



## Production, purification and characterization of an extracellular inulinase from *Kluyveromyces marxianus* var. *bulgaricus*

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The yeast *Kluyveromyces marxianus* var. *bulgaricus* produced large amounts of extracellular inulinase activity when grown on inulin, sucrose, fructose and glucose as carbon source. This protein has been purified to homogeneity by using successive DEAE-Trisacryl Plus and Superose 6HR 10/30 columns. The purified enzyme showed a relative molecular weight of 57 kDa by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and 77 kDa by gel filtration in Superose 6 HR 10/30. Analysis by SDS-PAGE showed a unique polypeptide band with Coomassie Blue stain and nondenaturing PAGE of the purified enzyme obtained from media with different carbon sources showed the band, too, when stained for glucose oxidase activity. The optimal hydrolysis temperature for sucrose, raffinose and inulin was 55 °C and the optimal pH for sucrose was 4.75. The apparent  $K_m$  values for sucrose, raffinose and inulin are 4.58, 7.41 and 86.9 mg/ml, respectively. Thin layer chromatography showed that inulinase from *K. marxianus* var. *bulgaricus* was capable of hydrolyzing different substrates (sucrose, raffinose and inulin), releasing monosaccharides and oligosaccharides. The results obtained suggest the hypothesis that enzyme production was constitutive. *Journal of Industrial Microbiology & Biotechnology* (2000) 25, 63–69.

**Keywords:** inulin; inulinase; *Kluyveromyces marxianus* var. *bulgaricus*; extracellular enzyme

### Introduction

The extracellular glycoprotein, inulinase, catalyzes hydrolysis of the polyfructoside inulin outside the cell wall. The enzyme also hydrolyses sucrose, both within and outside the cell wall [23]. For a microorganism to utilize inulin as its carbon and energy source, production of an extracellular inulinase is required to hydrolyse the fructan into fermentable monosaccharides. Fructans are polyfructose molecules that can be subdivided into two categories, the inulin and the levan type. The inulin fructans are found as carbohydrate reserves in several plants, including chicory, dandelion, dahlia, and Jerusalem artichoke. They consist of a linear chain of (2-1)-linked fructose molecules and, depending on the plant source, may have between 5 and 45 fructose monomers [2]. The levan fructans produced by many bacterial species consist of branched molecules with predominantly (2-6)-linkages branched at C1 [10,11]. Inulinases have been purified from yeast [7,21,25,31], molds [9,27] and bacteria [1,3,8,29,30] and have potential uses in the preparation of fructose syrups from inulin and invert sugar in the food industry [19,28]. The results of the study on the production and purification of an extracellular inulinase by *Kluyveromyces marxianus* var. *bulgaricus* are presented.

### Materials and methods

#### Strain

*K. marxianus* var. *bulgaricus* ATCC 16045 was obtained from the Bioengineering Laboratory, Campinas State University, São Paulo,

Brazil. The strain was maintained on solid medium at 4 °C. The medium composition (g/l) was comprised of the following: Difco yeast extract 10.0; Difco Bactopeptone 20.0; glucose 20.0 and Difco agar 20.0. Cells were harvested from 18 slants and used to inoculate liquid medium.

#### Culture media

Liquid media with 1% (w/v) glucose, sucrose or fructose were obtained from Reagen and raffinose, inulin from dahlia tubers and, inulin from Jerusalem artichoke were obtained from Sigma. Media were supplemented with 1% yeast extract and the pH was adjusted to 3.0 with orthophosphoric acid.

#### Culture conditions

The yeast was inoculated into 250 ml Erlenmeyer flasks containing 50 ml of medium incubated at 30 °C for 48 h in a temperature-controlled shaker (New Brunswick Scientific, USA) operated at 200 rpm. The culture was pelleted by centrifugation at 10,000 ×g for 10 min.

#### Fractionation of cultures for inulinase activity assays

**Supernatant enzyme:** The organism was grown at 30 °C in a continuous laboratory fermenter with a working volume of 600 ml, aeration of 0.2 vvm, pH 3.0 and sucrose as carbon source. One hundred milliliters of culture was harvested by centrifugation at 4 °C (10 min, 10,000 ×g). The supernatant was used as a source of extracellular enzyme.

**Cell wall enzyme:** The cell pellet obtained after centrifugation was suspended and incubated for 1 h at 30 °C in 10 ml of enzyme

Correspondence: Dr J Contiero, Laboratório de Biotecnologia Industrial, Instituto de Química de Araraquara - UNESP, P.O. Box 355, Rua Prof. Francisco Degni S/N, Araraquara 14801-970, SP, Brazil.  
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# Production Purification And Characterization Of Inulinase

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Deshmukh, B.N. Johri**



## **Production Purification And Characterization Of Inulinase:**

Marine Enzymes Biotechnology: Production and Industrial Applications, Part III - Application of Marine Enzymes, 2017-02-17 Approx 216 pages Approx 216 pages      **Progress in Food Engineering Research and Development** Jerrod M. Cantor, 2008 This book presents new and significant research in the growing field of food engineering which refers to the engineering aspects of food production and processing Food engineering includes but is not limited to the application of agricultural engineering and chemical engineering principles to food materials Genetic engineering of plants and animals is not normally the work of a food engineer Food engineering is a very wide field of activities Among its domain of knowledge and action are Design of machinery and processes to produce foods Design and implementation of food safety and preservation measures in the production of foods Biotechnological processes of food production Choice and design of food packaging materials Quality control of food production      **Industrial Biotechnology** Mukesh Yadav, Vikas Kumar, Nirmala Sehrawat, 2019-10-08 Industrial Biotechnology summarizes different aspects of plant biotechnology such as using plants as sustainable resources phytomedical applications phytoremediation and genetic engineering of plant systems These topics are discussed from an academic as well industrial perspective and thus highlight recent developments but also practical aspects of modern biotechnology      **Enzyme Technology** Ashok Pandey, 2006-04-28 Publisher Description      New and Future Developments in Microbial Biotechnology and Bioengineering Joginder Singh Panwar, Praveen Gehlot, 2020-06-16 New and Future Developments in Microbial Biotechnology and Bioengineering Recent Advances in Application of Fungi and Fungal Metabolites Environmental and Industrial Aspects provides a comprehensive overview of recent development and applied aspects of fungi and its metabolites in environmental and industrial settings Fungi and fungal metabolites have great prospects for developing new products in a wide range of sectors Many fungal metabolites are environmentally friendly clean non toxic agents used for environmental management practices This book offers a systems approach and provides a means to share the latest developments and advances about the exploitation of fungal products including their wide uses in the field of environment and industry Introduces the aspects and advances of fungi and fungal metabolites in environmental and industry perspectives Discusses the potential of fungi and its metabolites in environmental management Includes a description of traditional uses and the modern practices of harnessing the potential of fungi and its metabolites in solving environment issues Provides details about usage of fungi and its metabolites for environmental management and industrial purposes

**Role of Materials Science in Food Bioengineering** Alexandru Mihai Grumezescu, Alina Maria Holban, 2018-03-29 The Role of Materials Science in Food Bioengineering Volume 19 in the Handbook of Food Bioengineering presents an up to date review of the most recent advances in materials science further demonstrating its broad applications in the food industry and bioengineering Many types of materials are described with their impact in food design discussed The book provides insights into a range of new possibilities for the use of materials and new technologies in the field of food bioengineering This is an

essential reference on bioengineering that is not only ideal for researchers scientists and food manufacturers but also for students and educators Discusses the role of material science in the discovery and design of new food materials Reviews the medical and socioeconomic impact of recently developed materials in food bioengineering Includes encapsulation coacervation techniques emulsion techniques and more Identifies applications of new materials for food safety food packaging and consumption Explores bioactive compounds polyphenols food hydrocolloids nanostructures and other materials in food bioengineering

**Microbial Enzyme Technology in Food Applications** Ramesh C. Ray, Cristina M. Rosell, 2017-03-27 The aim of food processing is to produce food that is palatable and tastes good extend its shelf life increase the variety and maintain the nutritional and healthcare quality of food To achieve favorable processing conditions and for the safety of the food to be consumed use of food grade microbial enzymes or microbes being the natural biocatalysts is imperative This book discusses the uses of enzymes in conventional and non conventional food and beverage processing as well as in dairy processing brewing bakery and wine making Apart from conventional uses the development of bioprocessing tools and techniques have significantly expanded the potential for extensive application of enzymes such as in production of bioactive peptides oligosaccharides and lipids flavor and colorants Some of these developments include extended use of the biocatalysts as immobilized encapsulated enzymes microbes both natural and genetically modified as sources for bulk enzymes solid state fermentation technology for enzyme production Extremophiles and marine microorganisms are another source of food grade enzymes The book throws light on potential applications of microbial enzymes to expand the base of food processing industries

**Enzymes in Food Technology** Mohammed Kuddus, 2018-11-19 The integration of enzymes in food processing is well known and dedicated research is continually being pursued to address the global food crisis This book provides a broad up to date overview of the enzymes used in food technology It discusses microbial plant and animal enzymes in the context of their applications in the food sector process of immobilization thermal and operational stability increased product specificity and specific activity enzyme engineering implementation of high throughput techniques screening of relatively unexplored environments and development of more efficient enzymes Offering a comprehensive reference resource on the most progressive field of food technology this book is of interest to professionals scientists and academics in the food and biotech industries

**Fungal Biomolecules** Vijai Kumar Gupta, Robert L. Mach, S. Sreenivasaprasad, 2015-04-20 Fungi have an integral role to play in the development of the biotechnology and biomedical sectors The fields of chemical engineering Agri food Biochemical pharmaceuticals diagnostics and medical device development all employ fungal products with fungal biomolecules currently used in a wide range of applications ranging from drug development to food technology and agricultural biotechnology Understanding the biology of different fungi in diverse ecosystems as well as their biotrophic interactions with other microorganisms animals and plants is essential to underpin effective and innovative technological developments Fungal Biomolecules is a keystone reference integrating branches of fungal product research into a

comprehensive volume of interdisciplinary research As such it reflects state of the art research and current emerging issues in fungal biology and biotechnology reviews the methods and experimental work used to investigate different aspects of fungal biomolecules provides examples of the diverse applications of fungal biomolecules in the areas of food health and the environment is edited by an experienced team with contributions from international specialists This book is an invaluable resource for industry based researchers academic institutions and professionals working in the area of fungal biology and associated biomolecules for their applications in food technology microbial and biochemical process biotechnology natural products drug development and agriculture

### **Current Developments in Biotechnology and Bioengineering**

Ashok Pandey,Sangeeta Negi,Carlos Ricardo Soccol,2016-09-17 Current Developments in Biotechnology and Bioengineering Production Isolation and Purification of Industrial Products provides extensive coverage of new developments state of the art technologies and potential future trends focusing on industrial biotechnology and bioengineering practices for the production of industrial products such as enzymes organic acids biopolymers and biosurfactants and the processes for isolating and purifying them from a production medium During the last few years the tools of molecular biology and genetic and metabolic engineering have rendered tremendous improvements in the production of industrial products by fermentation Structured by industrial product classifications this book provides an overview of the current practice status and future potential for the production of these agents along with reviews of the industrial scenario relating to their production Provides information on industrial bioprocesses for the production of microbial products by fermentation Includes separation and purification processes of fermentation products Presents economic and feasibility assessments of the various processes and their scaling up Links biotechnology and bioengineering for industrial process development

### ***Technologies in Food Processing***

Harish Sharma,Parmjit Panesar,2018-07-17 With the unprecedented increase in the world s population the need for different foodprocessing techniques becomes extremely important And with the increase in awareness of and demand for food quality processed products with improved quality and better taste that are safe are also important aspects that need to be addressed In this volume experts examine the use of different technologies for food processing They look at technology with ways to preserve nutrients eliminate anti nutrients and toxins add vitamins and minerals reduce waste and increase productivity Topics include among others applications of ohmic heating cold plasma in food processing the role of biotechnology in the production of fermented foods and beverages the use of modification of food proteins using gamma irradiation edible coatings to restrain migration of moisture oxygen and carbon dioxide natural colorants as opposed to synthetic coloring which may have toxic effects hurdle technology in the food industry the unrecognized potential of agro industrial waste

### ***The Handbook of Microbial Bioresources***

Vijai Kumar Gupta,Gauri Dutt Sharma,Maria G Tuohy,Rajeeva Gaur,2016-06-27 Microbial technology plays an integral role in the biotechnology bioengineering biomedicine biopharmaceuticals and agriculture sector This book provides a detailed compendium of the methods biotechnological routes and processes used to

investigate different aspects of microbial resources and applications It covers the fundamental and applied aspects of microorganisms in the health industry agriculture and environmental sectors reviewing subjects as varied and topical as pest control health and industrial developments and animal feed      Industrial Applications of Microbial Enzymes Pankaj Bhatt,2022-08-17 Microbial enzymes are important because they can be used for a wide variety of industrial purposes There is dispersed and scanty information available with respect to microbial enzymes and their industrial applications In this edited book leading scientists have covered the various aspects of microbial enzymes and their industrial applications Using microbial enzymes can help expedite various manufacturing processes and contribute to sustainable development which is a priority worldwide Research gaps in the entrainment of microbial enzymes with their direct application in product development are a major focus of this volume Key Features Covers microbial enzymes with comprehensive and in depth information Benefits students by describing recent advancements into microbial enzymology Provides updates regarding microbial enzymes for researchers and industrial scientists Includes findings on the microbial actions for better life

*Developments in Fungal Biology and Applied Mycology* Tulasi Satyanarayana,Sunil K. Deshmukh,B.N. Johri,2017-12-29 This book explores the developments in important aspects of fungi related to the environment industrial mycology microbiology biotechnology and agriculture It discusses at length both basic and applied aspects of fungi and provides up to date laboratory based data Of the estimated three million species of fungi on Earth according to Hawksworth and coworkers more than 100 000 have been described to date Many fungi produce toxins organic acids antibiotics and other secondary metabolites and are sources of useful biocatalysts such as cellulases xylanases proteases and pectinases to mention a few They can also cause diseases in animals as well as plants and many are able to break down complex organic molecules such as lignin and pollutants like xenobiotics petroleum and polycyclic aromatic compounds Current research on mushrooms focuses on their hypoglycemic anti cancer anti pathogenic and immunity enhancing activities This ready reference resource on various aspects of fungi is intended for graduate and post graduate students as well as researchers in life sciences microbiology botany environmental sciences and biotechnology      *Advances in Applied Microbiology* ,1983-10-01 Advances in Applied Microbiology

**Extraction of Natural Products from Agro-industrial Wastes** Showkat Ahmad Bhawani,Anish Khan,Fasihuddin Badruddin Ahmad,2023-01-06 Extraction of Natural Products from Agro industrial Wastes A Green and Sustainable Approach focuses on the different techniques used in this type of extraction such as ultrasound assisted microwave assisted supercritical and other green extraction techniques The book compiles the expertise of authors with diversified backgrounds in analytical chemistry natural product chemistry and separation technology It will be of interest to researchers working in the fields of Analytical Chemistry Separation Science Green extraction techniques and Agro Industrial wastes Readers will find quantitative descriptions and reliable guidelines that reflect the maturation and demand of the field and the development of new green methods Includes an introduction to natural products Describes

various extraction techniques Includes extraction of natural products from Agro Industrial waste **New and Future Developments in Microbial Biotechnology and Bioengineering** Vijai G. Gupta,Susana Rodriguez-Couto,2018-02-06 New and Future Developments in Microbial Biotechnology and Bioengineering Penicillium System Properties and Applications covers important research work on the applications of penicillium from specialists from an international perspective The book compiles advancements and ongoing processes in the penicillium system along with updated information on the possibilities for future developments All chapters are derived from current peer reviewed literature as accepted by the international scientific community These important fungi were found to secrete a range of novel enzymes and other useful proteins and are still being extensively studied and improved for specific use in the food textile pulp and paper biocellulosic ethanol production and other industries The book caters to the needs of researchers academicians dealing with penicillium spp related research and applications outlining emerging issues on recent advancements made in the area of research and its applications in bioprocess technology chemical engineering molecular taxonomy biofuels bioenergy research and alternative fuel development In addition the book also describes the identification of useful compound combinations enzyme cocktails and the fermentation conditions required to obtain them at an industrial scale Finally the book provides updated information on the best utilization of these fungi as a natural tool to meet the next challenges of biotechnology Compiles the latest developments and current studies in the penicillium system Contains chapters contributed by top researchers with global appeal Includes current applications in bioindustry and lists future potential applications of these fungi species Identifies future research needs for these important fungi including the best utilization of them as a natural tool to meet the next challenges of biotechnology Isolation, Modification, and Characterization of the Constituents (Cellulose, Hemicellulose, Lignin, et al.) in Biomass and Their Bio-based Applications Caoxing Huang,Chunlin Xu,Xianzhi Meng,Lei Wang,Xin Zhou,2022-05-31 **Mining, Designing, Mechanisms and Applications of Extremophilic Enzymes** Junpei Zhou,Massimiliano Fenice,Sunil Khare,2021-12-29 **Studies of Fructosyltransferase and Inulinase from Aspergillus Niger NRRL 2270** Kuor-Yarng Jang,1996

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## **Table of Contents Production Purification And Characterization Of Inulinase**

1. Understanding the eBook Production Purification And Characterization Of Inulinase
  - The Rise of Digital Reading Production Purification And Characterization Of Inulinase
  - Advantages of eBooks Over Traditional Books
2. Identifying Production Purification And Characterization Of Inulinase
  - Exploring Different Genres
  - Considering Fiction vs. Non-Fiction
  - Determining Your Reading Goals
3. Choosing the Right eBook Platform
  - Popular eBook Platforms
  - Features to Look for in an Production Purification And Characterization Of Inulinase
  - User-Friendly Interface
4. Exploring eBook Recommendations from Production Purification And Characterization Of Inulinase
  - Personalized Recommendations
  - Production Purification And Characterization Of Inulinase User Reviews and Ratings
  - Production Purification And Characterization Of Inulinase and Bestseller Lists



5. Accessing Production Purification And Characterization Of Inulinase Free and Paid eBooks
  - Production Purification And Characterization Of Inulinase Public Domain eBooks
  - Production Purification And Characterization Of Inulinase eBook Subscription Services
  - Production Purification And Characterization Of Inulinase Budget-Friendly Options
6. Navigating Production Purification And Characterization Of Inulinase eBook Formats
  - ePub, PDF, MOBI, and More
  - Production Purification And Characterization Of Inulinase Compatibility with Devices
  - Production Purification And Characterization Of Inulinase Enhanced eBook Features
7. Enhancing Your Reading Experience
  - Adjustable Fonts and Text Sizes of Production Purification And Characterization Of Inulinase
  - Highlighting and Note-Taking Production Purification And Characterization Of Inulinase
  - Interactive Elements Production Purification And Characterization Of Inulinase
8. Staying Engaged with Production Purification And Characterization Of Inulinase
  - Joining Online Reading Communities
  - Participating in Virtual Book Clubs
  - Following Authors and Publishers Production Purification And Characterization Of Inulinase
9. Balancing eBooks and Physical Books Production Purification And Characterization Of Inulinase
  - Benefits of a Digital Library
  - Creating a Diverse Reading Collection Production Purification And Characterization Of Inulinase
10. Overcoming Reading Challenges
  - Dealing with Digital Eye Strain
  - Minimizing Distractions
  - Managing Screen Time
11. Cultivating a Reading Routine Production Purification And Characterization Of Inulinase
  - Setting Reading Goals Production Purification And Characterization Of Inulinase
  - Carving Out Dedicated Reading Time
12. Sourcing Reliable Information of Production Purification And Characterization Of Inulinase
  - Fact-Checking eBook Content of Production Purification And Characterization Of Inulinase
  - Distinguishing Credible Sources
13. Promoting Lifelong Learning

- Utilizing eBooks for Skill Development
- Exploring Educational eBooks

### 14. Embracing eBook Trends

- Integration of Multimedia Elements
- Interactive and Gamified eBooks

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