparaginase synthesizer. Various scientists have described the role of L-asparaginase in the treatment of cancer and cancer biology. A vast amount of investment has been made for the enzyme to explore the new effective ways. This research focuses on the better understanding of the L-asparaginase production by marine actinomycetes.

**Methodology**

The isolation and identification of actinomycetes from marine sediments sample was collected from Gulf of Mannar costal region, Kayalpatinam, located at Tuticorin district, Tamil nadu, India. The marine sediments were collected and processed by serial dilution. One ml of diluted sample was permitted in to the starch casein agar medium supplemented with cyclohexamide 10µg/ml. Suspected actinomycetes were purified and identified by spore and biochemical properties.

**L-Asparaginase Production by Plate Assay** (Gulati et al., 1997).

Screening of marine actinomycetes for L-asparaginase production was performed on minimal medium incorporated with phenol red indicator by plate assay. This is the qualitative method used for L-asparaginase. The enzyme L-asparaginase was acted as substrate for L-asparaginase production. After incubation, the phenol red indicator turns pink zone around the colonies due to formation of ammonia at alkaline pH which indicates the production L-asparaginase.

**Optimization of L-Asparaginase Production**

The effect of carbon sources such as glucose, starch, lactose, mannitol and xylose on L-asparaginase production with peptone and yeast extract nitrogen sources was evaluated by submerged fermentation. The minimal media was prepared with 1% concentration of above mentioned carbon source. Two set up was prepared and one set up was supplemented with peptone (0.1%) and another one with yeast extract. The growth rate and enzyme assay were performed after 24 h.

**Purification of L-Asparaginase**

The purification of enzyme was carried out by using crude enzyme extract (Distasio et al., 1982).The enzyme purification was performed by ammonium sulphate precipitation. Finely powdered ammonium sulfate was added to the crude extract. The L-asparaginase activity was associated with the fraction precipitated at 80% saturation. The precipitated was collected by centrifugation at 9,000 rpm for 15 min and dissolved in 1M Tris-HCl buffer and dialyzed against the same buffer.

**Total Protein Estimation**

Estimation of protein was determined by using lowery et al method. A stock solution of standard protein, BSA at a concentration of 1000 µg/ml was made. From these aliquots, 0.2-1 ml of working standard at concentration of 100 µg/ml was taken in a test tube. All the test tubes were made up to 1ml with distilled water.1 ml of FC reagents was added to each test tube. After 30 min of incubation, the absorbance was measured at 660 nm by using UV-Vis spectrophotometer.

**Enzyme Assay**

The enzyme assay performed on M-9 broth medium for L-asparaginase enzyme production. The selected marine actinomycetes were sub cultured and inoculated in to M-9 broth medium. All the production flasks were kept at rotary incubator shaker for 72 h. After incubation, 10 ml of the cell suspension was taken and centrifuged. Quantitative detection was carried out by Nessler reagent method. 0.5 ml of cell suspension,1 ml of 0.1M sodium borate buffer(pH 8.5) and 0.5ml of 0.04M L-asparaginase solution were mixed and incubated at 37 C for 10 min. The reaction was then stopped by the addition of 0.5 ml of 0.1N trichloroacetic acid. The precipitated protein was removed by centrifugation and the liberated ammonia was determined by direct nesslerization. Each sample was individually mixed with 1ml of 1N NaOH and 0.2 ml of 0.1M EDTA was added. After 2 min, 0.5 ml of nessler’s reagent was added and mixed well. After 5 min, the addition of nessler’s reagent to the sample, the yellow color was read on 117 UV-Vis spectrophotometer (Systronic) and the optical density of the sample was recorded at 450 nm.

**Results and Discussion**

Totally 10 marine actinomycetes were isolated and identified as Micromonaspora sp, Streptomyces sp and Micropolyspora sp (Table 1). All the marine actinomycetes were differing morphologically based on the color of colony, types of mycelium, spore and pigmentation. Among the ten isolates, six isolates were belongs to Streptomyces sp(60%) that are predominantly present in marine environments followed by three isolates were belongs to Micromonaspora sp(30%) and one isolate to Micropolyspora sp (10%).The dominance of Streptomyces sp is plentiful source in marine sediments and are reported by many workers (Lu et al., 2009; Hassan et al., 2011). Most of the strains produced spiral chains of spore are often spirally coiled and two of them were produced retractile spiral spore on substrate mycelium indicates the genera of Streptomyces sp. Mono spore was observed on actinomycetes colonies were belongs to the genera of Micromonaspora sp was produced branched, non-motile and mono spore present only on substrate mycelium. The aerial mycelium was unstained by sudan black and the spore surface was smooth whereas the substrate mycelium was rough and stained by sudan black. The aerial mycelia were initially white and turned to ash, grey and dull white or chalky white on starch casein agar. The Micropolyspora sp KPMS 4 produced chain of spore with fragmented mycelium. All the isolated marine actinomycetes found in gram positive cell wall type. The group of actinomycetes
was currently updated with spore forming gram positive bacteria that form extensive branching substrate and aerial mycelia (Waksman, 1961).

Species among the genera were differentiated based on the physiological properties (Table 2). Among the *Streptomyces* sp, three were found in catalase positive (KPMS2, KPMS5 & KPMS8). All *Streptomyces* sp were utilized starch and in oxidase except KPMS3. Similarly, *Streptomyces* sp of KPMS3 and KPMS6 were urease positive others were failed to obtain urease reaction. Among the *Micromonospora* sp, KPMS1 were positive all types of physiological reaction. Similarly, KPMS9 was positive only in urease reaction and KPMS10 was negatively found in starch utilization. *Micropolyspora* sp KPMS4 was negatively found in catalase, oxidase, urease and partially positive in starch. Isolation and characterization of promising strain of marine actinomycetes research is a major area for many years in worldwide (Laidi et al., 2006). In this purpose, identification of genera and species of actinomycetes, besides morphological and physiological properties were performed for the potent identification of actinomycetes isolates (Kuster, 1972; Abbas, 2006). Out of 10 isolates, 3 *Streptomyces* sp designated as KPMS 5, KPMS 6, KPMS 8 and one *Micromonospora* sp KPMS 10 were produced melanoid pigments. Actinomycetes are characterized by the production of various pigments on natural or synthetic media. The pigments may be dissolved in to the medium or it may be retained in the mycelium. Actinomycetes were known to be produced number of secondary metabolites and moreover these metabolites included many pigments. Production of pigments by marine actinomycetes has been utilized as an important cultural characteristic in describing the organisms (Miyaura and Tatsumi, 1960).

### Table 1: Morphology of Isolated Marine Actinomycetes

| S.N. | Strain code | Spore morphology                  | Color of aerial mycelium | Cell wall type | Isolated Genus     |
|------|-------------|-----------------------------------|--------------------------|----------------|-------------------|
| 1    | KPMS1       | Septate hyphae with monospore      | Greenish grey            | Gram positive | *Micromonospora* sp |
| 2    | KPMS2       | Spiral chain of spore              | White                    | Gram positive | *Streptomyces* sp |
| 3    | KPMS3       | Long chain of spore                | Ash                      | Gram positive | *Streptomyces* sp |
| 4    | KPMS4       | Chain of spore on fragmented hyphae| Ash                      | Gram positive | *Micropolyspora* sp |
| 5    | KPMS5       | Spiral chain of spore              | Dark greenish ash        | Gram positive | *Streptomyces* sp |
| 6    | KPMS6       | Moderate length of chain of spore  | Grey                     | Gram positive | *Streptomyces* sp |
| 7    | KPMS7       | Long chain of spore                | Sandal white             | Gram positive | *Streptomyces* sp |
| 8    | KPMS8       | Rarely branched spiral spore       | Light grey               | Gram positive | *Streptomyces* sp |
| 9    | KPMS9       | Septate hyphae with monospore      | Dull white               | Gram positive | *Micromonospora* sp |
| 10   | KPMS10      | Septate hyphae with monospore      | Whites ash               | Gram positive | *Micromonospora* sp |

### Table 2: Physiological characteristics of Isolated Marine Actinomycetes

| S.N. | Name of the Isolates | Catalase | Oxidase | Starch | Urease | Melanoid pigments |
|------|-----------------------|----------|---------|--------|--------|-------------------|
| 1    | *Micromonospora* sp KPMS1 | +        | +       | +      | +      | -                 |
| 2    | *Streptomyces* sp KPMS2 | +        | +       | +      | -      | -                 |
| 3    | *Streptomyces* sp KPMS3 | -        | -       | +      | +      | -                 |
| 4    | *Micropolyspora* sp KPMS4 | -        | -       | ±      | -      | -                 |
| 5    | *Streptomyces* sp KPMS5 | +        | +       | +      | -      | +                 |
| 6    | *Streptomyces* sp KPMS6 | -        | +       | +      | +      | +                 |
| 7    | *Streptomyces* sp KPMS7 | -        | +       | +      | -      | -                 |
| 8    | *Streptomyces* sp KPMS8 | +        | +       | +      | -      | +                 |
| 9    | *Micromonospora* sp KPMS9 | -        | -       | +      | -      | -                 |
| 10   | *Micromonospora* sp KPMS10 | +        | +       | -      | +      | +                 |

+ : Positive; - : Negative; ± : Partial
All the ten actinomycetes were screened for L-asparaginase production at 37°C for 24-72h. Among 10 isolates, two isolated were showed L-asparaginase activity on plate assay method by pink colour zones formation around the actinomycetes growth which were belongs to genera of Streptomyces sp KPMS5 and Micromonospora sp KPMS10. Further, these isolates were subjected to secondary screening of enzyme activity. The production of L-asparaginase by various microorganisms using phenol red dye for screening techniques has been reported by other researcher (Sarquis et al., 2004). Marine Streptomyces is serving as good source of L-asparaginase enzyme and converts L-asparagine to L-aspartic acid and ammonia. It has been used as a chemotherapeutic agent (Fisher and Wray, 2002). Among them, Micromonospora sp KPMS10 showed higher activity. To the best of our knowledge, this is the first report on the production of L-asparaginase. The results was presented as marine actinomycetes are a good source of L-asparaginase production. Our reports was accordance with other worker (Savitri et al., 2003).

The Streptomyces sp KPMS5 was able to use different carbon and nitrogen sources. Each carbon source and nitrogen source were incorporated in to basal medium at 1% and 0.1% concentration respectively. Among carbon sources, the maximum growth rate was found on glucose given an OD 1.58 with yeast extract by Streptomyces sp KPMS5 and least growth rate in xylose (OD 0.12). Among carbon sources supplemented with peptone, maximum growth of Streptomyces sp KPMS5 was 1.28 OD value and a minimum of 0.1 OD value respectively for glucose and xylose (Fig. 1).

Similarly, Micromonospora sp KPMS10 to obtain the maximum growth rate was found on glucose at 1.18 OD value by and less rate in xylose at 0.11 OD value with yeast extract as nitrogen source (Fig. 2). In various carbon sources along with peptone, the maximum growth rate was observed in 1.02 OD value and a minimum of 0.1 OD value respectively for glucose and xylose. As previously described, yeast extract has been used as a potent nitrogen source for production of enzymes L-asparaginase by Streptomyces albidoilavus and Streptomyces ginsengisoli. The significant growth rate of Micromonospora sp KPMS10 and Streptomyces sp KPMS5 were showed on glucose with yeast extract. Production of enzymes L-asparaginase was highly found in the presence of glucose as carbon substrate by a river isolate of Streptomyces ginsengisoli (Neelima Deshpande et al., 2014). Among the carbon source, glucose, lactose and starch were found to be suitable for growth of actinomycetes whereas xylose was significantly affect the growth rate.

The enzyme assay of Nessler’s method reveals that the strain Micromonospora sp KPMS10 was found to be maximum extracellular L-asparaginase producer than Streptomyces sp KPMS5 under submerged conditions. The maximum enzymatic activity of Micromonospora sp KPMS10 was found in 1.4mM on glucose followed by 1.12mM on starch and minimum of 0.1mM on xylose with yeast extract. In peptone source, Micromonospora sp KPMS10 was found in 1.13mM on glucose followed by 0.96mM on starch and minimum of 0.1mM on xylose (Fig. 3). The Streptomyces sp KPMS5 was found a maximum of 1.25mM on glucose followed by 0.46mM on lactose and least of 0.13mM on xylose with yeast extract source. In peptone source, Streptomyces sp KPMS5 was found 0.77mM on glucose followed by 0.46 mM on lactose and least of 0.13 mM on xylose (Fig. 4). Micromonospora sp are potential actinomycetes to produce secondary metabolite with diverse chemical structure and biological activity. Currently, diazepinomicin is a unique farnesylated...
dibenzodiazepine and used as anticancer metabolite are derived from marine actinomycetes *Micromonospora* sp (Charan et al., 2004). The potential actinomycetes exhibited both intra and extracellular production of L-asparaginase by *Streptomyces longsporusflavus* (Abdel-Fattah and Olama, 1998).

![Fig. 4: L-asparaginase assay of Micromonospora sp KPMS10 with yeast extract and peptone.](http://nepjol.info/index.php/IJASBT)

Difference on production of L-asparaginase with yeast extract and peptone was observed and evaluated at 24h. Among the two nitrogen sources, maximum enzymatic activity was found on yeast extract source given by *Micromonospora* sp KPMS10 and *Streptomyces* sp KPMS5. Yeast extract has been reported as a good nitrogen source for the production of L-asparaginase by *Streptomyces albidoflavus* (Narayana et al., 2008). L-asparaginase activity in *Streptomyces* sp isolated from the foregut and hindgut region of clam, *V. cyprinoids* (Mathew et al., 1994; Koshy et al., 1997). The maximum rate of L-asparaginase production by *Amycolatopsis* CMU-H002 was amended with starch as the carbon source (Khamna et al., 2009). Among the carbon source, glucose and starch showed the immediate conversion of L-asparagines into ammonia was observed within 24 h under submerged condition. The higher enzymatic activity occurred in submerged fermentation by *Streptomyces* sp (Sahu et al., 2007). Carbon sources except xylose and other sugar molecules showed L-asparaginase enzymes activity to be found after 48 h of incubation. The least L-asparaginase activity was found on xylose as from 0.1mM by both tested actinomycetes.

The L-asparaginase was partially purified by ammonium sulphate precipitation and analyzed further for characterization of protein. The total protein content was found to be 2 mg/ ml and 1.8 mg/ml for *Streptomyces* sp KPMS5 and *Micromonospora* sp KPMS10. The production and purification of L-asparaginase by marine actinomycetes *Streptomyces tendae* TK-VL-333 isolated from sediment samples of Parangipettal and Cochin coastal region in South India has been reported (Kavitha and Vijayalakshmi, 2010). The isolate PDK7 and PDK8 from Parangipettal and Cochin coastal region in South India, were gave potential L-asparaginase activity (Dhaval et al., 2006). Similarly, among 10 actinobacterial strains, three isolate S3, S4 and S8 belongs to *Streptomyces* sp were showed extracellular production of anti-leukemic enzyme L-asparaginase (Basha et al., 2009). Several terrestrial *Streptomyces* are capable of producing detectable amounts of L-asparaginase. There are limited reports on production of L-asparaginase from marine *Streptomyces* like *Streptomyces aurantiacus* (Gupta et al., 2007). *Streptomyces* and *Micromonospora* sp are distributed in both marine and terrestrial environments. They have unique features to produce novel bioactive metabolites such as antibiotics, enzymes and antitumor compounds (Mitchell et al., 2004).

**Conclusion**

Marine actinomycetes are one of the most eminent secondary metabolites synthesizers and are very important in industrial point of view. Enzyme after antibiotics are most important metabolites derived from actinomycetes. This study concludes that *Streptomyces* sp and *Micromonospora* sp is an efficient producer of L-asparaginase. Marine actinomycetes are well organized synthesizer of both extracellular and intracellular L-asparaginase and holds potential for treatment of acute lymphocytic leukemia and cancer in other types. With the increasing advancement in modern science, there would be a greater demand for new metabolites synthesized from marine action bacteria in future.

**Acknowledgement**

We thank the Department of Microbiology, Jamal Mohamed College (Autonomous), Tiruchirappalli-620020, for supporting and fulfilling all the needs to carry out this work.

**References**

Abbas IH (2006) A biological and biochemical studies of Actinomycetes isolated from Kuwait saline soil-Kuwait. *Journal Applied Science Research* 2: 809-815.

Abdel-Fattah YR and Olama ZA (1998) Studies on the asparaginolytic enzymes of *Streptomyces*. *Egypt Journal of Microbiology* 30: 155-159.

Amena S, Vishalakshi N, Prabhakar M, Dayanand A and Lingappa K (2010) Production, purification and characterization of L-asparaginase from *Streptomyces galbargensis*. *Brazilian Journal of Microbiology*. 41(1): 173-178. DOI: 10.1590/S1517-83822010000100025

Basha SN, Rekha R, Komala M and Ruby S (2009) Production of extracellular anti-leukaemic enzyme L-asparagasename from marine actinomycetes by solid-state and submerged fermentation: Purification and Characterization. *Tropical Journal of Pharmaceutical Research* 8: 353-360. DOI: 10.4314/tjpr.v8i4.45230

Charan RD, Schlingmann G, Janso J and Carter GT (2004) Diazepinomicin, a new antimicrobial alkaloid from a marine *Micromonospora* sp. *Journal of Nature Product*. 67: 1431-1433. DOI: 10.1021/mp040042r

Dejong PJ (1972) L-asparaginase production by *Streptomyces griseus*. *Applied Microbiology* 23(6): 1163-1164.
Deokar VD, Vetal MD and Lambert R (2010) Production of intracellular L-asparaginase from Erwinia caratovora and its statistical optimization using response surface methodology. International Journal of Chemical Science and Application 1(1): 25-26.

Dhavala P, Krasotkina J, Dubreuil C and Papageorgiou AC (2006) Isolation and characterization of L-asparaginase from marine actinomycetes. Indian Journal of Biotechnology 5: 514-520.

Distasio JA, Salazar AM, Nadji M and Durden DL (1982) Glutaminase-free asparaginase from Vibrio succinogenes: an antilymphoma enzyme lacking hepatotoxicity. International Journal of Cancer. 30(3):343-347. DOI: 10.1002/ijc.2910300314

Fisher SH and Wray LV (2002) Bacillus subtilis L168 contains two differentially regulated genes encoding L-asparaginase. Journal of Bacteriology. 184: 2148-2154. DOI: 10.1128/JB.184.8.2148-2154.200

Geckil H and Gencer S (2004) Production of L-asparaginase in Enterobacter aerogenes expressing Vireoscilla hemoglobin for efficient oxygen uptake. Applied Microbiology and Biotechnology. 63(6):691-697. DOI: 10.1007/s00253-003-1482-5

Gulati R, Saxena RK and Gupta R(1997) A rapid plate assay for screening L-asparaginase producing microorganisms. Letters in Applied Microbiology. 24(1):23-26. DOI: 10.1046/j.1472-765X.1997.00331.x

Gunasekaran S, Mc Donald L, Manavathu M, Manavathu E and Gunasekaran M(1995) Effect of culture media on growth and L-asparaginase production in Nocardioides asteroidis. Biomedical Letter. 52(207): 197-201.

Gupta N, Mishra S and Basak UC (2007) Occurrence of Streptomyces aurantiacus in mangroves of Bhitarankanika. Malaysian Journal of Microbiology. 3:7-14.

Hassan AA, El-Barawy AM, Mohkat EL and Nahed M (2011) Evaluation of biological compounds of Streptomyces sp for control of some fungal diseases. Journal of American Science. 7:752-60.

Jha SK, Pasrija D, Sinha RK, Singh HR, Nigam VK and Vidyarthi AS (2012) Microbial L-asparaginase: a review on current scenario and future prospects. International Journal of Pharmaceutical Sciences and Research 3(9): 3076.

Joseph B and Rajan SS (2011) L-Lysine alpha oxidase from fungi as an anti tumor enzyme agent. Advanced Biotechnology 10 (8):27-30.

Kavitha A and Vijayalakshmi M (2010) Optimization and purification of L-asparaginase produced by Streptomyces tendae TK-VL. Z Naturforsch. 65c:528-531.

Khamma S, Yokota A and Lumyong S (2009) L-asparaginase production by actinomycetes isolated from some Thai medicinal plant rhizosphere soils. International Journal of Integrative Biology 61(1): 22-26.

Koshey A, Dhvedendaran K, Geogekutty MI and Natarajan P (1997) L-asparaginase in Streptomyces pilatus isolated from the alimentary canal of the fish, Gerres filamentosus (Cuvier). Journal of Marine Science. 5:181-185.

Kuster E (1972) Simple working key for the classification and identification of named taxa included in the international Streptomyces project. International Journal of Systematic Bacteriology 22: 139-148. DOI: 10.1099/00207713-22-3-139

Laidi RF, Kansoh AL, Elshafei AM and Cheikh B (2006) Taxonomy, Identification and biological activities of novel isolate of Streptomyces tendae. Arab Journal of Biotechnology. 9: 427-436.

Lu Y, Dong X and Liu S (2009) T-murolol sesquiterpenes from the marine Streptomyces sp M491 and revision of the configuration of previously reported amorphanes. Journal of Natural Product. 72:99-101. DOI: 10.1021/np8006843

Mathew A, Dhvedendaran K, Geogekutty MI and Natarajan P (1994) L-asparaginase activity in antagonistic Streptomyces sp associated with clam villorita cyprinoides (Hanley). Indian Journal of Marine Science. 23:204-208.

Mitchell SS, Nicholson B, Teisa S, Lam KS and Potts BC(2004) Aureoverticillactam, a novel 22-atom macrocyclic lactam from the marine actinomycetes Streptomyces aureoverticillatus. Journal of Natural Product. 67:1400-1402. DOI: 10.1021/np049970g

Miyaura J and Tatsumi C (1960) Studies on the antibiotics from actinomycetes an antibiotics pigments from Streptomyces F-23b. Bull Univ Osaka Pref Ser B. 1: 129-137.

Narayana KJP, Kumar KG and Vijayalakshmi M (2008) L-asparaginase production by Streptomyces albidoflavus. Indian Journal of Microbiology. 48:331-336. DOI: 10.1007/s12088-008-0018-1

Neelima Deshpande, Prachi Choubey and Manasi (2014) Ashagie studies on optimization of growth parameters of L-asparaginase production by Streptomyces ginsengisoli. Scientific World Journal. 89:51-67.

Peela S, Kurada VVSNP and Terli R (2005) Studies on antagonistic marine actinomycetes from the bay of Bengal. World Journal of Microbial Biotechnology. 21:583-585. DOI: 10.1007/s11274-004-3493-9

Pieters R, Hunger SP, Boos J, Rizzari C, Silverman L and Baruchel J (2011) L-asparaginase treatment in acute lymphoblastic leukemia: a focus on Erwinia asparaginase. Cancer. 117(2):238-242. DOI: 10.1002/cncr.25489

Sahu MK, Sivakumar K, Poorani E, Thangaraju T and Kannan L(2007) Studies on L-asparaginase enzyme of actinomyces isolated from estuarine fishes. Journal of Environmental Biology. 28(2S):465-474.

Sarquis MI, Oliveira EM, Santos AS and Costa GL (2004) Production of L-asparaginase by filamentous fungi. Mem Inst Oswaldo Cruz. 99:489-492. DOI: 10.1590/S0074-02762004000500005

Savitti, Neeta Astana and Wamik Azmi (2003) Microbial L-asparaginase:a potent anti tumor enzyme. Indian Journal of Biotechnology. 2:184-194.

Waksman SA (1961). The Actinomycetes: Classification, Identification and Description of genera and species. Baltimore: Lippincott Williams and Wilkins. 2:377-375.