Effects of Carbon Sources on Production of Leucine Aminopeptidase and Activities of Sugar Metabolic Enzymes Produced by *Bacillus cereus* CZ

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Abstract: Leucine aminopeptidase (LAP) produced by *Bacillus cereus* CZ was investigated in this study. Results indicated that L-arabinose was the best carbon source for producing LAP, followed by D-galactose and lactose, whereas glucose, maltose, sucrose and sodium bicarbonate obviously inhibited LAP production. The production of LAP was also reduced largely when L-arabinose was mixed with other simple sugars, or Lactose/ D-galactose was mixed with glucose. The mixture of lactose and glucose was the optimal carbon sources for cell growth.When using 2% lactose, D-galactose and L-arabinose as carbon sources, LAP activities did not reach the maximum values after 48 h fermentation. For the sugar metabolic enzymes, β-galactosidase can be strongly induced by lactose. L-Arabinose had certain induction effect, while D-galactose had a strong depression effect. Galactokinase was induced significantly by D-galactose, followed by L-arabinose and lactose. L-arabinoseisomerase was induced strongly by L-arabinose, moderately by other simple sugars and mixed sugars. LAP production reached the highest with 4% L-arabinose and D-galactose. Extracelluar galactokinase decreased with increasing the sugar concentration, but intracellular enzymes showed the reverse trend. L-arabinoseisomerase increased with increasing concentration of L-arabinose. LAP production reached the highest when fermentation for 56 h with 4% L-arabinose. However, its production increased continuously with fermentation time when 4% D-galactose was used.

Key words: leucine aminopeptidase; β-galactosidase; galactokinase; L-arabinoseisomerase; *Bacillus cereus*.

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碳源对 *Bacillus cereus* CZ 氨肽酶产量及糖代谢酶活性的影响

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摘要： 本文对 *Bacillus cereus* CZ 生产亮氨酸氨肽酶（LAP）进行了研究。结果表明，L-阿拉伯糖最利于 LAP 生产，D-半乳糖和乳糖也有较好效果，葡萄糖、麦芽糖、蔗糖或碳酸氢钠则强烈抑制 LAP 产生。L-阿拉伯糖和单糖混合、乳糖或半乳糖与葡萄糖混合均大大降低 LAP 产量。乳糖、葡萄糖有利于细胞生长。2%的乳糖、半乳糖或阿拉伯糖作为碳源，发酵 48 h 时 LAP 产量并没有达到最高。对于三种糖代谢酶，β-半乳糖苷酶受乳糖强烈诱导，D-半乳糖则强烈抑制该酶的活性，半乳糖激酶受 D-半乳糖强烈诱导。L-阿拉伯糖异构酶受 L-阿拉伯糖强烈诱导。当 L-阿拉伯糖和半乳糖的浓度为 4%时，LAP 产量达到最高。胞外半乳糖激酶随 D-半乳糖增加而降低，而胞内正好相反。L-阿拉伯糖异构酶活性随 L-阿拉伯糖浓度增加而增加。当 L-阿拉伯糖为 4%时，LAP 产量在发酵 56 h 达到最高，但在半乳糖为 4%时，LAP 产量随时间延长而增加。

关键词： 氨肽酶;β-半乳糖苷酶；半乳糖激酶；L-阿拉伯糖异构酶；蜡样芽孢杆菌

Proteolytic enzymes are ubiquitous in occurrence, being found in all living organisms [1]. Proteolytic enzymes include two kinds of enzymes, exopeptidase (a group of enzymes that catalyze the hydrolysis of single amino acids from the end of a polypeptide chain) and endopeptidase (a group of enzymes that catalyze the cleavage of internal bonds in a polypeptide or protein) [2]. Aminopeptidases (APases) are a large and diverse group

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of enzymes that catalyze the cleavage of amino acids from the amino terminal of proteins or peptide substrates. APases play important roles in the maintenance of cell metabolism, such as participate in the presence of MHC I, affect the formation of bioactive peptides, guarantee the normal cycle of amino acids, combine with DNA to regulate transcription and control the biosynthesis of pyrimidine, alginate and cholera toxins, act an important role in meiosis and so on [3].

According to the different optimum reaction substrates, aminopeptidases are divided into leucine aminopeptidase, valine aminopeptidase, alanine aminopeptidase, phenylalanine aminopeptidase, proline ammonia peptide enzymes and so on. Leucine aminopeptidases (LAPs), a class of exoproteases that preferably catalyze the hydrolysis of leucine residues from the amino-termini of proteins or peptides [4], usually show broad substrate specificity. In view of their specificity, structure and wide distribution, LAP has become the most typical representative of the family aminopeptidases.

Several LAPs from many tissues and organs have been isolated [5] and characterized and a substantial amount of knowledge about bacterial and mammalian aminopeptidases is available. LAPs are exopeptidases that belong to a class of zinc-requiring metalloproteases [6]. Some LAPs are exceptional enzymes that require cobalt ions for full enzyme activity [7]. Several genes encoding for LAP also have been isolated and their amino acid sequences have been determined [8]. Much information on primary sequences for bacterial or mammalian aminopeptidases is available. Three-dimensional structures that complex with bestatin and metal-substituted enzyme kinetics are also well defined [9]. Aminopeptidases are highly similar in structures and recognized to constitute an enzyme superfamily, having evolutionarily conserved regions [10].

B. cereus has a wide distribution in nature, frequently isolated from soil and growing plants, and it is also well adapted for growing in the intestinal tract of insects and mammals [11]. *B. cereus* produces several toxins, so often considered to be a kind of pathogenic bacteria [12], but some strains of *B. cereus* not involved in foodborne illness outbreaks have been isolated from foods. *B. cereus* can be used as antagonistic bacteria in biological control, thereby having caused widespread attention. *B. cereus* itself can be prepared as micro-ecological agents and microbial fertilizers [13], the European Union has authorized *B. cereus* var. *toyoi* strain NCIMB 40112 (Toyocerin) as feed additive for swine, bovine, poultry and rabbit [14]. *B. cereus* can also produce a varieties of bioactive substances, including cereins [15,16], peptide antibiotics [17,18], enzymes [19-22], insecticidal exotoxins [23,24] and hemolysin [25].

Few studies were reported about LAP from *B. cereus*. We have selected a *B. cereus* strain (designated CZ) from a probiotics product used as feed additive. This paper described our findings on the influence of various carbon sources on the LAP production by *B. cereus* CZ. We also analyzed metabolite enzymes of various carbon sources in the process of LAP fermentation.

1 Materials and Methods

1.1 Bacterial strains and media

B.cereus CZ was obtained from a probiotics product used as feed additive and preserved in our laboratory. An incline medium (pH 7.0) containing yeast extract (10 g/L), peptone (10 g/L), glucose (6 g/L), K_2HPO_4 (2 g/L) and agar (20 g/L) was used to activate the strain. The seed medium (pH 7.0) was composed of peptone (10 g/L), yeast extract (5 g/L) and NaCl (10 g/L). The basal fermentation medium (pH 7.0) contained glucose (20 g/L), $MgSO_4$(1 g/L), yeast extract (10 g/L), peptone (10 g/L) and K_2HPO_4 (2 g/L). Fermentation was performed under the following conditions: 50 mL of fermentation medium with 3% inoculation volume, 37 ℃, 200 r/min. The carbon source was varied during fermentation.

1.2 LAP production with different carbon sources and in fermentation time course

To evaluate the effect of different carbon sources on LAP production, glucose, lactose, D-galactose, maltose, soluble starch, sodium bicarbonate and sodium citrate were used. The concentrations of carbon sources were all 20 g/L. After fermentation, the activity of LAP was measured. Each fermentation was performed in three replicates. Fermentation flasks containing different carbon sources were sampled every 8 h. OD_{600}, the activities of LAP and the metabolic key enzymes of different sugars were determined respectively.

1.3 Effect of mixed sugars on LAP production

Two kinds of sugars were mixed at a ratio of 1:1. LAP production and the metabolic key enzymes of

different sugars were compared under the mixed sugars and four single sugars in the fermentation media.

1.4 LAP production with different concentrations of sugars and in fermentation time course

The better two of all mixed and single sugars for LAP production were selected. Varying concentrations (10 g/L, 20 g/L, 30 g/L, 40 g/L, 50 g/L) of the two carbon sources were prepared in three parallel samples for LAP production. Fermentation flasks containing one of the best two carbon sources were sampled every 8 h. OD_{600}, the activities of LAP and the metabolic key enzymes of these two sugars were determined.

1.5 Aminopeptidase activity assay

Fermentation broth was collected and centrifuged for 15 min at 5000 r/min and 4 ℃. The supernatant was used for enzyme activity assay. The reaction mixture contained 0.5 mL supernatant, 6 mL Tris-HCl buffer and 0.5 mL 26 mmol/L LNA(L-leucine-*p*-nitroanilide) ethanol solution, incubated for 10 min at 37 ℃, then placed in ice bath for 5 min. OD_{405} of the reaction solution was measured. One unit of enzyme activity was defined as the amount of enzyme that catalyzed LNA to produce 1 μmol of *p*-nitroaniline per minute.

1.6 Activity assay of key enzymes in metabolic pathway of sugar

For extracellular enzymes activity assay, fermentation broth was collected and centrifuged for 15 min at 5000 r/min and 4 ℃. The supernatant was used for enzyme activity assay. For intracellular enzyme activity assay, fermentation broth was collected and centrifuged for 15 min at 5000 r/min and 4 ℃, the pellet was suspended in lysis buffer, then disrupted by uitrasonication for 10 min. The cell debris was removed by centrifugation at 12000 r/min for 40 min, the supernatant was used for enzyme activity assay.

Activity assay of β-galactosidase. The reaction mixture contained 0.1 mL supernatant with the appropriate dilution, 0.7 mL 0.6% ONPG (ortho-nitrophenyl-beta-D-galactopyranoside) solution, after incubated for 10 min at 37 ℃, the reaction solution was added by 0.8 mL 5% Na_2CO_3 for the color development, then the above solution was added by 6.4 mL phosphate buffer (pH 7.0), shaked evenly and then OD_{420} value was measured. One unit of activity was defined as the amount of enzyme that catalyzed ONPG to produce 1 μmol of ONP per minute [26,27].

Activity assay of Galactokinase. The reaction system (100 μL) was composed of 50 mmol/L Tris-HCl (pH 8.0), 10 mmol/L ATP, 8 mmol/L D-Gal (D-galactose), 5 mmol/L $MgC1_2$, 10 μL supernatant and 50 μL ddH_2O, incubated for 3 h at 45 ℃ and added 3 mL DNS (3,5-Dinitrosalicylic acid) reagents to stop the reaction, after treated in boiling water bath for 10 min, put into ice bath for 2 min, and then OD_{575} value of the solution was measured. One unit of galactokinase activity was defined as the amount of enzyme that catalyzed Gal and ATP to produce 1 μmol of glucose per minute [28,29].

Activity assay of L-arabinoseisomerase. The reaction mixture contained 0.1mL supernatant with appropriate dilution, 0.9 mL 0.5 mol/L D-galactose solution, incubated for 1 h at 50 ℃, then boiled for 10 min to stop the reaction. Then the reaction solution was added by 6 mL H_2SO_4 (13 mol/L), 0.2 mL 15% cysteine hydrochloride, 0.2 mL 0.12% carbazole alcohol solution in turn, incubated for 30 min at 25 ℃ and OD_{540} value was measured. One unit of L-arabinoseisomerise activity was defined as the amount of enzyme that catalyzed D-galactose to produced 1 μg tagatose per minute [30].

2 Results and Discussion

2.1 LAP production with different carbon sources

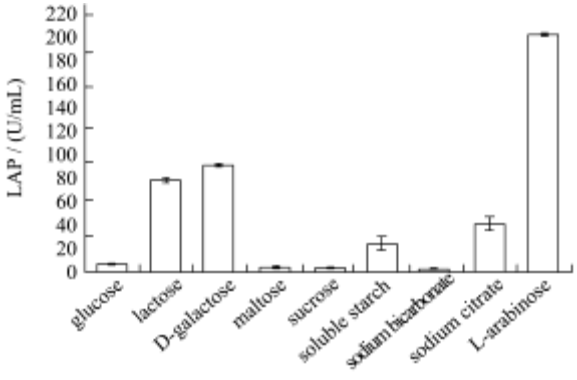

Fig.1 LAP production with different carbon sources

Note: The concentration of carbon sources was 2%, fermentation time was 48 h. Data are the mean of three replicates, and the error bars represent the standard deviation.

To determine the optimal carbon source for LAP production, eight different carbon sources were used. Among various carbon sources, L-arabinose showed the highest LAP production (202.77 ± 1.19 U/mL), followed by D-galactose, lactose and sodium citrate, but glucose,

maltose, sucrose, and sodium bicarbonate exhibited very low LAP production (Fig. 1). The same finding is reported by Yang [31], they find that protease production by *Bacillus subtilis* is greatly enhanced by addition of lactose or arabinose into the medium and that 1% (*m/V*) arabinose is the most effective substrate for protease production. In our study, glucose showed carbon catabolite repression on LAP production (Fig. 1). Similarly, for Marine *Aspergillus flavus*, the minimum aminopeptidase activity was found to be produced with the use of glucose, but arabinose, lactose, sucrose inhibited LAP production, the maximum aminopeptidase activity was with the use of maltose [32]. Transcription of the yeast vacuolar aminopeptidase yscI-encoding gene (*APE1*) is regulated by the carbon source, responding to glucose catabolite repression [33]. On the contrary, there are reports, which show that glucose stimulates the aminopeptidase production from *Lactococcus lactis* subsp. lactis and cremoris [34], *thermophilic Streptomyces* strains [35] and the marine bacterium Vibrio SA1 [36]. After 48 h of fermentation, 202.77 ± 1.19 U/mL of LAP was obtained, and the level of LAP production was close to so far the highest LAP production (376.9 U/mL after 72 h of fermentation) reported by Li Bin et al. [37].

2.2 Several simple sugars on LAP production and the activities of their metabolic enzymes

In this experiment, L-arabinose, D-galactose and lactose for better LAP production were used as carbon sources. In order to know the relationships between LAP production and the metabolic enzymes of these three sugars, activities of β-galactosidase, galactokinase and L-arabinoseisomerase were measured. LAP production displayed a slow increasing trend before 40 h, but fast increased from 40 h to 48 h with three sugars as carbon sources. L-arabinose was the optimal carbon source for LAP production, followed by D-galactose and lactose, but glucose exhibited a strong inhibitory effect on LAP production (Fig. 2a).

β-galactosidase, one of three enzymes encoded by lactose operon, cleaves β-linked galactose residues from various compounds and is commonly used to cleave lactose into galactose and glucose [38]. β-galactosidase showed mainly intracellular enzyme activity, and increased quickly from 8 h to 40 h, and rapidly from 40 h to 48 h. The intracellular β-galactosidase production reached to the highest value (73.49 ± 9.21 U/mL) at 48 h, but the extracellular enzyme activity did not appear until 32 h, only 15.81 ± 1.47 U/mL at 48 h (Fig. 2b), which maybe come from cell autolysis .

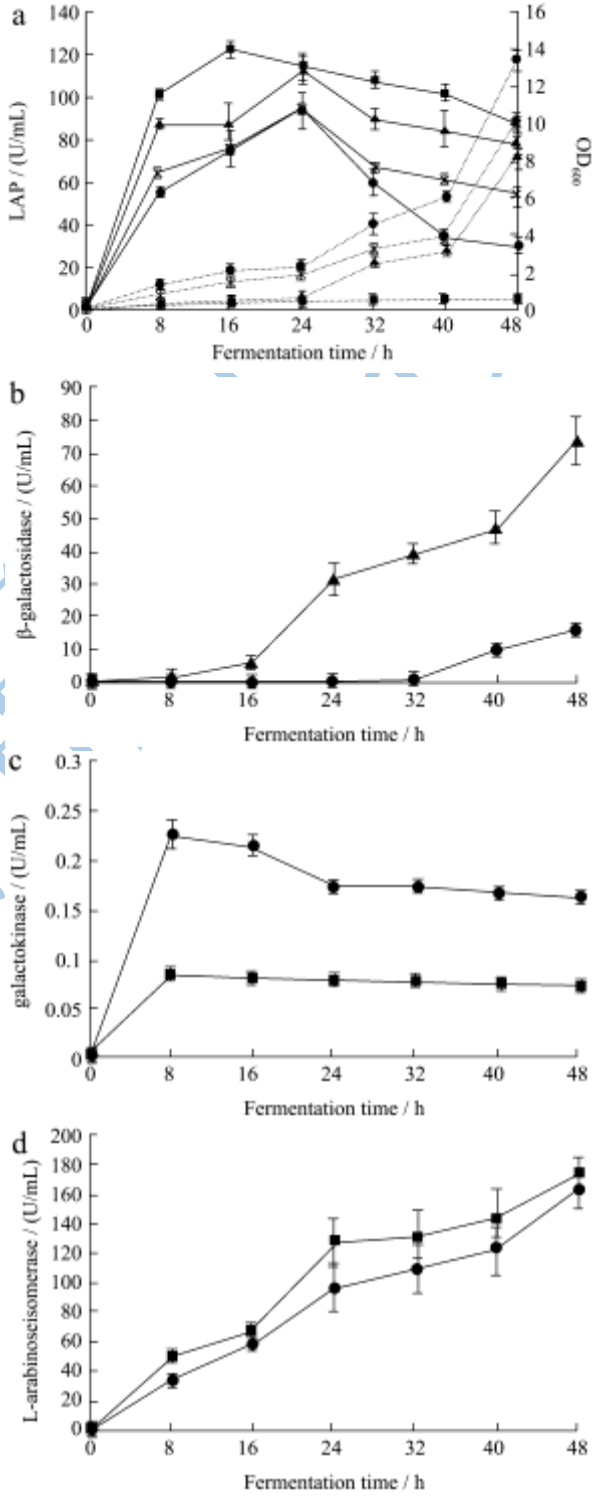

Fig.2 LAP production, cell biomass and activities of carbon metabolic enzymes in time course

Noet: The concentrarion of sugars was 2%, fermentation time was 48 h; (a) LAP production and cell biomass. Real lines standed for cell biomass, broken lines standed for LAP production; (■)

glucose, (▲) lactose, (×) D-galactose, (●) L-arabinose; (b) the activities of galactosidase. (●) extracelluar activities, (▲) intracelluar activities; (c) the activities of galactokinase. (●) extracelluar activities, (■) intracelluar activities; (d) the activities of L-arabinoseisomerase. (●) extracelluar activities, (■) intracelluar activities. Data are the mean of three replicates, and the error bars represent the standard deviation.

Galactokinase, one of three enzymes encoded by galactose operon, is an ATP-dependent enzyme that catalyzes the phosphorylation of galactose to form galactose-1-phosphate. The conversion of D-galactose to the more metabolically useful glucose-1-phosphate is accomplished by the action of four enzymes in the Leloir pathway [39]. *B. cereus* CZ produced both intracellular and extracellular galactokinase with very low activities. Galactokinase production increased rapidly from 0 h to 8 h, the intracellular part remained stable from 8 h to 48 h, the extracellular part remained stable from 8 h to 32 h, but increased rapidly again from 32 h to 48 h. The activity of extracellular part was one time higher than that of the intracellular part (Fig. 2c).

L-arabinoseisomerase is one of three enzymes encoded by arabinose operon. *B. cereus* CZ produced high activity of L-arabinoseisomerase. The activities of extracellular part and intracellular part showed an uptrend from 0 h to 48 h, reached to 164.33 ± 2.01 U/mL and 175.11 ± 7.42 U/mL at 48 h respectively (Fig. 2d).

The four sugars were all well utilized by *B. cereus* CZ. Glucose was the most advantageous to cell growth and the highest cell biomass was reached at 16 h, but the worst for LAP production. Lactose was the second better carbon source for cell growth, followed by D-galactose and L-arabinose, cell biomass all reached the highest at 24 h with the later three sugars.

2.3 Effect of simple sugars and mixed sugars

LAP production of *B. cereus* CZ reached the highest amount with L-arabinose as carbon source, whereas displayed the lowest amount (only 4.20 ± 0.05 U/mL) with glucose as carbon source. Compared with L-arabinose alone, L-arabinose mixed with glucose, lactose or galactose reduced largely LAP production. Especially, L-arabinose mixed with glucose resulted in about seven times reduction, lactose and galactose mixed respectively with glucose had a similar impact on LAP production. Cell biomass attained the highest with lactose + glucose as carbon sources, followed by glucose, lactose + L-arabinose, D-galactose + glucose, L-arabinose + glucose, D-galactose + L-arabinose, L-arabinose, OD_{600} values were all above 10. But lactose, lactose+D-galactose, especially D-galactose seemed disadvantageous to cell growth.

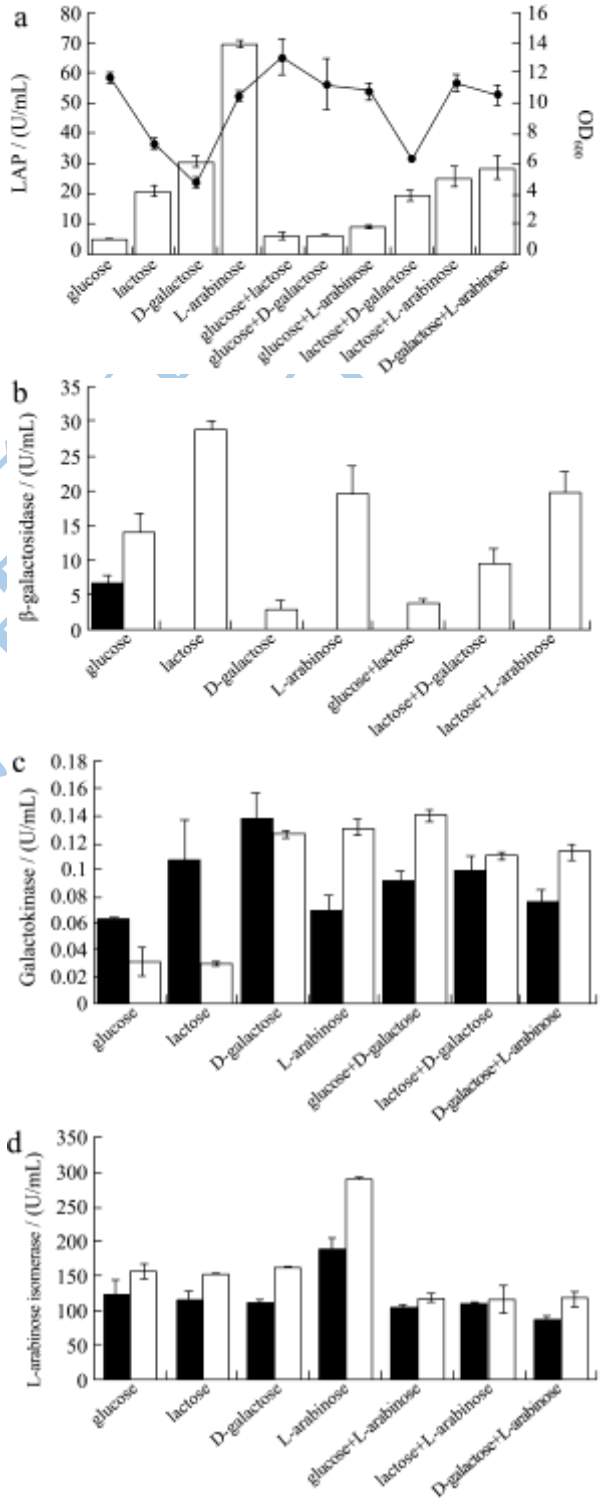

Fig.3 LAP production, cell biomass and activities of carbon metabolic enzymes with simple sugars and mixed sugars

Note: The total concentration of sugars was 2%, mixed sugars contained two simple sugars (according to the ratio of 1:1), fermentation time was 48 h. (a) LAP production and cell biomass. Line chart standed for cell biomass, bar chart standed for LAP production; (b) the activities of galactosidase. Black column chart standed for extracellular activities, blank column chart standed for intracellular activities; (c) the activities of galactokinase. Black column chart standed for extracellular activities, blank column chart standed for intracellular activities; (d) the activities of L-arabinoseisomerase. Black column chart standed for extracellular activities, blank column chart standed for intracellular activities. Data are the mean of three replicates, and the error bars represent the standard deviation.

The result showed glucose, L-arabinose and their mixtures with other sugars were better utilized (Fig. 3a). Our result is different from *Corynebacterium glutamicum* ATCC 31831 which grows on L-arabinose as the sole carbon source at a specific growth rate that is twice that on D-glucose [40]. The effects of the above several sugars on β-galactosidase, galactokinase and L-arabinoseisomerase were studied. Among simple sugars, lactose was the optimal carbon source for β-galactosidase production (28.84 ± 1.32 U/mL), L-Arabinose had certain induction on β-galactosidase. But galactose displayed a strong depressing action, followed by glucose+lactose in *B. cereus* CZ (Fig. 3b). Similarly, lactose is the best carbon source for production of β-galactosidase from *Bacillus licheniformis* E66, whereas glucose and galactose repress moderately on the production of β-galactosidase [41]. For *Aspergillus parasiticus* MTCC-2796, β-galactosidase synthesis is strongly repressed by glucose, followed by galactose [42]. For the thermophilic archaeon *Sulfolobus solfataricus*, L-arabinose has a strong induction on β-galactosidase, but β-galactosidase is repressed by galactose, followed by glucose [43]. For three mixed sugars in our study, β-galactosidase production was higher with lactose + galactose as carbon source, followed by lactose + L-arabinose and lactose + glucose, but all lower than that of lactose as carbon source. In *Escherichia coli*, the addition of galactose exhibits a decreasing induction in β-galactosidase, glucose also displays lower β-galactosidase activity [44]. In *Trichoderma reesei*, L-arabinose is also known to induce the lactose degrading enzyme [45]. Galactokinase, catalyzes the second step of the Leloir pathway, a metabolic pathway has been found in most organisms for the catabolism of β-D-galactose to glucose 1-phosphate [46]. In our study, galactokinase was induced strongly by D-galactose, reached 0.26 ± 0.02 U/mL (the extracellular activity 0.14 ± 0.02 U/mL, the intracellular activity 0.13 ± 0.00 U/mL). L-arabinose, lactose + D-galactose, glucose + D-galactose and L-arabinose + D-galactose had similar inducing effects on galactokinase production (about 0.20 U/mL). Galactokinase production was lower with lactose and glucose as carbon sources, only 0.14 ± 0.03 U/mL and 0.09 ± 0.01 U/mL, respectively (Fig. 3c). These results showed that galactokinase from *Bacillus cereus* CZ was not entirely an inducible enzyme. Comparatively, the activity of galactokinase coded by the gal operon of *Lact. rhamnosus* TCELL-1 is significantly enhanced in the presence of galactose, however, such an induction is remarkably suppressed by the same amount of glucose added [47].

L-arabinoseisomerase reached the highest production with L-arabinose as carbon source, the extracellular and intracellular activities were 187.89 ± 14.53 U/mL and 289.67 ± 2.90 U/mL respectively. Higher L-arabinoseisomerase activities (the total activities about 270 U/mL) were also obtained with the other three simple carbon sources, but much lower than that with L-arabinose. L-arabinoseisomerase production with L-arabinose + lactose, L-arabinose + D-galactose and L-arabinose + glucose as carbon sources were slightly lower than that with the above three simple sugars (Fig. 3d). These results showed that glucose did not repress L-arabinoseisomerase synthesis, L-arabinoseisomerase might be not only a constitutive enzyme, but also an inductive enzyme or there were more than one L-arabinoseisomerise in *B. cereus* CZ. But in *Escherichia coli*, the arabinose operon is often used as an example of a tightly controlled operon, and transcription from the *ara* promoter is very low in the absence of inducer [48]. In *Salmonella typhimurium*, the induced synthesis of L-arabinoseisomerase is also subject to glucose catabolite repression [49]. Our results also showed that L-arabinoseisomerase induced by D-Galactose was much less than that by L-arabinose. But in *Mycobacterium smegmatis*, D-Galactose and L-arabinose is found to display a similar induction on

L-arabinoseisomerase [50].

L-arabinoseisomerase is also referred to as D-galactose isomerase due to their ability, in vitro, to isomerize D-galactose to D-tagatose. Currently, isomerase enzymes are becoming increasingly significant because they play a key role in the synthesis of uncommon sugars, simply termed as rare sugars [51]. *B. cereus* CZ has a good application prospect in the synthesis of rare sugars because of higher production of L-arabinose isomerase.

2.4 Effects of concentrations of L-arabinose and D-galactose

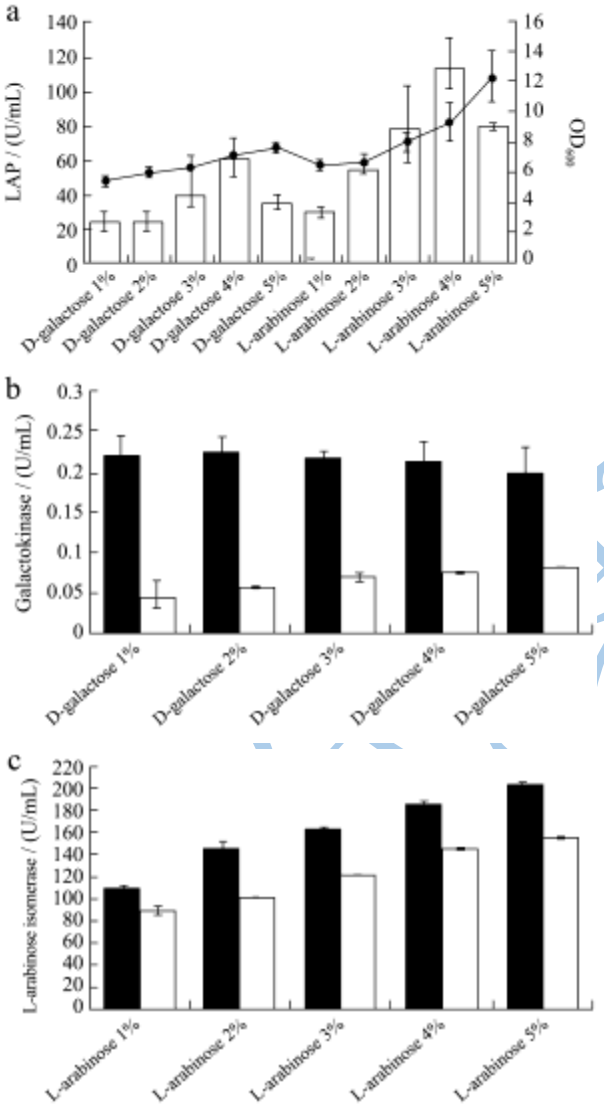

Fig.4 LAP production, cell biomass and activities of two sugars metabolic enzymes with different concentrations of D-galactose and L-arabinose

Note: The concentrations of sugars were varied from 1% to 5%, fermentation time was 48 h. (a) LAP production and cell biomass. Line chart standed for cell biomass, bar chart standed for LAP production; (b) the activities of galactokinase with different concentrations of D-galactose. Black column chart standed for extracellular activities, blank column chart standed for intracellular activities; (c) the activities of L-arabinoseisomerase with different concentrations of L-arabinose. Black column chart standed for extracellular activities, blank column chart standed for intracellular activities. Data are the mean of three replicates, and the error bars represent the standard deviation.

In this experiment, L-arabinose and D-galactose were chosen as carbon sources because of their higher LAP production. LAP production was higher (61.86 ± 11.75 U/mL) when the concentration of D-galactose was 40 g/L. Cell growth raised with the rising of concentration, the excelluar galactokinase production decreased slightly with the rising of concentration, while the intracelluar production increased slightly with the rising of concentration, but total galactokinase production did not changed significantly. This result is significant different from Ainsworth and Coleman's research which the concentration of D-galactose is higher, the production of galactokinase is also higher in both protoplasts and whole bacteria. In each case, the maximum amount of enzyme is induced by 5 mmol/L D-galactose [52].

The highest yield of LAP reached 114.73 ± 15.24 U/mL when the concentration of L-arabinose was 40 g/L (Fig. 4a). Excellur and intracellar L-arabinoseisomerase production as well as cell growth showed an increasing tendency with the concentration rising (Fig. 4b).

2.5 LAP production, cell biomass and activities of sugar metabolic enzymes with 4% concentration of L-arabinose and D-galactose in time course

Fermentation experiments with 4% D-galactose and 4% L-arabinose were performed. The results showed that LAP production reached the maximum (89.89 ± 9.74 U/mL) at 56 h and then dropped down with 4% concentration of L-arabinose, whereas LAP production had gradually increased with the time course of fermentation with 4% D-galactose as carbon source, and reached 56.99 ± 0.51 U/mL at 72 h (Fig. 5a). Cell biomass reached respectively the maximum at 48 h and 72 h with L-arabinose and D-galactose as carbon source, OD_{600} values were 7.25 ± 0.42 and 6.59 ± 0.849, respectively (Fig. 5b).

Our results showed that between LAP production and the activity of galactokinase or the activity of

L-arabinoseisomerase possessed important association.

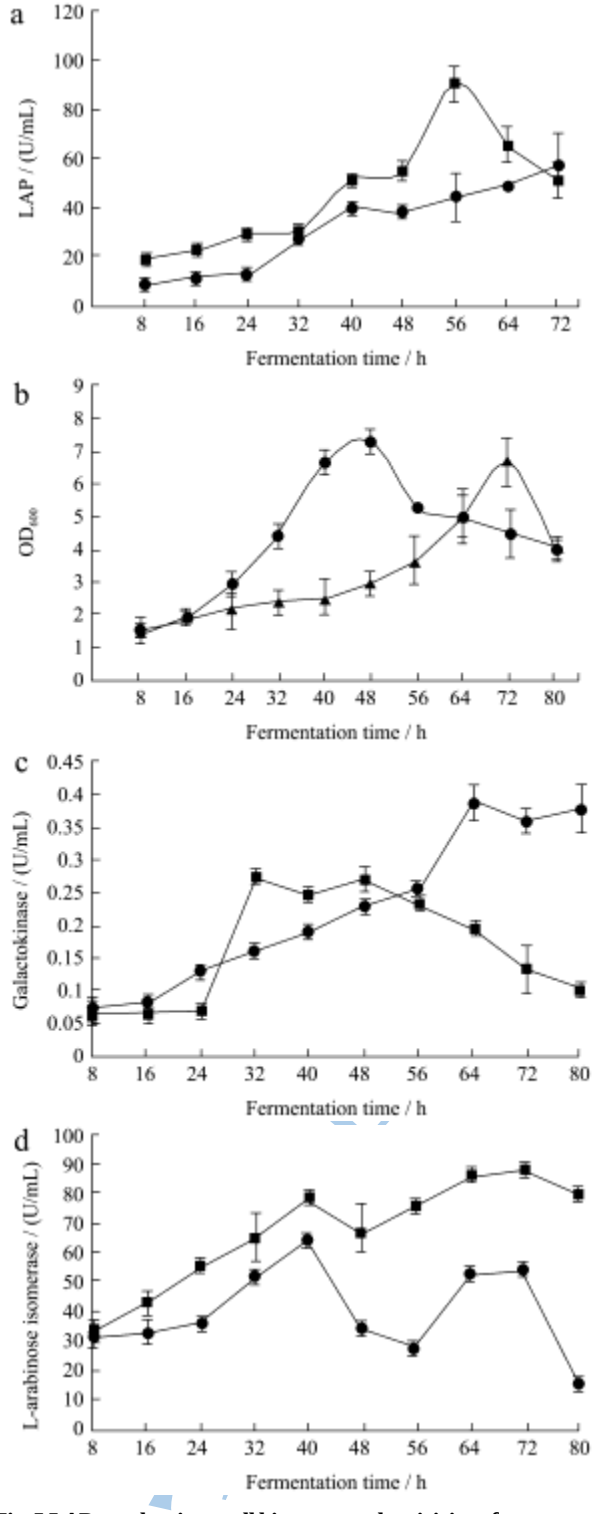

Fig.5 LAP production, cell biomass and activities of two sugars metabolic enzymes in time course

Note: The concentration of sugars was 4%, fermentation time was 80 h. (a) LAP production. (●) D-galactose, (■) L-arabinose; (b) cell biomass. (●) L-arabinose, (▲) D-galactose; (c) the activities of galactokinase with the concentration of 4% D-galactose. (●) extracelluar activities, (■) intracelluar activities; (d) the activities of L-arabinoseisomerase with the concentration of 4% L-arabinose. (●) extracelluar activities, (■) intracelluar activities.

3 Conclusion

Aminopeptidases are used in the production of cheese, beverages, flavorings, meat and milk products, also as an additive to animal feed to improve utilization of proteins for piglets and poultry. They can enhance and optimize aroma and flavor. In this study, the effects of carbon sources on the growth, LAP production of *Bacillus cereus* CZ and the activities of sugar metabolic enzymes during fermentation were studied. It was found that L-arabinose was the best carbon source for producing LAP and the optimum concentration was 4%. The metabolic enzymes of sugars were examined, β-galactosidase showed that lactose was the best inducer for it, galactokinase was induced strongly by D-galactose, and L-arabinoseisomerise was induced strongly by L-arabinose. These results will contribute to further study the molecular regulation mechanisms of carbon sources, especially L-arabinose.

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