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ORIGINAL PAPER

Interspecies variation of Kitasatospora recifensis endophytic from yam bean producing thermostable amylases in alternative media

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Abstract An endophytic actinomycete isolated from tubers of yam beam (*Pachyrhizus erosus* L. Urban) was classified as a novel species nominated *Kitasatospora recifensis* based in phenotypic and genotypic analysis (16S rDNA gene sequence). Monosporic culture using specific ISP2 media revealed three interspecies, which were identified by DNA southern hybridization (Wild strain 13817 W, Aerial Mycelium strain 13817 AM and Vegetative Mycelium strain 13817 VM). The strains were tested for the production of amylolitic enzymes in alternative media. Maximum yields for both enzymes were observed in starch-casein. Higher α-amylase was obtained with strain 13817 W in starch-urea, and amyloglucosidase with strain 13817 AM in starch-ammonium that are economic sources and may be important for industrial purposes. Type strain (DAUFPE 13817^{T} = KCTC 9972^{T} = DSM 44943^{T}).

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Introduction

There is much global interest in the discovery of new microorganisms for use in enzyme production, as well as in bacterial diversity in general, with an increasing number of new species now being identified. Amylases are among the most important classes of enzymes and are of great significance in present-day biotechnology, due to their widespread application in many fields, including clinical, medicinal and analytical chemistries, textile, paper, adhesive, and food industries, starch liquefaction and sugar production (Lynko and Wu 1993; Melo et al. 1994; Melo et al. 1996; Pandey et al. 2000). Accordingly, further research, aimed at identifying new strains of microorganisms for amylases production, is warranted.

According to Timothy et al. (1993) endophytic microorganisms are those that live within their host plants without causing any noticeable symptoms of disease. Indeed novel endophytic actinomycetes have been found in many plant species and their presence has been reported in leaves and roots of cultivated and native plants (McInroy and Kloepper 1991; Souza 1996). Yam bean (*Pachyrhizus erosus* L. Urban), a tuber legume, is a native of Central Mexico and the Northern Amazon Region (Sorensen 1996). Its tubers are used as a source of starch for various applications, particularly in the food industry (Melo et al. 1994).

Actinomycetes are one of the most widely investigated groups of microorganisms in industrial microbiology, since they constitute a potential source of biotechnologically interesting substances (Nolan and Cross 1988). The

taxonomical position of these microorganisms is well defined within the prokaryote group which includes filamentous, Gram-positive, aerobic and chemo-organotrophic microorganisms (Küster 1972). The genus *Kitasatospora* comprises a group of filamentous and aerobic Gram-positive bacteria with the aerial mycelium bearing long spore chains of more than 20 spores. These bacteria are phenotypically similar to *Streptomyces* strains and are distinguished by the presence of galactose in their whole-cell hydrolysates, as described by Omura et al.1983). At present, the genus comprises 19 species, including recently described K. *arboriphila*, *K. gansuensis*, *K. nipponensis*, *K. paranensis*, *K. terrestris* (Hankin and Anagnostakis 1985) and *K. viridis* (Groth et al. 2004; Liu et al. 2005).

Like *Streptomyces*, *Kitasatospora* species have been demonstrated to produce several antifungal and antibacterial agents (Chung et al. 1999), but there have been no previous reports about their production of amylolitic enzymes. However, since enzyme activity has been reported to depend on the composition of microbial growth media, and especially on nitrogen and carbon sources, the strategic selection of specific media might be considered important for enzymes with potential industrial and commercial application (Pridham et al. 1956/57).

This paper reports the production of simultaneous amylolitic enzymes by Kitasatospora *recifensis* isolated from yam bean tubers, a natural source of starch, classified on the basis of physiological characteristics and phylogenetic analyses of their genomic DNA 16S rRNA sequence. The simultaneous and thermostable production of amylases (α-amylase and amyloglucosidase) by three interspecies of *K. recifensis* was studied grown in alternative and economic media.

Materials and methods

Isolation and microbial culture

The endophytic strain (DAUFPE 13817^T) was isolated from tubers of yam bean (*Pachyrhizus erosus*) – a legume originating from the Amazon region (Sorensen 1996). These tubers were purchased from the Department of Agronomy of the Federal Rural University of Pernambuco, Recife, Brazil, which produces an average tuber yield 60 t/ha. The endophytic microorganism was isolated using methodology described by Souza et al. (1996), with slight modifications (Stamford et al. 1998). The tubers were initially washed in running water and then pre-treated with ethanol at 95 °C for 1 min and with $HgCl_2$ (1:1000) for 30 s (Stamford et al. 2002). Inoculated plates were incubated at 28 °C for 3–4 days, while representative colonies were grown on ISP2 agar plates. Stock cultures of the isolate were maintained on ISP2 agar slants with glycerol medium.

The type strain (DAUFPE 13817^T) was grown aerobically on alternative media and on yeast extract/malt agar plates (ISP2 medium) (pH 7.3) supplemented with 1% (w/v) agar starch. The plates were incubated for 7 days at 28 °C. Cultures were lyophilized at 4 °C, and maintained in the Collection Type Cultures of the Department of Antibiotics at the Federal University of Pernambuco (DAUFPE 13817^T). The type strain was also deposited in the Korea Collection Type Cultures (KCTC 9972^T) and in the German Resource Centre for Biological Material (DSM 44943^T).

Morphological and physiological characterization

The type strain was grown aerobically on medium ISP2 agar (Pridham et al. 1956/57) supplemented with 1% (w/v) agar starch slants and adjusted to pH 5.0. The plates were incubated for 7 days at 28 °C (Riddell 1950). *K. kifunensis*, which is its closest relative in terms of 16S rDNA similarity, was used as a reference strain and was obtained from KCTC and grown under the same conditions. The type strain DAUFPE 13817^T was characterized on the basis of its structural micromorphology in slant cultures and in permanently stained preparations under a light-microscope (Leitz, Wetzlar, Germany). Cellular dimensions were measured with an ocular (×10) and an objective (×100/1.25) lens. The strain was identified using the International Color Harmony Manual (Jacobson et al. 1948) and the Color Table (Baumanns Farbtonkarte Atlas), as described by Prauser (1964).

Culture characteristics, production of melanoid pigments, and the use of carbohydrate sources were investigated following Shirling and Gottlieb (1966). This revealed that growth was optimum in ISP2 medium supplemented with 1% soluble starch (w/v). Standard chromatographic procedures were used for the analyses of sugars and diaminopimelic acid as per Staneck and Roberts (1974), with a mixture of isomers α, ε (Sigma 1377). Sugar patterns, nutritional and physiological tests were conducted according to Hopwood et al. (1985).

Phylogenetic analyses

Growth under anaerobic conditions was determined after incubation for 7 days in anaerobic Gaspak jars (BBL) containing an atmosphere of 80% N_2, 10% CO_2 and 10% H_2. Isoprenoid quinones were extracted from 100 mg of freeze-dried cells, according to the methods described previously by Collins and Jones (1981), and purified via preparative thin-layer chromatography (TLC, silica gel F254; Merck). The ubiquinone fraction was also analyzed

by high-performance liquid chromatography (HPLC, Hitachi L-5000) using a reverse-phase column (YMC pack ODS-AM; YMC Co.). Bacterial strains grown on ISP2 media for 96 h at 28 °C were used for the analysis of fatty acid methyl esters (FAMEs). The FAMEs were then extracted and prepared, according to the standard protocols provided by the MIDI/Hewlett Packard Microbial Identification System (Sasser 1990). Chromosomal DNA was extracted and purified, according to the method described by Sambrook et al. (1989). DNA G+C content was assessed by the Tamaoka and Komagata (1984) methodology. The DNA was hydrolyzed, and the resultant nucleotides were analyzed via HPLC using a reverse-phase column (Supelcosil LC-18-S; Supelco). The 16S rDNA was amplified by PCR using two universal primers, and sequencing of the amplified 16S rDNA and phylogenetic analysis were performed according to the methods described by Yoon et al. (2003).

Southern hybridization analysis of genomic DNA was carried out with the three interspecies strains by using a capillary system with a nylon membrane (Hybond N^+, Amershan Biosciences) at 42 °C for 16 h. DNA probes for hybridization were digoxin-labelled with a DIG DNA Labeling and Detection Kit (Roche) and subsequent color detection was by enzyme immunoassay.

Production of α-amylase and amyloglucosidase

Amylases were produced under shaking flask cultivation using a dense spore suspension. The spores were washed with physiologic serum and centrifuged at 4,000 rpm for 10 min. The sedimented spores were resuspended in glycerol (2 mg l^{-1}) and stored at –4 °C (Groth et al. 2004). Viable cells were determined before dilution and transfer to ISP2 medium.

In order to obtain microbial suspensions of high cellular density (pre-inocula), the strains were prepared in 250 ml Erlenmeyer flasks containing 50 ml of the following culture media supplemented with starch (10 g/l): (a) Lechevalier (Vigal et al. 1991), (b) ammonium (Shirling and Glottlieb 1966), (c) casein (casein 1.0 g/l, K_2HPO_4 0.5 g/l), (d) urea (Pridham et al. 1956/57), (e) Czapek ($NaNO_3$ 2.0 g/l, K_2HPO_4 1.0 g/l, $MgSO_4.7H_2$) 0.5 g/l, KCl 0.5 g/l, $FeSO_4.7H_2O$ 0.01 g/l), all of which had been previously sterilized at 121 °C for 15 min. Inoculation was performed with a spore suspension containing 3.6 to 4.0×10^8 colony forming units (CFU) ml^{-1}. The culture was then shaken at 180 rpm, for 48 h at 28 °C. 5.0 ml aliquots of pre-inocula were added to 50 ml of each medium. Flasks containing the three strains (wild -13817W, aerial mycelium -13817AM and vegetative mycelium -13817VM) and *Streptomyces griseus* (reference culture) were then incubated with shaking at 180 rpm at 28 °C. At intervals of 24, 48 and 72 h, enzymatic activity and protein content were determined. The culture was filtered, centrifuged at 4,000 rpm for 15 min, and the supernatant fluid, containing the extra cellular enzyme, was stored at –4 °C. Glucose levels were determined as described by Bernfeld (1955) using 3,5 dinitrosalicilic acid (0.1 ml) as a standard, and by maintaining the solution at 100 °C, for 10 min.

One unit of α-amylase or amyloglucosidase (U ml^{-1}) was estimated as the amount of enzyme necessary to produce a reducing sugar equivalent of 1 μmol of glucose min^{-1}. Starch solution (0.1 g l^{-1}), previously gelatinized in 0.1mol l^{-1} citrate-phosphate buffer, was used to evaluate α-amylase (pH 6.5) and amyloglucosidase (pH 4.5) activities at 37 °C, as described by Pandey et al. (2000). The specific activity was expressed as units per mg of protein. Total protein content in the cellular extracts and in enzymatic crude extracts was estimated according to Lowry et al. (1951) with bovine serum albumin (Sigma, V-98) as a standard.

Results and discussion

Isolation and microbial culture

The Strain DAUFPE 13817^T grew well in media ISP2, ISP3, ISP4 and ISP5, especially in ISP2 supplemented with starch (data not shown). The type strain showed characteristics of instability and after intensive cultivation with monosporic colonies, three strains that showed different and consistent growth patterns were distinguished (Fig. 1). When grown in several distinct media (ISP2, ISP3, ISP4, ISP5 Difco) at 28 °C for 10–15 days the type culture (DAUFPE 13817^T) exhibited aerial mycelium, which is a characteristic of the wild-type strain (13817 W); however, the second strain (13817 VM) only formed vegetative mycelium and the remaining strain (13817 AM) produced aerial mycelium (Fig. 1).

Isolation of monosporic colonies revealed that 30% produced grey aerial mycelium (AM), 10% produced vegetative mycelium (VM), and the majority (60%) were of the wild (W) type with white aerial mycelium. Cullum et al. (1989) previously described the occurrence of genetic instability in actinomycetes as observed in this study.

The characteristics of the *Kitasatospora recifensis* (strains W, AM and VM) were investigated on media ISP2, ISP3, ISP4, and ISP5. The strains showed regular growth in casein (sub-type W and VM), but no growth with Czapek (strains W, AM and VM). None of the strains produced soluble pigment in the culture media over a 14 day incubation period and the different sources produced unstable effects on the growth of the three strains (data not shown).

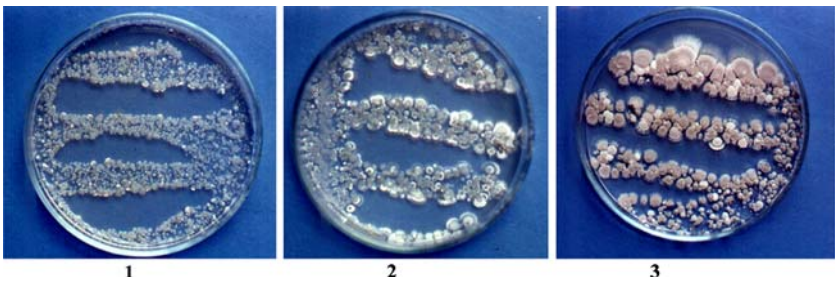

Fig. 1 Morphology of the three strains of *Kitasatospora* sp. (1. Vegetative mycelium 13817 VM; 2. Wild 13817 W and 3. Aerial mycelium 13817 AM) grown in ISP2 medium supplemented with 1% starch for 5 days at 28 °C, in monosporic culture assays

Morphological, physiological and phylogenetic analyses

The isolated actinomycetes showed the same micromorphology and were identified as *Kitasatospora sp*., *Thermomonospora* group, with well-developed mycelium and long, branched, aerial hyphae (20 or more spores), which were fragmented into coccoid or bacillaris elements (data not shown). Chemical analysis of the cellular wall revealed the presence of meso 2,6 diaminopimelic acid (cellular wall type II), which has previously been described by Zhang et al. (1997) as the main characteristic for the identification of the genus *Kitasatospora*. He proposed to revive the genus *Kitasatospora* as it forms a significant monophyletic clade and contains differential chemotaxonomic markers, with notably larger amounts of meso-DAP.

The 16S rDNA sequence of strain DAUFPE 13817^{T} was 1379 bp long, corresponding to the regions between positions 28 and 1523 (Accession number NCBI AY494737). The 16S rDNA of *Escherichia coli*. Phylogenetic trees based on 16S rRNA gene sequences from members of the genus *Kitasatospora* showed that strain DAUFPE 13817^{T} falls within the cluster comprising *Kitasatospora* species. DNA-DNA homology studies (Fig. 2) were performed to determine the genomic relationship between the strain DAUFPE 13817^{T} and the type strains of the closest *Kitasatospora* species. According to the phenotypic, phylogenetic and genotypic characteristics of the isolate, it was concluded that strain DAUFPE 13817^{T} belongs to the genus *Kitasatospora.* According to the 16S rDNA sequence and phylogenetic analysis this actinomycete was classified as a novel species (*Kitasatospora recifensis*) deposited in the Gen Bank and EMBL databases, with accession numbers AY494737 and AJ 616217, respectively.

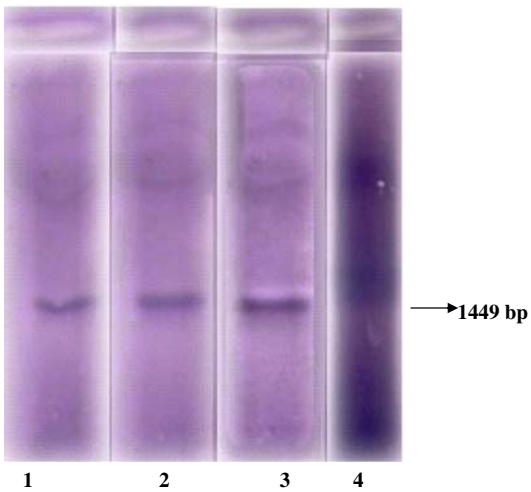

Fig. 2 DNA-DNA Southern Hybridization (with probe 16S rDNA (1449 pb) labeling with DIG. 1. Vegetative mycelium; 2. Cells (Wild); 3. Aerial mycelium; 4. Dig Probe 16S rDNA (1449 pb)

Amylolitic activity

The type strain (DAUFPE 13817^{T} = KCTC 9972^{T} = DSM 44943^{T}) produced simultaneously two amylases (α-amylase and amyloglucosidase) that showed thermostable properties. These desirable enzyme characteristics indicate that this strain has great potential for use in biotechnological applications (Cullum et al. 1989; Pandey et al. 2000).

The α-amylase activities produced when the type strain was grown in different media are presented in Fig. 3. After 72 h fermentation, a yield of 37.3 U ml^{-1} (strain type W) was observed in starch urea and 40.0 U ml^{-1} (strain VM) in casein starch, showing that there was no significant effect of the growth medium on enzyme production. These results were higher than those obtained for *Streptomyces griseus* (30.0 U ml^{-1}) in Lechevalier medium, or in ammonium starch (32.0 U ml^{-1}). These results are considered both relevant and promising since there are no previously published reports on the use of ammonium as a nitrogen source in α-amylase production by *Streptomyces griseus* or other amylolitic bacteria.

The production of amyloglucosidase using different sources of nitrogen is shown in Fig. 4. Optimal results for strain 13817 VM were obtained with starch casein medium (40.0 U ml^{-1}) after 72 h of growth. According to previous reports (Hankin and Anagnostakis 1975, Kelly et al. 1980), ammonium sulphate is the best nitrogen source for the synthesis of amyloglucosidase in the fungi *Aspergillus*, *Rhizopus* and *Endomyces*.

Streptomyces griseus did not grow in starch urea medium, showing that urea was inhibitory. Similar results have

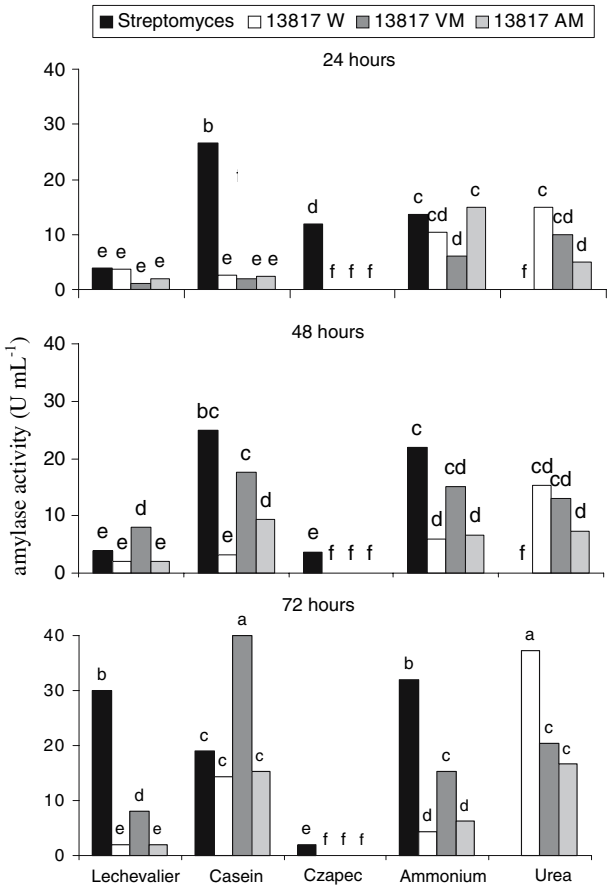

Fig. 3 α amylase activity (U ml^{-1}) by sub- types of *Kitasatospora* sp. and *Streptomyces griseus* grown on alternative culture media and times of growth. Coefficient of Variation (C.V.) = 14,48% and L.S.D. = 3,25

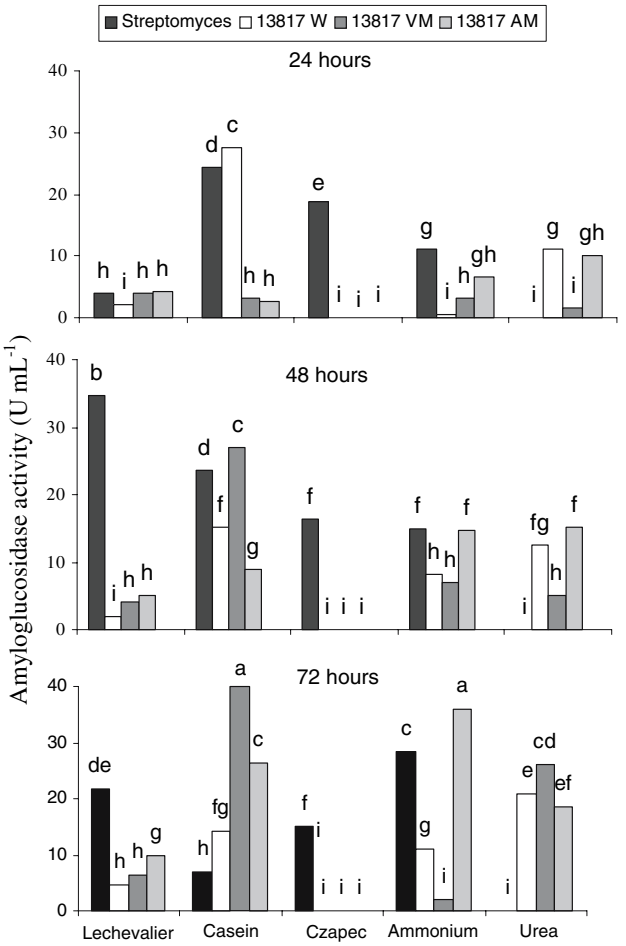

Fig. 4 Amyloglucosidase activity (U ml^{-1}) by *Streptomyces griseus* and subtypes of *Kitasatospora* sp. on alternative culture media and times of growth. Coefficient of Variation (C.V.) = 16.55% and L.S.D. = 3.42

been obtained with *Streptosporangium*, an endophytic actinomycete isolated from maize leaves (Stamford et al. 2002). Urea and ammonium were used by the three strains of *Kitasatospora recifensis*, but they did not grow in Czapek medium which employs nitrate as a nitrogen source.

The results clearly indicate that casein starch medium results in the simultaneous production of α-amylase and amyloglucosidase with optimal yield. After 72 h of growth, strain 13817 VM showed a similar yield of both enzymes. The simultaneous production of amylolitic enzymes was also reported by Kelly et al. (1980) with *Bacillus amyloliticus* and by Castro et al. (1992) with *Bacillus* sp. The simultaneous production of α amylase and amyloglucosidase by the three strains of *Kitasatospora recifensis* may be useful for starch hydrolyzation. Additionally, the production of higher amylases in urea and ammonium media could be important because these are economically viable sources of nitrogen. It is important to report that the three strains produced highly active α-amylase and amyloglucosidase enzymes that were thermostable, suggesting that they may hold great potential in agricultural and industrial applications.

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References

Bernfeld P (eds) (1995) Methods in enzymology. Academic Press Inc., New York, pp 149–150

Castro RR, Ferreri MA, Abate BS, Mendez BS, Siñeriz F (1992) Simultaneous production of alpha and beta amylases by *Bacillus subtilis* MIR – 5 in batch and continuous culture. Biotechnol Let 14:49–54

Chung YR, Sung KC, Mo HK, Young son D, Nam JS, Chun J, Bae KS (1999) *Kitasatospora cheerisanensis* sp. nov., a new species of the genus *Kitasatospora* that produces an antifungal agent. *Intern*. J Syst Bacteriol 45:753–758

Collins MD, Jones D (1981) Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implication. Microbiol Rev 45:316–354

Cullum J, Flett F, Piendl W (1989) Genetic Instability, deletions and DNA simplification in *Streptomyces* species. In: Genetics and molecular biology of industrial microorganisms. Amer. Soc. Microbiol. p 203

Groth I, Rodriguez Schütze B, Schmitz P, Leistner E, Goodfellow M (2004) *Kitasatospora putterlickiae* sp. nov., isolated from rhizosphere soil, transfer of *Streptomyces kifunensis* to the genus *Kitastospora* as *Kitasatospora kifunensis* comb. Nov., and emended description of *Streptomyces aureofaciens* Duggar 1948. Int J Syst Evol Microbiol 53:2033–2040

Hankin L, Anagnostakis SL (1975) The use of solid media for detection of enzyme production by fungi. Mycol 67:597–607

Hopwood DA, Bibb MJ, Chater KF, Kiese T, Gruton CJ, Kieser HM, Lydiate DJ, Smith CP, Ward JM, Schirempe H (1985) Genetic manipulation of Streptomyces a laboratory manual, John Innes Inst., p 302

Jacobson E, Granville WC, Foss CE (1948) Colour harmony manual, 3rd edn. Container Corp. Amer., p 58

Kelly CT, Hefferman ME, Fogarty WM (1980) A novel α-amyloglucosidase produced by *Bacillus amylolyticus*. Biotechnol Let 2:351–356

Küster E (1972) Simple working key for the classification and identification of named taxa included in the International *Streptomyces* Project. Intern J System Bacteriol 22:139–148

Linko Y, Wu SY (1993) Improvement and estimation of enzymic starch saccharification process. Biotechnol Technol 7:551–556

Liu Z, Rodriguez CR, Wang L, Cui Q (2005) *Kitasatospora viridis* sp. nov., a novel actinomycete from soil. Int J Syst Evol Microbiol 55:707–711

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folium phenol reagent. J Biol Chem 193:265–275

McInroy JA, Kloepper JW (1991) Analysis of population sanities and identification of endophytic bacteria of maize and cotton in the field. Kew Bull 14:328–331

Melo EA, Krieger N, Stamford TLM (1994) Physico-chemical properties of jacatupé (*Pachyrhizus erosus* L Urban). Starch 46:245–247

Melo EA, Vieira R, Krieger N, Guerra NB, Silva MPC, Kennedy JF (1996) Enzymatic hydrolysis of starch from jacatupé (*Pachyrhizus erosus* L Urban) by thermostable amylolitic enzymes. Starch 48:101–104

Nolan RD, Cross D (1988) Actinomycetes in biotechnology. Acad. Press. Inc., San Diego, California, p 204

Omura S, Takahashi Y, Iwi Y, Tanaka H (1983) *Kitasatospora setalba* sp. nov. In Validation of the publication of new names and new combinations previously effectively published outside the IJSB, List no. 11. Int J Syst Bacteriol 33:672–674

Pandey A, Nigam P, Soccol CR, Soccol VT, Singh D, Mohan R (2000) Advances in Microbial Amylases. Biotechnol Appl Biochem 31:135–152

Prauser H (1964) Aptness and application of colour codes for exact description of colour of *Streptomyces*. Zeitschrift Allag Mikrobiol 4:95–98

Pridham TG, Anderson P, Foley C, Lindenfelser LA, Hesseltine CW, Benedict RG (1956/57) A selection of media for maintenance and taxonomic study of Streptomyces. Antibiotics Ann, 947–953

Riddell RW (1950) Permanent stained mycological preparation obtained by slide culture. Mycol 42:265–270

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a Laboratory Manual, 2nd ed. Cold Spring Harbor, New York

Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. DE MIDI Inc., Newark

Shirling EB, Glottlieb D (1966) Methods for characterization of *Streptomyces* species. Int J Syst Bacteriol 16:313–340

Sorensen M (1996) Yam bean: *Pachyrhizus* D.C. International Plant Genetic Resources Institute, Rome p 141

Souza AO (1996) Bactérias endofíticas de milho (*Zea mays*. L.) e sua variabilidade genética analisada por RAPD. Piracicaba, 85p. MSc., thesis. Escola Superior de Agricultura ''Luiz de Queiroz'', Universidade de São Paulo

Stamford TLM, Araújo JM, Stamford NP (1998) Atividade enzimática de microrganismos isolados do jacatupé (*Pachyrhizus erosus* L. Urban). Ci Tecnol Alim 18:382–385

Stamford TML, Stamford NP, Coelho LCB, Araújo JM (2002) Production and characterization of a thermostable amyloglucosidase from *Streptosporangium* sp. endophyte of maize leaves. Biores Technol 83:105–109

Staneck JL, Roberts GD (1974) Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. Appl Microbiol 2:226–231

Tamaoka J, Komagata K (1984) Determination of DNA base composition by reverse-phase high-performance liquid chromatography. FEMS Microbiol Lett 25:125–128

Timothy L, Mc Cutcheon N, Carrol GA (1993) Genotypic diversity in populations of a fungal endophyte from Douglas Fir. Mycol 82:180–186

Vigal T, Gil JF, Daza A, Garcia-Gonzalez MD, Martin JF (1991) Cloning characterization and expresión o fan α amylae gene from *Streptomyces griseus* IMRU 3570. Mol Gen Genet 225:278–288

Yoon JH, Kang KH, Park YH (2003) *Halobacillus salinus* sp. nov., isolated from a salt lake on the coast of the East Sea in Korea. Int J Syst Evol Microbiol 53:687–693

Zhang Z, Wang Y, Ruan J (1997) A proposal to revive the genus *Kitasatospora* (Omura, Takahashi, Iwai and Tanaka, 198). Int J Syst Bacteriol 47:1048–1054