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RESEARCH ARTICLE

PRODUCTION OF L-GLUTAMINASE FROM MARINE ECOSYSTEMS AND OPTIMAL CONDITIONS FOR MAXIMAL PRODUCTION BY ACTINOMYCETES

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Abstract

L-glutaminase is an enzyme produced by various microorganisms which are currently used for the treatment of leukemia, as flavour enhancer and also as enzyme sensors. Actinomycetes have been recognize as rich source of glutamaminase production enzyme. Even though L-glutaminase activity was reported in various microorganisms, L-glutaminase from actinomycetes in general and marine actinomycete in particular is very scanty. Attempts are now being made to replace enzymes which traditionally have been isolated from animal tissues and plants with enzymes from microorganisms. Solid state fermentation is economically advantageous. Actinomycetes from terrestrial and marine ecosystems have long been recognized either as organism of academic curiosity and organism of antibiotic producers and marine Actinomycetes are effective in enzyme production. Among different substrates Wheat bran was the best substrate for induction of L-glutaminase.The strain of Actinomycetes was used for glutamic acid production under optimum growth conditions. L-glutaminase was assayed by direct Nesslerization measured at 480 nm using a spectrophotometer. Actinomycetes was used for enhancing glutamic acid.The strain gave maximum production at pH 7, temperature 30°C, time 96hours and salinity 3.5%.

Introduction

L-glutaminase (L-glutamine amidohydrolase E.C. 3.5.1.2) catalyses the hydrolysis of L-glutamine to L-glutamic acid and ammonia. This is an essential enzyme for the synthesis of various nitrogenous metabolic intermediates. Glutaminase is synthesized by various bacteria, fungi, yeast, moulds and filamentous fungi. Glutaminase has tissue-specific isoenzymes. Glutaminase has an important role in glial cells.

Glutaminase catalyzes the following reaction:

Glutamine + H_2O → Glutamate + NH_3

Glutaminases belong to a larger family that includes serine-dependent beta-lactamases and penicillin-binding proteins. Many bacteria have two isozymes. This model is based on selected known glutaminases and their homologs within prokaryotes, with the exclusion of highly-derived (long-branch) and architecturally varied homologs, so as to achieve conservative assignments. A sharp drop in scores occurs below 250, and cutoffs are set accordingly.

This enzyme belongs to the family of hydrolases, those acting on carbon-nitrogen bonds other than peptide bonds, specifically in linear amides. The systematic name of this enzyme class is protein-L-glutamine amidohydrolase.

Other names in common use include peptidoglutaminase II, glutaminyl-peptide glutaminase, destabilase, and peptidylglutaminase II.

Microbial sources like actinomycetes are well recognized to produce a variety of chemical structures, several of which are most valuable pharmaceuticals, agrochemicals and industrial products like enzymes. Actinomycetes are considered to be preferred enzymes sources due to their production of extracellular enzymes. They act as decomposers of complex animal and plant materials resulting in release of simple substances, especially carbon and nitrogen which is easily utilized by other organisms, thus performing a vital role in life cycle.

I. Role of Glutamase

This enzyme is involved in glutamine catabolism in micro-organisms. Mammalian cells also synthesis this enzyme which is involved in the generation of the energy using glutamine as the major respiratory fuel. Thus, many types of tumor cells as well as actively dividing normal cells exhibit high rates of glutamine utilization.

Cancer cells, especially Lymphatic tumor cells cannot synthesize L-glutamine and hence require large amount of L-glutamine for their rapid growth. Thus these cells depend on the exogenous supply of L-glutamine for their survival and rapid cell division.Hence, the use of amidases deprives the tumor cells from L-glutamine and causes selective death of L-glutamine dependant tumor cells.

II. Application of l-glutaminase

The terrestrial bacterial source of L-asparginase is currently used for the treatment of leukemia but this is known to cause a lot of side effects and hence there is a need for an alternative enzyme drug which is more compatible to human blood and induces less or no side effects in patients. The marine environment particularly sea water, which is saline in nature and chemically closer to human blood plasma, can provide microbial enzymes that are safe with no or less side effects when administered for human therapeutic application. Yet another important fact about marine microbial enzyme is that they show high level of salt tolerance. Hence there is an increasing interest in the marine micro-organism for therapeutic purposes.

The action of glutaminase plays a major role as therapeutic agent in cancer and HIV. L-glutaminase has inculcated significant buzz in food industry as potential flavor .modulating agent, imparting a savory flavor as it increases food.s glutamic acid content. Attempts to increase the glutamate content of soya sauce using salt and thermo tolerant glutaminase have drawn much attention. It also plays an important role in biosensor as a monitoring agent for glutamine level measurement.

Another important application of L-glutaminase is in food industry as flavor enhancing agent. It increases the glutamic acid content of the fermented food there by imparting a unique flavor.

III. Advantages of microbial production of enzymes

Short fermentation time, inexpensive media, ease of developing simple screening procedures, fast growth of microbes, biochemical diversity, enzyme concentration may be increased by environmental and genetic manipulation, flexibility of choice of fermentation conditions, higher production rate.

IV. Microbes for the production of l-glutaminase

Almost all living cells produce L-glutaminase but only certain microbial strains have the potential for industrial production of this enzyme. It is ubiquitous from the presence point of view in plants, animals and microbes both in prokaryotes and eukaryotes. Among some well studied genera in microbes worth mentioning from study perspective are E. coli, Pseudomonas sp., Brevibacterium sp., Vibrio costicola, Streptomyces rimosus, Streptomyces avermitilis and Streoptomyces labedae, Streptomyces gresius, Hypocrea jecornea, Zygosaccharomyces sp., Bacillus sp. and Micrococcus luteus k, Acinetobacter species, Hansenula, Cryptococcus, Candida, Aspergillus oryzae and Beuveria bassiana etc.,

V. Role of actinomycetes the production of l-glutaminase

Since the sources for Lglutaminase are limited, the search for potential strains that hyper-produce enzyme with novel properties for their industrial production is being pursued all over the world. Actinomycetes are well

recognized to produce a variety of chemical structures , which are most valuable for pharmaceuticals, agrochemicals and industrial products like
enzymes . The value of actinomycetes to society in terms of providing useful drugs especially antibiotics and anticancer agent and to the pharmaceutical industry for revenue generating discovery platform, is indisputable.
Actinomycetes are aerobic gram positive bacteria with high G+C content and are widely distributed in both terrestrial and aquatic habitats. Among the actinomycetes producing bioactive compounds, 73% are Streptomyces and 27% are rare actinomycetes.
Three genera of actinomycetes: *Actinomyces*, *Nocardia*, and *Streptomyces*. These organisms have been shown to be higher bacteria, but they were thought to be fungi for many years because they have filamentous forms, 0.5 to 0.8 microns in diameter, which appear to branch.

VI. Characteristics of actinomycetes

Actinobacteria include some of the most common soil life, freshwater life, and marine life, playing an important role in the decomposition of organic materials, such as cellulose and chitin, and thereby playing a vital part in organic matter turnover and the carbon cycle. In the soil, this replenishes the supply of nutrients and is an important part of humus formation. Other genuses of Actinobacteria inhabit plants and animals, and include some well-known pathogens;
for example the genus *Mycobacterium*, includes the species *M. tuberculosis* which causes tuberculosis and *M. leprae* which causes leprosy; *Corynebacterium*, includes *C. diphtheriae* causing diphtheria; *Nocardia* which has several pathogenic species commonly causing nocardiosis and *Rhodococcus* which has two pathogenic species a major one affecting the tobacco plant and one which is largely an equine pathogen affecting foals.
Actinobacteria are well known as secondary metabolite producers and hence of high pharmacological and commercial interest. In 1942 Selman Waksman discovered that the soil bacteria he was studying made actinomycin, a discovery for which he received a Nobel Prize. Since then, hundreds of other naturally occurring antibiotics have been discovered in these terrestrial microorganisms, especially from the genus *Streptomyces*.
Some Actinobacteria form branching filaments, which somewhat resemble the mycelia of fungi, among which they were originally classified under the older name Actinomycetes. Most members are aerobic, but a few, such as *Actinomyces israelii*, can grow under anaerobic conditions. Unlike the Firmicutes, the other main group of Gram-positive bacteria, they have DNA with a high GC-content, and some Actinomycetes species produce external spores.
Some types of Actinobacteria, the Actinomycetes are responsible for the peculiar odor emanating from the soil after rain (petrichor), mainly in warmer climates. The chemical that produces this odor is known as geosmin.
The *Actinomycetes* are also known to form intracellular inclusions of polyhydroxyalkanoates under certain environmental conditions (e.g. lack of elements such as phosphorus, nitrogen, or oxygen combined with an excessive supply of carbon sources).

VII. Sources of actinomycetes

Rhizosphere soils of Ginger, marine environments like marine sediments, Agricultural fields, Industrial areas, Grave yard, Tree bark, coastal sand, Pink lime stone quarry, Gray lime stone quarry, Majority of the studies carried out on extremophilic organisms.

VIII. Sample collection and pretreatment

Marine sediment sample was collected from sdifferent sources.The surface layer of the sediment was removed and central portions of sediment, approximately 0.5 kg was collected and transferred in to sterile plastic bag. The collected sediment sample was dried at room temperature for a week. 10 gm of sediment sample was transferred to sterile petriplate and kept at 55°C for 10 minutes. The pretreated sample was used for the isolation of actinomycetes.

IX. Isolation of l-glutaminase producing actinomycetes

About 5 gm of sample was taken and suspended in 95 ml of sterile distilled water in a 250 ml conical flask and kept in a rotary shaker with 120 rpm for 30 minutes for the thorough mixing of the sediment sample. About 1 ml of mixed sediment suspension from conical flask was transferred in to 9 ml of sterile distilled water. The sample was serially diluted up to 10 dilutions. Islolation medium was prepared and used for the isolation of L-glutaminase

producing marine actinomycetes. Sterilize the medium.After sterilization, the medium was supplemented with two filter sterilized antibiotics viz., cycloheximide (20 µg/ml) and nalidixic acid (100 µg/ml), in order to retard the growth of fungi and bacterial populations were taken and spreaded on medium by using sterile L-rod. Plating was done in triplicates. All the plates were incubated at 28°C for 1 month. One uninoculated plate was kept as control. All the plates were observed from second day of incubation.

X. Selection of l-glutaminase producing actinomycetes

During incubation, morphologically different actinomycete colonies which showed powdery or leathery consistency were selected. To obtain pure culture, the selected colonies were streaked on selection medium by phase streaking and incubated at 28oC for 7 days. After incubation, morphologically different actinomycetes colonies were selected and sub cultured on yeast extract malt extract medium.

XI. Effect of pH, temperature and salinity on the production of l-glutaminase

Actinomycete inoculum was prepared by inoculating spores of potential actinomycete strain and kept in shaker for 48 hours with 120 rpm. Each 5 ml of broth culture was used as inoculum. 5 ml of potential actinomycete strain was inoculated in to series of flasks containing 100 ml of medium with different pH (6, 6.5, 7, 7.5 and 8). All the flasks were incubated at 28°C in rotary shaker with 120 rpm for 120 hours. To study the effect of temperature, 5 ml of inoculum was inoculated in to 100 ml medium and incubated at different temperature (25°C, 30, 35, 40 and 45°C) for 120 hours in rotary shaker with 120 rpm. To study the effect of salinity on glutaminase production, MSG medium was prepared with different concentration of sodium chloride (0, 1, 2, 2.5, 3 and 3.5 %). 5 ml of inoculum was added in to each flasks and incubated at 28°C in rotary shaker with 120 rpm for 120 hours. All the extracts obtained from above parameters are studied for its L-glutaminase activity.

XII. Different media used for growth, isolation, screening of actinomycetes and production of glutaminase

Mineral salt glutamine (MSG) medium (pH 7) in 250 ml conical flask. Components of MSG medium include (grams/litre) 1.0 KH2Po4; 0.5 MgSo4; 0.1 CaCl2; 0.1 NaNo3; 0.1 tri sodium citrate; 25 NaCl; 10 glucose. All the flasks were incubated at 28oC for 72 hours in a rotary shaker at 120 rpm. Each 100 ml of MSG medium with phenol red (0.012 %) at pH 7 was prepared in 500 ml Erlen Meyer flask and used for the production of L- glutaminase enzyme.

Minimal glutamine agar (MGA) medium was prepared and used for the isolation of L-glutaminase producing marine actinomycetes. Components of MGA(gram/litre) include 0.5 KCl; 0.5 MgSo4; 1.0 KH2Po4; 0.1 FeSo4; 0.1 ZnSo4; 25 NaCl; 10 L-glutamine; 0.012 phenol red in which L-glutamine act as carbon and nitrogen source and phenol red act as pH indicator.
McBeth-Scales starch-mineral agar - MBS (starch 10 g, $CaCO_3$ 3 g, K_2HPO_4 1 g, $(NH_4)_2SO_4$ 2 g, $MgSO_4.7H_2O$ 1 g, NaCl 1 g, agar 25 g, pH 7.0), R2A agar (Difco, pH 7.2) and Krainsky was used and actinomycete colonies were randomly isolated after 7-14 days of incubation at 28 °C.
Nutrient Agar medium (peptone, 5 g; yeast extract, 1.5 g; beef extract, 1.5 g; NaCl, 5 g; agar, 16 g; distilled water 1000 ml; pH 7.2) medium with 4% keratin as carbon source.

Potato Dextrose Agar medium for maintaining in house culture of Actinomycetes species and Nutrient medium for growing Actinomycetes in different nutrient medias.
For thepreparation of Potato Dextrose Agar medium, the potatoes are scrubbed but didn't peeled and cut into 12mm cubes. Boiled 200g in 1litre of water for 60 min. Squeezed as much of the pulp as possible through a fine sieve. Added 20 g of Agar and boiled until it gets dissolved. Added 20g Dextrose and made up to 1litre. Autocla ve at 115oC for 30 min. Cooled to 50oC and poured approximately 20ml amounts into Petri dishes. For the preparation of Nutrient medium, Meat Extract 1.5 g and Peptone 5 g dissolved in 500ml water. The pH of medium is adjusted to 7.4. For the purpose of pH adjustments the 0.1N NaOH and 0.5N HCl are used. All prepared medias are sterilised in autoclave at 115oC for 30 min. The Actinomycetes species sample are gifted by Modern College of Biotechnology, Pune. The all glass accessories including pipettes and beake rs are sterilised in the oven for 30 minutes at 150oC.

Starch casein nitrate(SCN) agar medium (Himedia, Mumbai, India) was used for isolation and enumeration of actinomycetes. The medium was supplemented with 10 µg/ml amphotericin and 25 µg/ml streptomycin (Himedia, Mumbai, India) to inhibit fungal and bacterial contamination respectively.In conventional dilution plate technique, 10g of marine soil samples were suspended in 100 ml of sterile sea water and 0.5 ml of suspension from this was spread over 50% sea water starch casein agar medium (15) and incubated for 7-9 days at 28oC. After incubation the actinomycete colonies were purified and sub-cultured on SCN
agar plates and stored for further assay.
Czapek Dox's medium containing (g/L distilled water) glucose, 2; L-glutamine 10; KH2PO4, 1.52; KCl, 0.52; MgSO4.7H2O, 0.52; CuNO3.3H2O, trace; ZnSO4.7H20, trace FeSO4, trace; agar, 20.0. Modified Czapek Dox's medium was supplemented with different concentrations of the dye. A 2.5% stock of the dye was prepared in ethanol and the pH was adjusted to 7.0 using 1M NaOH. The stock solution of the dye ranging from 0.04 ml to 0.3 ml was added to 100 ml of modified Czapek Dox's medium, giving final dye concentration of 0.2% with a final pH of 7.0.

Among them Starch casein nitrate(SCN) agar medium, Minimal glutamine agar (MGA) medium, Potato Dextrose Agar medium are mostly used for the isolation of actinomycetes.
Mineral salt glutamine (MSG) medium is mainly required for production and McBeth-Scales starch-mineral agar – MBS, Nutrient Agar medium, Potato Dextrose Agar medium , Czapek Dox's medium are mostly used for screening.

XIII. Types of fermentations for production of l-glutaminase

Different methods of fermentation technology can be applied for the production of L-glutaminase. Commercially, L-glutaminase has been produced by submerged fermentation technique, but in recent years, it is also being produced under solid state fermentation technique, using natural (E.g. brans, husks, oil cakes etc) and inert solid materials (E.g. polystyrene beads).

XIV. Production of l-glutaminase by submerged fermentation

After 96 hours of fermentation, most of the strains changed the color of the medium from yellow to pink which shows that the extracellular L-glutaminase production by the actinomycete strains. The intensity of pink color was increased up to 120 hours of incubation. Submerged fermentation is the routinely used method for L-glutaminase production from various microorganisms. But there is no encouraging report on L-glutaminase production from actinomycetes in general and marine actinomycetes in particular by adopting submerged and solid state fermentation. For this reason, in this present study actinomycete strain was inoculated in two flasks of mineral salt glutamine media in which one is added with phenol red as a pH indicator. During incubation, color of the phenol red supplemented media was changed from yellow to pink after 96 hours and increased up to 120 hours but not latter. Based on this observation, it is easy to conclude the color change was due to the production of L-glutaminase which liberated ammonia from L-glutamine. Thus the color change from yellow to pink indicated the production and increase of Lglutaminase level respectively.

XV. Production of l-glutaminase by submerged fermentation

The effect of process parameters on enzyme production was determined by incubating at different agro industrial residues (blackgram husk (BG), bengalgram husk (BH), corn cob (CC), cottonseed (CS), groundnut oilcake(GC), greengram husk (GH), gingelly oilcake, (GOC), lemon peel (LP), orange peel (OP), rice bran (RB), tea dust (TW) and wheat bran (WB), pH (3 to11 adjusted with 1 N HCl or 1 N NaOH), temperature (25 to 50°C), moisture content (10 to 60%), incubation period (3 to 7 days), additional carbon sources (sucrose, glucose, lactose, galactose and dextrose at 1% w/v), nitrogen sources (peptone, yeast extract, urea, casein and albumin) and metal ions (CaCl**2**, KCl, MgSO**4**, NaCl and ZnSO**4**).

XVI. Economical and industrial advantages of ssf

Solid state fermentation offers several advantages over other conventional fermentations, such as submerged fermentation etc. Solid state fermentation was conducted for the production of L-glutaminase using different agro-

industrial byproducts including wheat bran, groundnut residues, rice hulls, soya bean meal, corn steep, sesamum oil cake, cotton seed residues and lentil industrial residues as solid substrates. Wheat bran was the best substrate for induction of L-glutaminase. The major advantages include higher product yields, lower capital and recurring expenditure, lower waste water output/less water need, reduced energy requirement, absence of foam formation, simplicity, high reproducibility, simpler fermentation media, Lesser fermentation space, absence of rigorous control of fermentation parameters, economical to use even in smaller scales, easier control of contamination, applicability of using fermented solids directly, storage of dried fermented matter, lower cost of downstream processing.

Problems commonly associated with solid state fermentation are heat build-up, bacterial contamination, scale-up, biomass growth estimation and control of process parameters.

XVII. Test for activity of glutaminase

L-glutaminase was assayed by direct Nesslerization. The enzymatic reaction mixture contains 1 ml of 1% L-glutamine in citrate-phosphate buffer(pH 7.0) and 1ml of the crude enzyme incubated at 30°C for 1 h. The enzymatic acitivty was stopped by adding 0.5 ml of 1.5 M trichloroacetic acid. The reaction mixture was centrifuged at 5000 rpm for 5 min to remove the precipitated protein. The released ammonium was determined using 0.5 ml Nessler reagent, after 15 min the developed color was measured at 480 nm using a spectrophotometer (Spekol-Spectrocolorimeter). Enzyme and substrate blanks were used as controls. The ammonium concentration of the reaction was determined by inference from the standard curve of ammonium sulphate. One unit (U) of L-glutaminase was defi ned as the amount of enzyme that liberates 1µ mol of ammonia under optimal assay conditions. Specifi c activity of L-glutaminase was expressed as the activity of the enzyme (U) per mg protein released. Enzyme yield was expressed as the activity of L-glutaminase per grams dry substrate (U/gds).

XVIII. Optimum conditions

The physicochemical parameters were studied for the optical conditions required for the maximal production of L-glutaminase are- pH 7-9, temperature 27-30°C, time 96hours and salinity of 3.5% showed optimal enzymatic activity. The maximal activity of L-glutaminase at 3.5% NaCl also confirms the marine nature of the actinomycete strain.

XIX. Conclusion

From this study, it is clearly indicated that marine soils can provide a rich source of L-asparaginase-producing actinomycetes. The current study was focused on the evaluation of the potentiality of *Actinomycetes* for utilization of different agro-industrial byproducts namely; wheat bran, groundnut residues, rice hulls, soya bean meal, corn steep, sesamum oil cake, cotton seed residues and lentil industrial residues as substrates for production of L-glutaminase under solid state fermentation. L-glutaminase an enzyme of super therapeutic and technological values that could be sustained by conventional lower economical/higher efficiency productive bioprocesses. Therefore, solid state fermentation seems to be the prospective technique for large-scale production of microbial metabolites of biotechnological importance. Actinomycetes are the main source of clinically impor-tant antibiotics, most of which are too complex to be synthesized by combinatorial chemistry and which are ecofriendly filamentous bacteria. Actinomycetes showed remarkable capacity to produce L-glutaminase enzyme and could, therefore, be potentially useful for industrial production for L-glutaminase enzyme. The enzyme from *Streptomyces avermitilis* possesses the positive property of salt-tole-rance which is often required and highly advantageous for food fermentation processes.

References

Ammon, H.L., Weber, I.T., Wlodawer, A., Harrison, R.W., Gilliland, G.L., Murphy, K.C., Sjolin, L. and Roberts, 1.(1988). Preliminary crystal structure of *Acinetobacter glutaminasificans* glutaminase –asparaginase. *Journal of Biological Chemistry, 263:*150-156.

DeJong PJ, (1972). L-Asparaginase production by *Streptomyces griseus.*. *Appl. Microbiol.*, **23**(6): 1163-1164.

Prabhu GN, Chandrasekaran M, (1997). Impact of process parameters on L-glutaminase production by marine *Vibrio costicola* under solid state fermentation using polystyrene as inert support. *Proce Biochemistry*. 32: 285-289.

Balagurunathan R., Radhakrishnan M. and Somasundaram S., (2010). L-glutaminase producing actinomycetes from marine sediments –selective isolation, semi quantitative assay and characterization of potential strain, *Aust. J. Basic Appl. Sci*., , 4: 698-705.

Yano T, Ito M, Tomita K, Kumagai H, Tochikura.T, (1988). Purification and properties of glutaminase from *Aspergillus oryzae*, Ferment Technology, 66:137–43.

Krishnakumar S., Alexis Rajan R. And Ravikumar S., (2011). Extracellular production of Lglutaminase by marine alkalophilic *Streptomyces* sp.-SBU1 isolated from Cape Comorin Coast. *Ind. J. Geo-Marine Sci.*, , 40: 717-721.

Sabu,A., Keerthi,T.R, S. Rajeev Kumar and M. Chandrasekaran, (1999). LGlutaminase production by marine *Beauveria* sp under solid state fermentation, *Process Biochemistry.* 53:705-710.

Sivakumar K., Sahu M.K., Manivel P.R. and Kannan L., (2006). Optimum conditions for Lglutaminase production by actinomycete strainisolated from estuarine fish *Chanos chanos*, *Ind. J. Exp. Biol*., 44, 256-258.

Moriguchi,M., Sakai, K., Tateyama, R, Furuta, Y and Wakayama, M.(1994). Isolation and characterization of salt tolerant glutaminase from marine *Micrococcusluteus* K-3., *J.Ferment. Bioengg.,* 77: 621-625.

Pisano M.A., Sommer M.J. and Lopez M.M., (1986). Application of pretreatment methods for the isolation of bioactive actinomycetes from marine sediments. *Appl. Microbiol*.,, 25:285-288.

Thongsanit J., Yano S., Tachiki T. and Wakayama M., (2008). Identification of a glutaminaseproducing bacterial strain isolated from Thai fermented pork sausage and characterization of glutaminase production. *Ann. Microbiol.*, 58: 671-675.

Jeya P., Poorani P., Anantharaman P. And Balasubramaniam T., (2009)L-glutaminase production and growth of marine bacteria. *Res. J. Microbiol*., , 4, 168-172.

Imada A., Igarasi S., Nakahama K. and Isono M.,(1973). Asparaginase and glutaminase activities of microrganisms. *J. Gen. Microbiol*., 76: 85-99.

Davidson L., Brear D.R., Wingard P., Hawkins J. and KittoG.B., (1977). Purification and Properties of an L-Glutaminase-LAsparaginase from Pseudomonas acidovorans. J. Bacteriol., 129: 1379-1386.