

ABSTRACT

A sporulating, aerobic *Bacillus subtilis* sp. was isolated from the Chimanimani hot spring in Zimbabwe. The microorganism was characterised using classical identification tools and molecular techniques. The microbial strain was designated *Bacillus subtilis* CHZ1. It produced an endoglucanase enzyme when cultured on M162 medium with an initial value of pH within the range 5.0 – 9.0 and a temperature range between 30°C and 50°C. The optimal conditions for the production of the endoglucanase enzyme were a pH value of 6.0 and temperature of 50°C. The enzyme was produced constitutively when the microorganism was cultured in M162 medium supplemented with either starch, cellobiose, carboxymethylcellulose, sucrose, glucose, galactose, Avicel, lactose, mannose or maltose as carbon sources.

The *Bacillus subtilis* CHZ1 was found to produce hydrolytic extracellular enzymes when cultured on a medium extracted from opaque beer brewery wastewater supplemented with defatted soya, spent yeast and malt flour. The medium from the food waste materials was named CWW medium. Elimination of one raw material component during CWW medium preparation revealed that all the components were essential for maximum enzyme production. The absence of spent yeast during CWW medium preparation greatly reduced the levels of enzyme production. In addition, the microorganism produced endoglucanase, amylase, polygalacturonase, xylanase and protease when cultured on CWW medium. All assayed enzymes reached high levels within 5 – 10 h during fermentation at 40°C and pH maintained at 6.0 in fermentor experiments. A cultivation temperature of 50°C resulted in a rapid drop in all the enzyme levels after 12 h of cultivation. High biomass and enzyme levels were obtained in fermentor cultivations compared to shake flask experiments. This showed that food wastes could be used to produce hydrolytic enzymes from *B. subtilis* CHZ1.

A proteolytic enzyme produced by a *B. subtilis* CHZ1 was purified using ammonium sulphate precipitation, gel filtration and cationic exchange on S-Sepharose fast flow column chromatography. Production of the protease enzyme was higher when the *Bacillus* strain was cultured in a synthetic medium, M162, supplemented with 0.3 % (w/v) organic compared to inorganic nitrogen sources. Enzyme production was growth associated and its production was highest when tryptone was used as the nitrogen source. When run on SDS-PAGE gel, the purified enzyme gave a 35 kDa band, suggesting that it consisted of a single polypeptide chain. High protease enzyme activity was observed in the pH range of 6 – 10 with a maximum value at pH 8.0 when 0.5 % (w/v) azocasein was used as the substrate for activity assay. Maximum temperature for protease activity was found to be 50°C, and the enzyme had considerable thermal stability for 5½ h retaining about 90 % residual activity at this temperature. At 2.5 mM concentration, PMSF, Ag⁺ and Hg⁺ caused marked reduction in activity on the protease enzyme. Natural biological metal cofactors that include Mn²⁺, Mg²⁺ and Fe²⁺ increased the enzyme activity, while Zn²⁺, Cu²⁺ and Ca²⁺ did not affect the enzyme's activity. The protease pH and thermal stability as well as high activity expressed by small amounts of the enzyme can be exploited for industrial applications. The endoglucanase gene (*celG*) from *B. subtilis* CHZ1 was amplified by the polymerase chain reaction, cloned and expressed in *Escherichia coli* DH5α. The amplified fragment with the full *celG* structural gene was 2 589 bp and its nucleotide sequence was determined and analysed. The amplified fragment had an open reading frame of 1 524 bp encoding a predicted protein of 56 472 daltons in size containing 508 amino acids. The most likely ribosomal binding site resembling that of *B. subtilis* σ⁴³ RNA polymerase was identified upstream of the highly likely initiation start codon. The gene contains a secretory signal sequence encoding a peptide of 38 amino acid residues. A BLASTN search using the 2 589 bp sequence showed 98 % identity to endo-β-1,4-glucanase genes

in the databases. Its predicted protein structure was that of an endoglucanase comprising a catalytic domain (CD) of 278 amino acids linked to a cellulose-binding domain (CBD-3) with 83 residues. The enzyme was identified as belonging to the family-5A glycosyl hydrolases. Amino acid alignment of the predicted protein with the aid of SWISSPro computer program gave high homology. The regular seven conserved regions of the endoglucanase enzymes were identified on Cel5G.

The *celG* gene was overexpressed in *E. coli* DH5 α and could be induced 10 fold with 1.0 mM IPTG. The cloned endoglucanase enzyme optimal activity conditions were the same as those of the wild type strain. It was however observed that about 70 % of the endoglucanase activity from the clone was cell bound.

Site directed mutagenesis at position 1168 of the *celG* endoglucanase enzyme resulted in the production of an inactive enzyme.

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ABBREVIATIONS

CBD	-	cellulose binding domain
CD	-	catalytic domain
CMC	-	carboxymethyl cellulose
CWW	-	chibuku waste water
DFP	-	diisopropyl fluorophosphate
DNA	-	deoxyribonucleic acid
EDC	-	ethyl-(3-dimethylamine propyl) carbodiimide
EDTA	-	ethylenediaminetetraacetic acid
EHEC	-	ethylhydroxyethyl cellulose
FPU	-	filter paper units
HEC	-	hydroxyethyl cellulose
HPMC	-	hydroxypropylmethyl cellulose
IPTG	-	isopropyl- β -D-thiogalactopyranoside
PCR	-	polymerase chain reaction
PMSF	-	phenyl methyl sulfonyl fluoride
PVA	-	polyvinyl alcohol
RNA	-	ribonucleic acid
rRNA	-	ribosomal ribonucleic acid
SCP	-	single cell protein
SDS-PAGE	-	sodium dodecyl sulphate polyacrylamide gel
TLCK	-	N-p-tosyl-L-lysine chloromethyl ketone
TPCK	-	N-tosyl-L-phenylalanine chloromethyl ketone
CWW	-	Chibuku waste water