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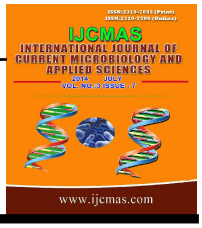

Original Research Article

Isolation and Characterization of β- Galactosidase Enzyme Producing Microbe and Optimization of its Enzyme Activity under different culture condition

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A B S T R A C T

Lactic acid bacteria (LAB) that used as starters for production of dairy products are the main factors of fermentation and protection of fermentative foods and also have a significant role in texture and flavour of food products. The isolated blue colony which have ability to hydrolyze X-Gal on MRS agar has been selected and were inoculated in different culture conditions (carbon source, nitrogen source, metal ions and natural substrates) and pH, Temperature for optimization of β - galalctosidase enzyme activity. As per the reaction of enzyme with its substrate ONPG (O-nitrophenyl β-D galactopyranoside), it was observed that among all the Carbon sources, Nitrogen , Natural sources , culture containing Metal ions, Maximum production of enzyme was obtained in Starch supplemented medium and in comparison with pH at 6 and Temperature at 45ºC. *Lactobacillus delbrueckii* was identified by Gram stain and biochemical methods. The antibiotic test was analyzed by single disc diffusion method, which shows zone of inhibition shown maximum in Tetracycline (2.9 mm), no zone of inhibition in isoniazid and gentamycin. This strain showed that it is ideal for lactose intolerant people and can be used for probiotics.

Keywords

Lactobacillus, MRS Agar, β-galactosidase, X-Gal, ONPG.

Introduction

β-Galactosidase is widely used in food industry to improve sweetness, solubility, flavor and digestibility of dairy products (Richmond, Gray, and Stine, 1981; Grosova et al. 2008a) . Enzymatic hydrolysis of lactose by β galactosidase is one of the most popular technologies to produce lactose reduced milk and related dairy products for consumption by lactose intolerant people (Ladero, Santos, and García-Ochoa, 2000; Ladero et al., 2001; 2002; Jurado et al., 2002; Sener, Apar, and Ozbek, 2006; Haider and Husain, 2008). β-galactosidase facilitates the reaction between the disaccharide molecules (Lactose) and water, thereby cleaving the oxygen bridge resulting in the production of two simple sugars (Glucose and Galactose). By hydrolyzing lactose with β galactosidase, the problems associated with whey disposal, lactose

crystallization in frozen concentrated deserts and milk consumption by lactose-intolerant individuals can be eliminated (Kim and Rajagopal, 2000; Bayramoglu et al., 2007).

β-Galactosidases are found in microorganisms (bacteria fungi, yeasts), plants especially in almonds, peaches, apricots, apples and animal organs (Nagy et al., 2001; Flood and Kondo, 2004; Haider and Husain, 2007a). β-Galactosidases from bacterial sources has been widely used for the hydrolysis of lactose because of the ease of fermentation, high activity of the enzyme and good stability (Picard et al., 2005). Lactic acid bacteria (LAB) which constitute a diverse group of lactococci, streptococci and lacto bacilli have become a focus of scientific studies for three particular reasons: (1) lactose maldigesters may consume some fermented dairy products with little or no adverse effects, (2) LAB are generally regarded as safe (GRAS) so the enzyme derived from them might be used without extensive purification, and (3) some strains have probiotic activity such as improved digestion of lactose (Vasiljevic and Jelen, 2002; Vinderola and Reinheimer, 2003).

Lactobacillus reuteri is a dominant strain of the hetero fermentative Lactobacilli in the gastrointestinal tract of humans and animals. Lactobacilli isolated from the stomach contents of piglets were used for the production of improved fermented milk products. Such Lactobacilli were found in large numbers on the stratified, squamous epithelium of the oesophagea. Porcine strains of Lactobacilli would probably be capable of fermenting lactose in bovine milk (Sung et al., 2003).

β galactosidase activity is also abundantly present in the colon of human beings. It catalyzes the first step of lactose fermentation in colon and is often measured as an indication of the capacity of colonic microbiota to ferment lactose present in the intestine (Jain, Gupta, and Jain, 2007). The organism was isolated and screened with Xgal and assayed to estimate the ONPG fermenting ability of β -galactosidase. Our main focus was on the optimization of the media based on the following factors: Temperature, pH, carbon and nitrogen sources, metal ions and natural substrates.

Materials and Methods

Collection of Sample: Culture of microbes were isolated from the curd sample which was collected from the domestic/residential area(Delhi), and maintained in the laboratory of Helix Biogensis, Noida which was used throughout the work.

Isolation of β - Galactosidase Producing Bacteria:

The curd sample were serially diluted and plated on MRS agar plate containing 20ul of X-Gal (20mg/ml in DMSO) on its surface. The isolated blue colonies has been selected which indicates the presence of β galactosidase enzyme producing bacteria. The isolated strain were primarily screened for an effective β-galactosidase production which is further selected for the detailed investigation regarding the optimization of its Enzyme production in different culture conditions (carbon source, nitrogen source, metal ions and natural substrates) and at different pH and Temperatures.

Inoculums Preparation For Enzyme Production:

One loop full of master culture was transferred to the MRS broth (Robert et al.2006). For effective production. it was carried out in shaker at 37ºC for 48 hours.

Culture Supplementation with various Sources and Stresses

The production medium was supplemented with different carbon sources (1% w/v Maltose, Dextrose, Sucrose, Lactose Starch), Nitrogen sources (1% w/v organic nitrogen sources such as Yeast, Beef, Peptone and inorganic nitrogen sources such as Urea, Sodium Nitrate, Sodium Chloride, Ammonium. sulphate, Ammonium nitrate, Potassium nitrate), Metal ions (1mM concentration $FeSO_4$, $ZnSO_4$, $MnSO_4$ and EDTA) and Natural sources (1% w/v Wheat Bran, Wheat Flour and Potato Starch) to investigate their effect on enzyme production. The effects of temperature and pH on the production of enzyme were also carried out by different temperature range (45-85ºC) and pH value (4-10).

Estimation of β -Galactosidase Enzyme Assay

All bacteria were inoculated into tubes containing ONPG and the production of yellow color was indicated positive ONPG results(Miller,1998). 0.1 ml of the culture pallet with 0.9 ml Z-Buffer was added with 100 ul chloroform and 50 ul of 1:9 ratio of Toluene: Acetone. After that 0.2 ml of ONPG (pre warm 4 mg/ml) was added and incubated at 37ºC for 15 min. The yellow color was observed and then reaction was stopped by adding 0.1 ml of 1M Na_2CO_3. Absorbance valued at 420 and 550nm were recorded for each micro tube and β-galactosidase value was calculated.

Identification of Isolated Bacteria:

Bacteria were examined by Gram stain, and identified by standard bacteriological and biochemical methods. Biochemical characterization of selected curd isolate were done by testing for IMVIC test, Urease, Salt concentration, Gelatin liquefaction, Starch hydrolysis, Catalase test, Oxidase test, Carbohydrate Assimilation Test and Mannitol Salt agar test. The bacterium which shown maximum enzyme activity was characterized based on Bergey's Manuel of Systematic Bacteriology.

Antibiotic Susceptibility of β-Galactosidase Producing Bacteria

The antibiotic susceptibility of *β-galactosidase* producing bacteria was analyzed by using standard single disc-diffusion method. The overnight culture of the test organism was seeded on MRS agar plate. Then various antibiotic-impregnated discs containing Gentamycin, Destone, Nalidixic, Isoniazid, Tetracyclin, Penicilin, Amphicilin (25 mg/ml) were placed on the seeded plate and the plates were incubated at 37°C. The zone of inhibition was determined after 48 hr.

Results and Discussion

β -galactosidase has been widely used for industrial as well as medical application. In dairy industries β-galactosidase has been used to prevent crystallization of lactose, to improve sweetness and to increase the solubility of the milk product (Kara, 2004). The organism was isolated and screened with X- gal and assayed to estimate the ONPG fermenting ability of β -galactosidase.

The isolated blue colonies has been selected which indicates the presence of β -galactosidase enzyme producing bacteria (Favier et al., 1996). Then selected colonies were inoculated in different culture conditions (carbon source, nitrogen source, metal ions, pH, Temperature and natural substrates) to optimized it for β -galactosidase enzyme production.

Effect of differences sources on β-gal activity

It was observed that, all the carbon sources (dextrose, maltose, lactose, sucrose and starch), nitrogen sources (yeast extract, beef extract, peptone, urea, sodium nitrate, ammonium nitrate, ammonium sulphate, sodium chloride and potassium nitrate) showed deep yellow coloration positive. Culture supplemented with natural sources showed vary faint yellow coloration and culture containing metal ions showed no coloration. In case of pH the intensity of color decreases from alkaline pH 10 to acidic medium pH 4 and when temperature increases the activity of enzyme decreases from 45ºC to 85ºc. and 45ºC and 55ºC temp shows faint yellow coloration as shown in the Fig. 1.

The carbon source is very essential for the effective production of the enzyme. Starch was found to be the most effective carbon source for the maximum production of enzyme. This is correlated with the findings of Akcon. In most of the organisms, both the organic and inorganic forms of nitrogen are metabolized within the cell to produce amino acids, proteins, nucleic acids, and cell wall components. Various nitrogen sources were used, of which sources such as Beef extract and Yeast extract had an influence over the enzyme production when compared to Urea, Peptone and Sodium chloride where the activity was reduced as shown in Fig. 2. These findings are related to El-Shebay et al. who worked on the production of *α-galactosidase* activity. When the culture supplemented with Natural substrates and Metal ions at 1% concentration, enzyme activity was not observed. The pH value is very important, the maximum production was observed at pH 6.Yapi et al., 2009 reported that Bacillus sp. can be grown maximally when the medium is maintained at pH of 7. At Temperature Enzyme activity was found to be maximum at the temperature 45ºC & 55ºC (Fig. 2). Chakraborti et al., 2003 showed that β-galactosidase was produced at maximum level when maintained at temperatures of 37 ºC. Comparative values of maximum activity against all Culture conditions can be observed in Fig. 3 and Table 1. The results detected by different biochemical methods (ONPG, X-gal methods) confirmed each other which were similar to previous works (Favier et al., 1996).

Antimicrobial Sensitivity: The antibiotic test was analyzed by single disc diffusion method. The zone of inhibition shown in Destine, Ampicillin, Penicillin, Tetracycline, Nalidixic Acid and no zone of inhibition in Isoniazid and Gentamycin as shown in Fig. 4 and Table 2.

Fig.1 The Effect of Different Sources (A- Carbon, B -Nitrogen, C- Natural and Metal) and Stresses (D- pH, E- Temperature) on β-gal Activity

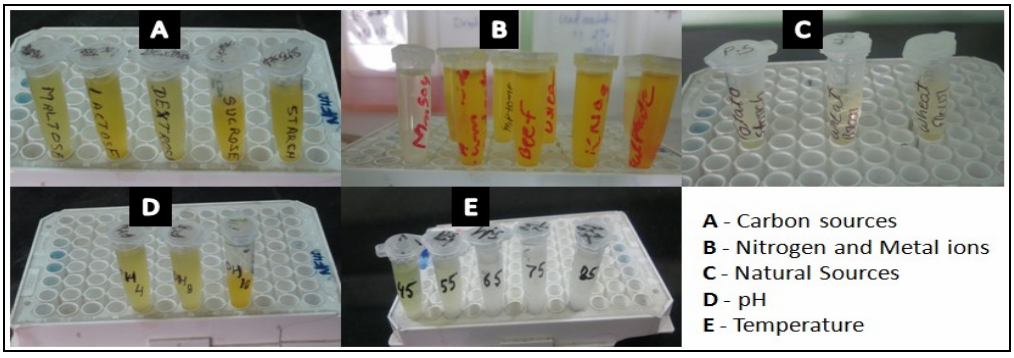

Fig.2 Comparison of Enzyme activity within supplemented sources and stress itself

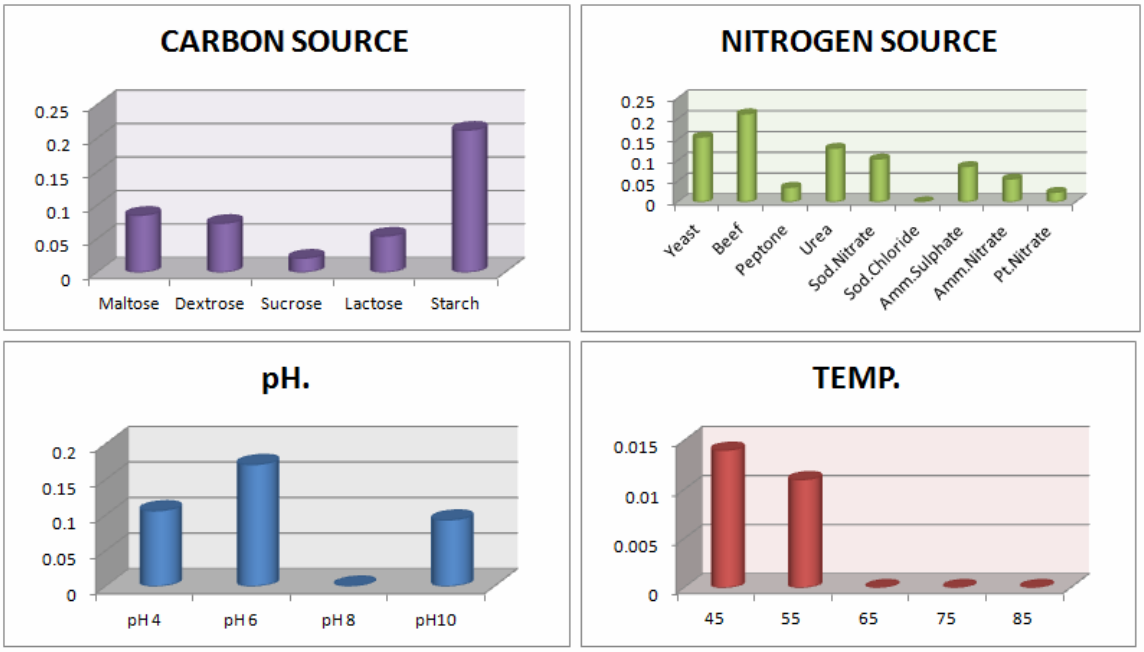

Fig.3 β- Galactosidase Activity Against All Sources and Stress Conditions

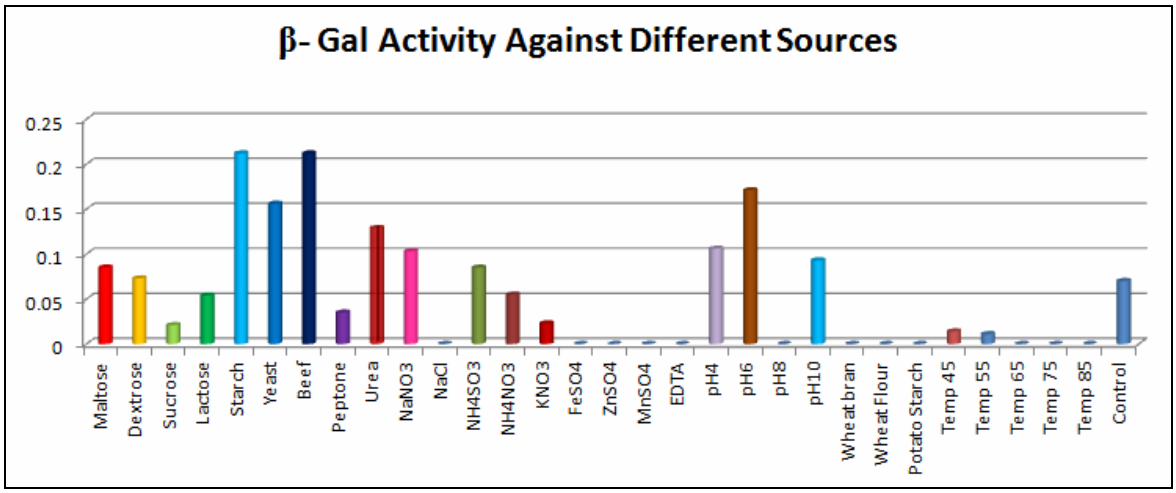

Table.1 Value of Enzyme activity after Supplementation of Different sources and stress Conditions

Maltose	**Dextrose**	**Sucrose**	**Lactose**	**Starch**	**Yeast Extract**	**Beef Extract**	**Peptone**	**Urea**	**$NaNO_3$**	**Control**
0.085	0.073	0.021	0.054	0.212	0.156	0.212	0.035	0.129	0.103	0.07
NaCl	**NH_4SO_3**	**NH_4NO_3**	**KNO_3**	**$FeSO_4$**	**$ZnSO_4$**	**$MnSO_4$**	**EDTA**	**pH_4**	**pH_6**	
0	0.085	0.055	0.023	0	0	0	0	0.106	0.171	
pH_8	**pH_{10}**	**Wheat bran**	**Wheat Flour**	**Potato Starch**	**Temp 45**	**Temp 55**	**Temp 65**	**Temp 75**	**Temp 85**	
0	0.093	0	0	0	0.014	0.011	0	0	0	

Fig.4 Antimicrobial Activity of Isolated Microbe Against Different Antibiotics.

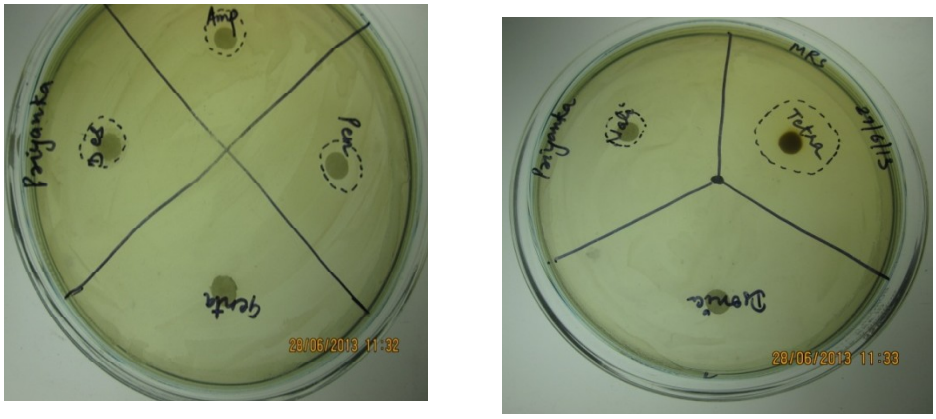

Table.2 Zone of Inhibition Produced by Antibiotics Against Isolated Microbe

Antibiotics	Zone of Inhibition (mm)	Sensitive/Resistant
Destine	1.1	++
Ampicillin	0.8	+
Penicillin	1.3	++
Tetracycline	2.9	+++
Nalidixic Acid	0.7	+
Isoniazid	0	-
Gentamycin	0	-

+++ Highly Sensitive (>2.0) , ++ Moderately Sensitive (1.0 -2.0) , + Less Sensitive (< 1) , - Resistant (0)

The curd isolate showed positive results in Indole and MR test , Starch, carbohydrates assimilation, where as negative results showed in citrate test, VP test, catalase, gelatin liquification, urease, oxidase and mannitol. Through the conclusion of these biochemical tests *Lactobacillus delbrueckii* was identified. Maximum production of enzyme was obtained in Starch supplemented medium and in comparison along pH at 6 and Temperature at 45º.

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References

Richmond ML, Gray JI, Stine CM. 1981. β galactosidase: review of recent research related to technological application, nutritional concerns, and immobilization. J Dairy Sci 64: 1759–1771

Ladero M, Santos A, García-Ochoa F. 2000. Kinetic modeling of lactose hydrolysis with an immobilized beta-galactosidase from Kluyveromyces

fragilis. Enzyme Microb Technol 27: 583–592.

Ladero M, Santos A, Garcia JL, Garcia-Ochoa F. 2001. Activity over lactose and ONPG of a genetically engineered β galactosidase from Escherichia coli in solution and immobilized kinetic modeling. Enzy Microb Technol 29: 181–193

Ladero M, Santos A, Garcia JL, Carrascosa AV, Pessela BCC, Garcia-Ochoa F. 2002. Studies on the activity and stability of β galactosidase from Thermus sp. strain T2 and from luveromyces fragilis. Enzy Microb Technol 30: 392–405

Jurado E, Camacho F, Luzon G, Vicaria JM. 2002. A new kinetic model proposed for enzymatic hydrolysis of lactose by a β galactosidase from Kluyveromyces fragilis. Enzy Microb Technol 31: 300–309.

Sener N, Apar DK, Ozbek B. 2006. A modeling study on lactose hydrolysis and β galactosidase stability under sonication. Proc Biochem 41: 1493–1500

Haider T, Husain Q. 2008. Concanavalin A layered calcium alginate-starch beads immobilized beta galactosidase as a therapeutic agent for lactose intolerant patients. Int J Pharm 359: 1–6.

Kim JW, Rajagopal SN. 2000. Isolation and characterization of beta galactosidase from Lactobacillus crispatus. Folia Microbiol (Praha) 45 29–34

Bayramoglu G, Tunali Y, Arica MY. 2007. Immobilization of β galactosidase onto magnetic poly (GMA-MMA) beads for hydrolysis of lactose in bed reactor. Cataly Comm 8: 1094–1101.

Nagy Z, Kiss T, Szentirmai A, Biró S. 2001. Beta-galactosidase of Penicillium chrysogenum: production, purification, and characterization of the enzyme. Protein Expr Purif 21: 24–29.

Flood MT, Kondo M. 2004. Toxicity evaluation of a beta-galactosidase preparation produced by Penicillium multicolor. Regul Toxicol Pharmacol 40: 281–292.

Haider T, Husain Q. 2007a. Preparation of lactose free milk by using ammonium sulphate fractionated proteins from almonds. J Sci Food Agric 87: 1278–1283

Picard C, Fioramonti J, Francois A, Robinson T, Neant F, Matuchansky C. 2005. Review article: bifidobacteria as probiotic agents -- physiological effects and clinical benefits. Aliment Pharmacol Ther 22: 495–512.

Vasiljevic T, Jelen P. 2002. Lactose hydrolysis in milk as affected by neutralizers used for the preparation of crude β galactosidase extracts from Lactobacillus bulgaricus 11842. Innov Food Sci Emerg Technol 3: 175–184.

Vinderola CG, Reinheimer JA. 2003. Lactic acid starter and probiotic bacteria: A comparative in vitro study of probiotic characteristics and biological barrier resistance. Food Res Int 36: 895–904.

Sung HW, Chen CN, Liang HF, Hong MH. 2003. A natural compound (reuterin) produced by Lactobacillus reuteri for biological-tissue fixation. Biomaterials 24: 1335–1347

Jain A, Gupta Y, Jain SK. 2007. Perspectives of biodegradable natural polysaccharides for site-specific drug delivery to the colon. J Pharm Pharm Sci 10: 86–128.

Akcan, N. 2011. High level production of extracellular -galactosidase from Bacillus licheniformis ATCC 12759 in submerged fermentation. African Journal of Microbiology Research. 5: 4615-4621.

Chakraborti, S., Sani, R.K., Sahoo, D.K., Banerjee, U.C. and Sobti, R.C. 2003. Production and Partial Characterization of a Novel beta Galactosidase from a newly isolated Bacillus polymyxa. Scientia Iranica. 10: 279-286.

Désiré Yapi, Assoi Yapi, Dago Gnakri, Sebastien Lamine Niamke and Lucien Patrice Kouame. 2009. Purification and Biochemical characterization of a specific -glucosidase from the digestive fluid of larvae of the palm weevil, Rhynchophorus palmarum. Journal of Insect Science. 9: 1-13.

El-Shebawy, K., Saleh, S., Afify, A. and El-Sayed, O.H. 2007. Production of A-Galactosidase from Local Isolated Strain of Bacillus circulans. Part 1: Optimization of the different physiological condition. N. Egypt. J. Microbiol. 17: 224-232.

Favier, C., Neut, C., Mizon, C., Cortot, A., Clombel, J.F., Mizon, J .1996. Differentiation and identification of human faecal anaerobic bacteria producing beta-galactosidase (a new methodology). J. Microbiol. Methods. 27: 25-31.

Kara, F. 2004. Release and Characterization of Beta galactosidase from Lactobacillus plantarum. M.Sc. Thesis, Middle East Technical University, Turkey.

Miller. J.H. 1998. Assay of α-galactosidase. In: Miller JH, ed. Experiments in molecular genetics. New York: Cold Spring Harbor Laboratory. 352-355.

Robert Kranz, Kathleen Weston-Hafer and Eric Richards. 2006. Identifying Unknown Bacteria Using Biochemical and Molecular Methods. Washington University press, Saint Louis.