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Characterization of a new keratinolytic bacterium that completely degrades native feather keratin

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Abstract A novel feather-degrading microorganism was isolated from poultry waste, producing a high keratinolytic activity when cultured on broth containing native feather. Complete feather degradation was achieved during cultivation. The bacterium presents potential use for biotechnological processes involving keratin hydrolysis. *Chryseobacterium* sp. strain kr6 was identified based on morphological and biochemical tests and 16S rRNA sequencing. The bacterium presented optimum growth at pH 8.0 and 30 °C; under these conditions, maximum feather-degrading activity was also achieved. Maximum keratinase production was reached at 25 °C, while concentration of soluble protein was similar at both 25 and 30 °C. Reduction of disulfide bridges was also observed, increasing with cultivation time. The keratinase of strain kr6 was active on azokeratin and azocasein as substrates, and presented optimum pH and temperature of 7.5 and 55 °C, respectively. The keratinase activity was inhibited by 1,10-phenanthroline, EDTA, Hg^{2+}, and Cu^{2+} and stimulated by Ca^{2+}.

Keywords Keratin · Proteolysis · Poultry waste · Chicken feather · Bacteria

Introduction

Feathers are produced in large amounts as a waste by-product at poultry processing plants, reaching millions of tons per year worldwide (Williams et al. 1991). Since feathers are almost pure keratin protein, feather wastes represent a potential alternative to more expensive dietary ingredients for animal feedstuffs (Shih 1993). However, feathers are currently utilized on a limited basis as a dietary protein supplement for animal feed because feather meal production is an expensive process, requiring significant amounts of energy. In addition this process destroys certain amino acids, yielding a product with poor digestibility and variable nutrient quality (Papadopoulos et al. 1986; Wang and Parsons 1997).

Keratin is the insoluble structural protein of feathers and wool and is known for its high stability (Bradbury 1973). The composition and molecular configurations of its constituent amino acids warrant structural rigidity. The keratin chain is tightly packed in the α-helix (α-keratin) or β-sheet (β-keratin) into a supercoiled polypeptide chain (Parry and North 1998), resulting in mechanical stability and resistance to common proteolytic enzymes such as pepsin, trypsin, and papain. In addition, cross-linking of protein chains by cysteine bridges confers high mechanical stability and resistance to proteolytic degradation of keratins. Nevertheless, feathers do not accumulate in nature, since structural keratin can be degraded by some microorganisms (Onifade et al. 1998). The current investigation has been focused on proteolytic microorganisms; however, reduction of cysteine bridges may significantly influence keratin degradation (Noval and Nickerson 1959; Kunert and Stransky 1988).

Keratinolytic enzymes may have important uses in biotechnological processes involving keratin-containing wastes from poultry and leather industries, through the development of non-polluting processes (Shih 1993; Onifade et al. 1998). After hydrolysis, the feathers can be converted to feedstuffs, fertilizers, glues, and films or used for the production of the rare amino acids serine, cysteine, and proline (Papadopolous et al. 1986; Yamauchi et al 1996). Known keratinases are mainly produced by mesophilic fungi and actinomycetes (Noval and Nickerson 1959; Kushwaha 1983; Böckle et al. 1995; Santos et al. 1996), but some thermophilic species of *Bacillus* produce feather-degrading enzymes (Williams et al. 1990; Kim et al. 2001). The use of keratinase to upgrade the nutritional value of feather meal has been described. Com-

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parable growth rates were observed between chickens fed with isolated soybean and those fed with feather meal fermented with *Streptomyces fradiae* plus methionine supplementation (Elmayergi and Smith 1971). The utilization of a *Bacillus licheniformis* feather-lysate with amino acid supplementation in test diets fed to growing broilers produced a weight gain identical to that achieved with soybean meal (Williams et al. 1991). The use of crude keratinase significantly increased the amino acid digestibility of raw feathers and commercial feather meal (Lee et al. 1991).

The aim of this study was to identify new keratinolytic bacteria showing high feather degradation at room temperature, with potential application in biotechnological processes. Such microorganisms will be less energy-consuming than the thermophilic strains usually used in feather processing. We have isolated mesophilic feather-degrading bacteria from industrial poultry waste. In a previous work, we described the characterization of the proteolytic *Vibrio* sp. strain kr2, which may be potentially useful for processes involving keratin hydrolysis (Sangali and Brandelli 2000). This report describes the identification, growth, and keratinase production by another novel feather-degrading isolate.

Materials and methods

Isolation of keratinolytic micro-organism

Feathers were collected from several sites at a local poultry industry. Feathers were flooded in peptone broth (5 g l^{-1})and incubated for 24 h at 30 °C. The suspension was used to streak feather-meal agar plates (10 g feather-meal l^{-1} , 0.5 g NaCl l^{-1} , 0.3 g $K_2HPO_4l^{-1}$, 0.4 g $KH_2PO_4l^{-1}$, and 15 g agar l^{-1}) which were incubated at 30 °C for 3 days. Single colonies were isolated and screened for their ability to hydrolyze keratin in feather-meal agar plates. Colonies producing clear zones in this medium were selected for further analysis.

Growth determination

The isolate from a 10^6 colony forming units (CFU) ml^{-1} culture was cultivated for 72 h in whole-feather medium (10 g whole feathers l^{-1} , 0.5 g NaCl l^{-1} , 0.3 g K_2HPO_4 l^{-1} , 0.4 g $KH_2PO_4l^{-1}$). Initial pH was set at 5.0, 6.0, 7.0 or 8.0. Cultures were grown in 500-ml flasks containing 100 ml of medium and incubated at either 25, 30 and 37 °C in an orbital shaker at 180 rpm. Bacterial growth was monitored by measuring the CFU ml^{-1}, as described elsewhere (Sangali and Brandelli 2000). The bacterial suspension was diluted to 10^{-8} in phosphate-buffered saline. The samples were then homogenized and loaded (20 µl) in triplicate onto nutrient agar plates, which were incubated for 24 h at 30 °C. Thirty to 100 colonies were subsequently counted.

Taxonomic studies

Bacteria were identified based on morphological and biochemical tests (MacFadin 2000), comparing the data with standard species and using an API 20E kit (Bio-Mérieux). Morphological and physiological characteristics of the isolated bacterium were compared with data from *Bergey's manual of systematic bacteriology* (Palleroni 1984).

The sequence of the 16S rRNA gene was determined after genomic DNA extraction, PCR amplification, and sequencing. Genomic DNA was extracted from one colony of isolate kr6, which was resuspended in 50 µl of distilled water and boiled for 10 min. Samples were centrifuged and DNA was amplified by PCR using 5 µl of supernatant, according to the method of Osborn et al. (1999). The bacterial 16S rRNA primers were 63f (5′ CAGGCCTAACACATGCAAGTC 3′), 907r (5′ CCGTCAATTCCTTTGAGTTT 3′) and 1389r (5′ ACGGGCGGTGTGTACAAG 3′), corresponding to *Escherichia col*i 16S rRNA gene position. PCR products were purified using the columns from Wizard PCR preps DNA purification systems (Promega). Sequencing reactions were carried out in a 7.5-µl reaction volume with 15 ng of purified DNA, 1.5 µl of 1-µM primer 63f, 3 µl of BigDye Terminator 3.0 (ABI Prism, PE Applied Biosystems), and 0.5 µl of distilled water. PCR was run for 25 cycles under the following conditions: 96 °C for 20 s, 55 °C for 10 s, and 60 °C for 4 min. An ABI PRISM 373 XL (PE Applied Biosystems) was used for sequencing. The 1,237-bp sequence was submitted to Genbank (accession number AY157745). The BLAST algorithm was used to search for homologous sequences in Genbank. The sequence was reversed, aligned, and compared to similar database sequences using the Genetics Computer Group package (Madison, Wis.). The phylogenetic tree was inferred from Jukes-Cantor distances using the neighbor-joining method (software PHYLIP 3.6 a2, Felsenstein 1989). The branching pattern was checked by 1,000 bootstrap replicates.

Enzyme production

The organism was cultivated for 72 h in whole-feather medium, from a 10^6 CFU ml^{-1} culture. Samples were centrifuged at 10,000×*g* for 5 min, and the supernatant was used as a crude enzyme preparation.

Synthesis of azokeratin

Azokeratin was synthesized based on the methodology described for azoalbumin (Tomarelli et al. 1949). Keratin was coupled with a diazotized aryl amine to produce a chromophoric derivative, sulfanilic acid-azokeratin. Fifteen grams of feather keratin were suspended in 1 l distilled water, and 100 ml of a 100 g $NaHCO_3$ l^{-1} solution were added with stirring. Simultaneously, 8.65 g of sulfanilic acid were dissolved in 200 ml of 0.12 M NaOH; then 1.7 g $NaNO_2$ were added. The solution was stirred and 10 ml of 5.0 M HCl added. The solution was stirred again for 2 min and then 10 ml of 5.0 M NaOH were added with stirring. This solution was mixed with the keratin suspension. The reaction mixture was stirred for 5 min and dialyzed against distilled water at 4 °C. The dialysed solution was freeze-dried. Azokeratin is a deep red-orange compound with an absorption maximum at 440–450 nm.

Enzyme assays

Keratinase activity was assayed with azokeratin as a substrate by the following method. The reaction mixture contained 100 µl of enzyme preparation and 800 µl of 10 g azokeratin l^{-1} in 50 mM tris buffer, pH 8.0. The mixture was incubated for 15 min at 50 °C; the reaction was stopped by the addition of trichloroacetic acid to a final concentration of 100 g l^{-1}. After centrifugation at 10,000×*g* for 5 min, the absorbance of supernatant was determined at 440 nm. One unit of enzyme activity was the amount of enzyme that caused a change of absorbance of 0.01 at 440 nm for 15 min at 50 °C. A similar protocol was used to determine enzyme activity on azocasein. Activity on benzoyl-arginine-*p*-nitroanilide (BAPNA) was carried out as described previously (Sangali and Brandelli 2000).

Determination of protein concentration

A cell-free supernatant of each feather culture was used for the determination of soluble protein by the Folin phenol reagent method (Lowry et al. 1951), with bovine serum albumin as standard.

Determination of thiol formation

Free thiol groups were determined essentially as described elsewhere (Sullivan et al. 1942). To 1 ml of sample were added 0.2 ml of NH_4OH, 1 ml of 0.5 g NaCN l^{-1} , and 1 ml of water. The mixture was incubated for 20 min at 25 °C, and then 0.2 ml of 0.5 g sodium nitroprusside l^{-1} were added. Absorbance at 530 nm was measured within 2 min.

Effects of chemicals on keratinase activity

Chemicals were added to the enzyme preparations and incubated for 15 min at 25 °C before being tested for keratinase activity. The protease inhibitors phenylmethylsulfonyl fluoride (PMSF), EDTA, and pepstatin, the detergents SDS and Triton X-100, the organic solvents dimethyl sulfoxide (DMSO) and isopropanol, and the reducing agent 2-mercaptoethanol were used at the working concentrations listed in Table 2. The metal ions tested were added to reach a working concentration of 10 mM.

Chemicals

Azocasein, BAPNA, PMSF, pepstatin, and Folin-Ciocalteau's reagent were from Sigma (St. Louis, Mo., USA). Other reagents were from Merck (Darmstadt, Germany) unless otherwise stated.

Table 1 Morphological and physiological characteristics of keratinase-producing bacterial strain kr6

Morphological characteristics	
Form	Rods
Size	0.5×1-2 µm
Gram stain	Negative
Spore	Non-sporulating
Cultural characteristics	
Feather meal agar colonies	Yellow color, circular, smooth, convex, undulate, moist
Physiological characteristics	
Catalase	Positive
Oxidase	Positive
Oxidation-fermentation test	Oxidative
Voges-Proskauer test	Negative
Citrate	Negative
Nitrate reduction	Positive
Gelatin liquefaction	Positive
Starch hydrolysis	Negative
Lipase	Positive
DNAse	Negative
Lysine decarboxylase	Negative
Ornithine decarboxylase	Negative
Arginine dihydrolase	Negative
Triple sugar iron agar	Acid slant/alkaline butt/no H_2S production
Motility	Negative

Results

Characterization of keratinase-producing strain

The feather-degrading microorganism kr6 isolated from feathers in decomposition demonstrated pronounced growth in whole-feather medium. This isolate was selected for identification and for its adaptation to feather degradation. Cells of the isolate were grown on whole feathers and transferred at frequent intervals to basal medium containing whole feathers. Feather barbules were completely degraded and rachis was also attacked by the bacterium (Fig. 1). The isolate was able to complete the disintegration of whole feathers.

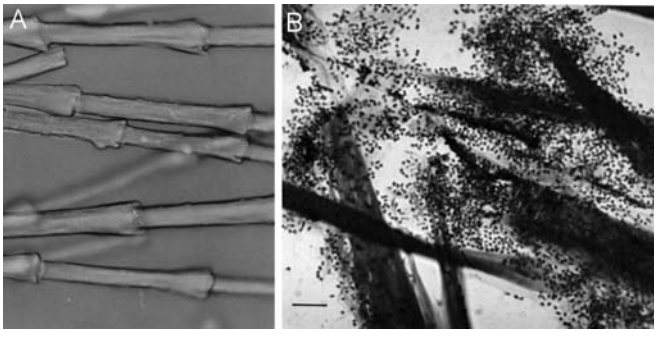

Fig. 1A, B Feather degradation by keratinolytic bacteria. Native feathers were autoclaved and incubated at 30 °C for 3 days in **A** the absence or **B** the presence of strain kr6. *Bar* 10 µm

The results of taxonomic studies on the isolated strain kr6 are summarized in Table 1. The identification of this bacterial isolate was based on cell and colony morphology, growth characteristics, several biochemical tests, API20E, and 16S rRNA sequence data. Microscopic observation of the isolate showed a gram-negative straight rod; the bacterium grew aerobically and formed typical yellow colonies. Together with physiological and API 20E profiling, these characteristics suggest the *Flavobacteriaceae* family, genus *Flavobacterium* (Palleroni 1984) and genus *Chryseobacterium* (Vandamme et al. 1994). In the genus *Chryseobacterium*, the new isolate was similar to *C. gleum* and *C. indologenes*.

The genus determination based on physiological traits was confirmed by phylogenetic analysis of the 16S rRNA gene. The 16S rRNA sequence of strain kr6 showed high similarities to those of a group consisting of several *Chryseobacterium* strains (similarity 95.6–99.2%). The isolate kr6 shared 99.2% sequence similarity with *Chryseobacterium gleum* ATCC 35910 and 98.6% similarity with *Chryseobacterium indologenes* ATCC 29897 (Fig. 2). Bootstrap analysis resulted in relatively high values for the branching of kr6 within the *Chryseobacterium* cluster. Other related members of the *Flavobacteriaceae* (*Bergeyella, Empedobacter, Capnocytophaga, Coenonia, Arenibacter, Aequorivita, Flavobacterium* species) shared 81.2–94.4% sequence similarity with strain kr6 (Fig. 2).

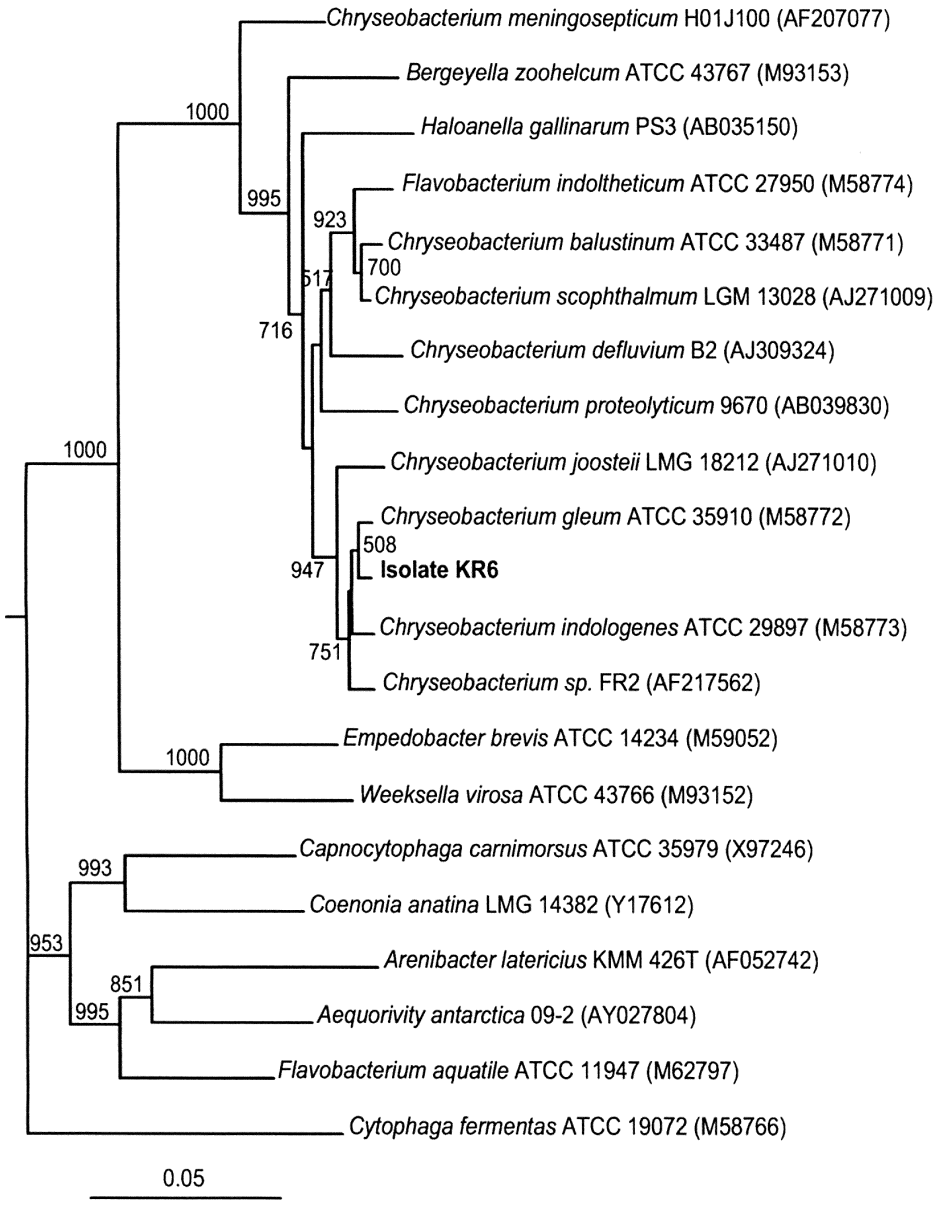

Fig. 2 Phylogenetic position of strain kr6 within the genus *Chryseobacterium* and allied bacteria. The branching pattern was generated by the neighbor-joining method. The Genbank accession number of the 16S rRNA nucleotide sequences used are indicated in *parentheses*. The number of each branch indicates the bootstrap values. *Bar* Jukes-Cantor distance of 0.05. *Cytophaga fermentas* was used as an outgroup

Optimal growth conditions

The newly isolated strain grew at pH 5.0–8.0 and 22–37 °C, with an optimum at pH 8.0 and 30 °C. *Chryseobacterium* sp. strain kr6 grew at approximately the same rate at 25–37 °C and from a starting pH of 5–8, although at neutral to alkaline pH, cells began to lyse soon after reaching stationary phase (data not shown).

Production of keratinase and soluble protein

The effects of pH and temperature on the production of keratinase and soluble protein were investigated. Maximum enzyme activity was observed during cultivation at 25 °C (96 U ml^{-1} at 48 h), followed by 30 °C (64 U ml^{-1} at 36 h) and then at 37 °C (40 U ml^{-1} at 48 h). Keratinase production was similar at starting pH values of 5.0–8.0, and soluble protein concentration was higher with increasing starting pH (data not shown). More efficient feather degradation was observed at 30 °C and initial pH 8.0.

Keratinase activity was followed during cultivation of strain kr6 in whole-feather medium at 30 °C and initial pH 8.0. Keratinase reached a maximum activity at 48 h, coinciding with the end of the exponential phase, then decreased, and increased again at 100 h (Fig. 3A). The kinetics of production of soluble protein and free amino acids are shown in Fig. 3B. Maximum values were reached at 36 h, at which the concentration of soluble protein was approximately five times higher than the amino acid concentration.

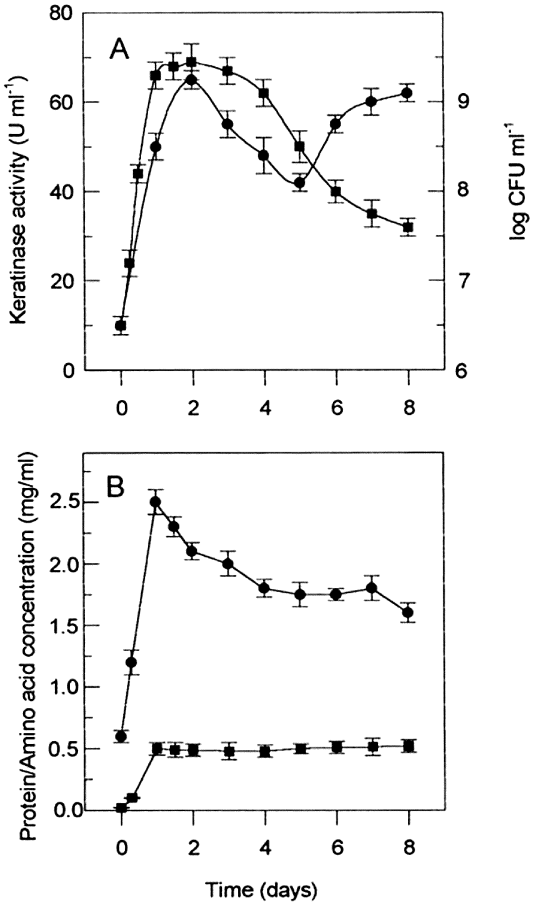

Fig. 3A, B Proteolytic activity during growth of *Chryseobacterium* strain kr6 in whole-feather medium at 30 °C and initial pH 8.0. **A** Keratinase activity measured using azokeratin as substrate(●) and viable cell counts (■). **B** Concentration of soluble protein (●) and free amino acids (■). Each point represent the mean±SEM of three independent experiments

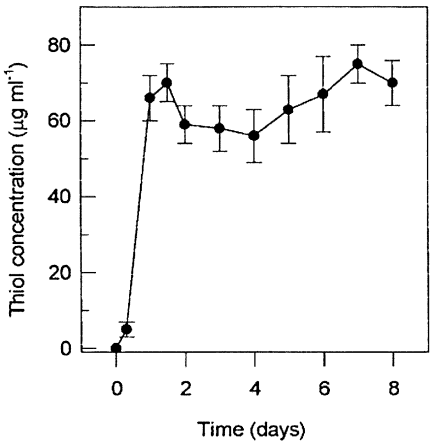

Fig. 4 Formation of extracellular thiol groups during growth of *Chryseobacterium* strain kr6 in whole-feather medium at 30 °C and initial pH 8.0. Each point represent the mean±SEM of three independent experiments

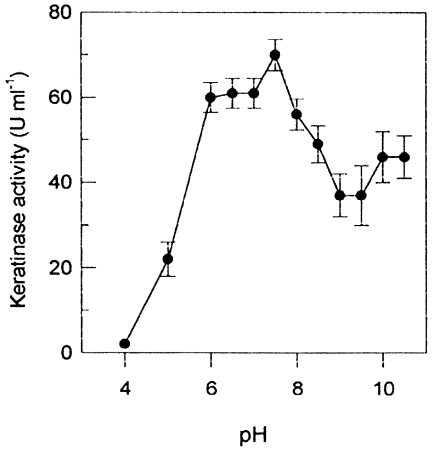

Fig. 5 Effect of pH on the activity of *Chryseobacterium* sp. kr6 keratinase. Keratinolytic activity was measured at different pH values. Each point represent the mean±SEM of three independent determinations

Reduction of disulfide bonds

The high stability of keratin proteins against mechanical and enzymatic attack is due, at least in part, by the occurrence of cysteine bridges. Reduction of disulfide bonds by strain kr6 was investigated. Thiol formation increased with cultivation time (Fig. 4), reaching a maximum at 40 h. The rate of thiol formation was maximum during the exponential phase.

Characterization of keratinase

The effect of pH on keratinase activity was determined. The enzyme was active in the range of pH 6–8 with a maximum at pH 7.5 (Fig. 5). The effect of temperature on keratinase activity is shown in Fig. 6. Activity was observed in the range of 45–85 °C with a maximum at 75 °C (Fig. 6A). The activity was stable for 30 min at 37 °C, with 80% of the initial activity remaining after 1 h and 60% after 2 h. At 55 °C and 70 °C, the enzyme maintained 50% its initial activity after 2 h of incubation (Fig. 6B).

The keratinase was active on azocasein but not on BAPNA as substrate. Keratinase activity was investigated after preincubation of the enzyme with several chemicals for 15 min. The effects of these chemicals on keratinase activity are shown in Tables 2 and 3. The enzyme was inhibited by 1,10 phenanthroline and EDTA (Table 2), although other protease inhibitors also had minor effects (14–24% inhibition). The enzyme maintained at least 50% of its activity after incubation with SDS or organic solvents. The use of the non-ionic detergent Triton X-100 and reducing agent caused important increases in enzyme activity (Table 2). The enzyme was totally inhibited by Hg^{2+} and Cu^{2+} (Table 3). Activity increased in the presence of Ca^{2+} and was partially inhibited by Zn^{2+} (Table 3).

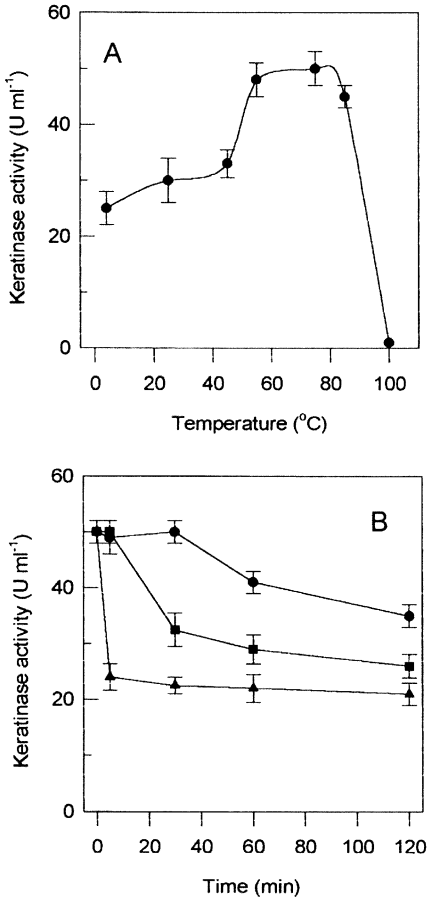

Fig. 6A, B Effect of temperature on the activity and stability of *Chryseobacterium* sp. kr6 keratinase. **A** Enzyme activity was determined at various temperatures. **B** Enzyme was incubated for indicated times at 37 °C (●), 55 °C (■), or 70 °C (▲) and residual activity was measured

Table 2 Effect of chemicals on keratinase activity of *Chryseobacterium* sp. strain kr6. Values are representative of three independent determinations

Chemical	Concentration	Residual activity (%)
Control	–	100
PMSF	1 mM	83
E-64	5 µM	86
Pepstatin	1 mM	76
EDTA	5 mM	43
EGTA	5 mM	60
1,10 phenanthroline	1 mM	12
SDS	0.1% (w/v)	50
SDS	0.5% (w/v)	88
Triton X-100	0.1% (v/v)	231
	0.5% (v/v)	77
DMSO	1% (v/v)	75
	5% (v/v)	68
Isopropanol	1% (v/v)	56
	5% (v/v)	56
2-Mercaptoethanol	0.1% (v/v)	238
	0.5% (v/v)	172

Table 3 Effect of ions on keratinase activity of *Chryseobacterium* sp. strain kr6. Values are representative of three independent determinations. Compounds were tested at a working concentration of 10 mM

Ion	Residual activity (%)
Control	100
$CaCl_2$	116
$BaCl_2$	96
Fe_2Cl_3	102
$HgCl_2$	0
$MgCl_2$	81
$CuSO_4$	0
$MnCl_2$	81
$ZnCl_2$	62

Discussion

A feather-degrading bacterium was isolated from a poultry-processing industry. Based on phenotypical and phylogenetical characteristics, strain kr6 belongs to the Flavobacteriaceae of the *Cytophaga-Flavobacterium* group and was identified as *Chryseobacterium* sp. strain kr6. The 16S rRNA sequence of strain kr6 showed 99% similarity to that of *Chryseobacterium gleum* ATCC 35910.

Chryseobacterium strains have been isolated from various ecosystems such as water, soil, fish, marine environments, and clinical specimens. Several *Chryseobacterium* strains produce highly proteolytic activities (Jooste and Britz, 1986; Yamaguchi and Yokoe 2000). However, the isolate kr6 is the first *Chryseobacterium* strain described as a feather degrader. In two earlier studies, two Cytophagales isolates were discovered that solubilized autoclaved feathers and wool, the organisms resembled *Cytophaga johnsoniae* but were never clearly classified (Reichembach 1992). Until recently, feather degradation by bacteria has been described only in gram-positive bacteria such as *Bacillus* and *Streptomyces* species (Williams et al., 1990; Bockle et al., 1995; Onifade et al. 1998; Zaghloul 1998; Kim et al. 2001). We described a *Vibrio* sp. presenting high feather-degrading activity (Sangali and Brandelli 2000). Lucas et al. (2003) also isolated feather-degrading strains from soils, belonging to the *Cytophaga-Flavobacterium* group. The isolate presented optimum growth at mesophilic temperatures, as expected from its environmental origin. Other previously described keratinolytic bacteria generally have optimum growth and feather degradation activity at highest temperatures (Williams et al. 1990; Nam et al. 2002).

Strain kr6 caused a significant increase in the pH of the medium during cultivation on raw feathers and was able to complete feather degradation, indicating its strong keratinolytic character. Organisms with higher keratinolytic activity alkalinize the media to a greater extent than those exhibiting lower keratinolytic activity (Kaul and Sumbali 1997). This tendency to alkalinize the medium results from the production of ammonia by means of the deamination of peptides and amino acids originating from keratin degradation. The resulting increase of pH is typical of microorganisms growing on protein substrates.

Strain kr6 produced an extracellular keratinase, the levels of which varied during cultivation on whole feathers. This pattern suggests that the enzyme is inducible and that substrate and metabolite levels in the extracellular milieu regulate its secretion. These results resemble those obtained with cultures of *Streptomyces pactum* (Böckle et al. 1995), *Trichophyton* species (Grzywnowicz et al. 1989), *Bacillus* sp. (Atalo and Gashe 1993) and *Vibrio* sp. (Sangali and Brandelli 2000) growing on keratin substrates.

Thiol formation by strain kr6 suggests the presence of disulfide reductase activity. Reduction of disulfide bridges was also observed for *Streptomyces pactum* grown on feathers (Böckle and Müller 1997), and *Streptomyces fradiae* on wool (Kunert and Stransky 1988). Kunert (1989) reported that keratin degradation by *S. fradiae* and *Microsporum gypseum* extracellular proteases follows sulfitolysis of cystine. In addition, the use of reducing agents to enhance keratin degradation by keratinases has been described (Böckle et al. 1995; Letourneau et al. 1998). The stimulatory effect of 2-mercaptoethanol on keratinolytic activity of strain kr6 may be explained by the reduction of disulfide bridges of azokeratin, allowing a more accessible substrate. Thus, screening for keratinolytic micro-organisms should also lead to the isolation of organisms possessing disulfide reductases.

The main proteolytic activity of keratinases is normally associated with serine proteinase activity (Böckle et al. 1995; Lin et al. 1995), The keratinases produced by *B. licheniformis* and *B. subtilis* are serine proteases (Lin et al. 1995; Suh and Lee 2001), and their genes (*kerA* and *aprAk*) show significant similarity with subtilisins, which are typical members of the serine-protease family (Lin et al. 1995; Zaghloul 1998). However, the keratinase produced by strain kr6 appears to belong to the metalloprotease type since it was inhibited by EDTA and 1,10-phenanthroline (Zn^{2+}-specific chelator) and did not hydrolyze the substrate BAPNA. These features resemble those of *Flavobacterium*/*Chryseobacterium* proteases. The two major proteases secreted by *Flavobacterium meningosepticum* are zinc metalloendopeptidases, one of them presenting unusual *O*-glycosylation (Tarentino et al. 1995). A protease purified from *Chryseobacterium indologenes* was inhibited by EDTA and 1,10-phenanthroline, and atomic absorption analysis showed that the enzyme contained Ca^{2+} and Zn^{2+} (Venter et al. 1999). The partial inhibition by EDTA and EGTA could be explained by the increased strength of zinc binding to the active site at neutral to alkaline pH (Auld 1995).The inhibition by 10 mM Zn^{2+} is also in agreement with the fact that several zinc peptidases are inhibited by excess zinc, particularly at neutral to alkaline pH (Auld 1995). Activation by Ca^{2+} and inhibition by Zn^{2+} is also a feature of keratinase of *Vibrio* kr2 (Sangali and Brandelli 2000) and of some calpains (Sorimachi et al. 1997). The results of inhibitor studies also suggest that gram-negative bacteria possess keratinases that differ from those previously isolated from gram-positive bacteria.

The new Cytophagale strain described here has high keratinolytic activity and is very effective in feather degradation, suggesting its potential use in biotechnological processes involving keratin hydrolysis. In addition, the keratinase produced by strain kr6 was active over a wide range of pH values and temperatures, and was relatively heat stable. These are interesting properties for regarding industrial use of the enzyme.

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