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• BRIEF REPORTS •

Recombinant *Helicobacter pylori* catalase

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Abstract

AIM: To construct a recombinant strain which highly expresses catalase of *Helicobacter pylori* (*H. pylori*) and assay the activity of *H. pylori* catalase.

METHODS: The catalase DNA was amplified from *H. pylori* chromosomal DNA with PCR techniques and inserted into the prokaryotie expression vector pET-22b (+), and then was transformed into the BL21 (DE3) *E.coli* strain which expressed catalase recombinant protein. The activity of *H. pylori* catalase was assayed by the Beers&Sizers.

RESULTS: DNA sequence analysis showed that the sequence of catalase DNA was the same as GenBank's research. The catalase recombinant protein amounted to 24.4 % of the total bacterial protein after induced with IPTG for 3 hours at 37 ℃ and the activity of *H. pylori* catalase was high in the BL21 (DE3) *E.coli* strain.

CONCLUSION: A clone expressing high activity *H. pylori* catalase is obtained, laying a good foundation for further studies.

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INTRODUCTION

Helicobacter pylori (*H.pylori*) is a bacillus first isolated from human gastric antral epithelium in 1982. It is recognized as a human-specific gastric pathogen that colonizes the stomachs of at least half of the world population[1,2]. *H.pylori* infection is the major cause of chronic gastritis and peptic ulcer[3-13], and is also closely related to adenocarcinoma of stomach and mucosa-associated lymphoid tissue (MALT) lymphoma and primary gastric non-Hodgkin's lymphoma[14-32]. This organism was recently categorized as a class I carcinoma by the World Health Organization[33], and direct evidence of carcinogenesis was recently demonstrated in an animal model[34-36]. In addition, seroepidemiologic studies indicate that *H.pylori* infection is also associated with the occurrence of circulatory, respiratory and alimentary (except stomach and duodenum) system diseases and autoimmune diseases[37-41]. With discoveries that *H.pylori* may play an important role in many diseases, *H.pylori* is being studied thoroughly, especially the mechanism of escaping from host killing and clearing. Because of only escaping from host killing and clearing, *H.pylori* can locate in body steadily and cause diseases. The effect of catalase is emphasized increasingly in escaping from killing of host's free radical and sustaining the balance of self-oxygen metabolism. No study in expressing high activity *H.pylori* catalase had been reported in China and abroad. In this study, PCR technology was performed to obtain catalase gene, and construct expressing vectors. The sequence analysis and activity detection were performed in order to obtain the clone, effective expression and activity evaluation of catalase gene, thus laying a good foundation for further studying the associated functions.

MATERIALS AND METHODS

Plasmids and strains and growth conditions

Plasmid pET-22b (+) was obtained from Novagen. *Escherichia coli* (*E.coli*) DH5α (Biodev) was used as a host for recombinant DNA manipulation, *E.coli* BL21 (DE3) was used for expression of the catalase gene. *H. pylori* was stored in this lab. *E.coli* was grown in Luria-Bertani medium containing 100 mg of ampicillin liter^{-1}.

Recombinant DNA techniques

All restriction enzyme digestions, ligations and other common DNA manipulations, unless otherwise stated, were performed by standard procedures. The genome of *H.pylori* was prepared from the cells collected from the colonies on the agar plate. The gene of *H.pylori* catalase was amplified from the genome of *H.pylori* by PCR (Techne PROGENE) using the primers cat1 (5' -TG GCC ATG GAT GTT AAT AAA GAT GTG AAA C-3') as upstream primer and cat2 (5' -AG TGC GGC CGC CTT TTT CTT TTT TGT GTG-3') as downstream primer as described in the literature[42]. Cat1 and cat2 contained *Nco* I and *Not* I sites, respectively. The PCR product was recovered from agarose gel, digested with *Nco* I and *Not* I, and inserted into the *Nco* I and *Not* I restriction fragment of the expression vector pET-22b(+) using T4 DNA ligase. The resulting plasmid pET-CAT was transformed into competent *E.coli* BL21 (DE3) cells using ampicillin resistance for selection. The insert was confirmed using *Xho* I digestion to check for a 1.5-kb increase in size and *Nco* I and *Not* I digestion to show a 1.5-kb fragment.

Microbiological manipulations

Strain BL21 (DE3) BL21 (DE3) containing pET-22b(+)-CAT, was incubated overnight at 37 ℃ while shaking in 5 ml LB with 100 μg/mL ampicilline. Fifty mL LB was inoculated and the cells grew until the optical density at 600nm reached 0.4-0.6. Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 1mM.

Enzyme assay

Catalase activity was assayed according to the modified method of Aebi. The assay was performed in a reaction mixture (1 ml) containing 10mM H_2O_2 in 50mM potassium phosphate buffer (pH 7.0, buffer B) at 30 ℃. The rate of disappearance of H_2O_2 was measured spectrophotometrically at 240nm using a

Shimadzu UV-3000 spectrophotometer (Kyoto). One unit of catalase activity was defined as the amount of enzyme that decomposed 1 μmol H_2O_2 per minute under the assay conditions.

Preparation of cell fractionation

E.coli cells from a 50 mL growth 5 h after induction were harvested by centrifugation at 12 000×g for 10 min and the pellet was resuspended in 1 ml 30mM Tris buffer (pH8.0) containing 1 mmol/L EDTA (pH8.0), 20 % sucrose. The suspension was put on ice for 10 min, then centrifuged for 10 min at 12 000×g, and the resulting supernatant contained proteins from the periplasm. The resulting pellet was resuspended in 5 mL 50mM Tris buffer (pH8.0) containing 2mM EDTA, 0.1 mg/mL lysozyme and 1 % Triton X-100. The suspension was incubated at 30 ℃ for 20 min and then sonicated on ice until it became clarified. The lysate was centrifuged at 12 000×g for 15 min at 4 ℃, and the resulting supernatant contained proteins from the cytoplasm, while pelleted proteins were derived from inclusion bodies.

Optimization of expression

Induction of glycolate oxidase expression as a function of the concentration of IPTG: Seven flasks each with 50 mL medium containing 100 μg/mL ampicillin were inoculated with BL21 (DE3) carrying plasmid pET-CAT. At OD600 of 0.6, expression of CAT was induced with IPTG, of which the concentrations were 0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0mM, respectively. After 5 hours the cells were harvested, crude extracts were compared by SDS-gel electrophoresis. CAT activity of the cells was determined as described above.

Induction of glycolate oxidase expression as a function of cell density: Three flasks each with 50 mL medium containing 100 μg/mL ampicillin were inoculated with BL21 (DE3) carrying plasmid pET-CAT. At OD_{600} of 0.4, 0.6 and 1.0, respectively, expression of CAT was induced with IPTG (final concentration 0.2mM). After 5 hours cells were harvested, crude extracts were compared and CAT activity was determined as described above.

RESULTS

Cloning of H. pylori catalase gene

The *H.pylori* catalase gene was amplified by PCR using the *H.pylori* genome as the template and cloned into plasmid pET-22b(+).The recombinant plasmids pET-CAT were all digested by Xho I, and by Nco I and Not I simultaneously, then digestive products were visualized on 10 g· L^{-1} agarose gel electrophoreses (Figure 1). It demonstrated that recombinant plasmid contained the objective gene. The DNA sequencing proved that the entire sequence of the gene was consistent with the results reported before[42].

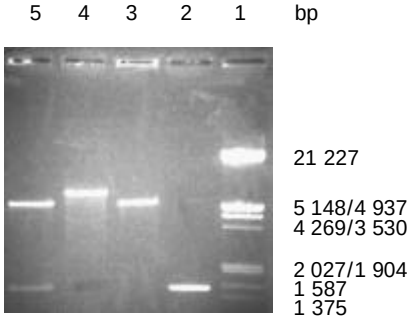

Figure 1 Agarose gel electrophoresis of PCR products and plasmid pET-CAT digested with restriction enzymes. Lane 1: DNA marker; Lane 2: PCR product; Lane 3: pET-22b (+)/ Xho I; Lane 4: pET-CAT/ Xho I; Lane 5: pET-CAT/ NcoI+NotI.

Expression of H. pylori catalase in E.coli

Following recombinant vector transformed into BL21 *E.coli* strains, recombinant *E.coli* strains expressing catalase were obtained. The expressed protein amounted to 24.4 % of the total bacterial protein after induced with IPTG for 3 h at 37 ℃. Its molecular mass was M_r 58 000 by 100 g/L SDS-PAGE gel analysis. After preparation of cell fractionation, the expressed protein amounted to 11.5 % of the bacterial periplasm protein, 14.9 % of the bacterial sonicate supernatant and 58.1 % of the bacterial inclusion body (Figure 2).

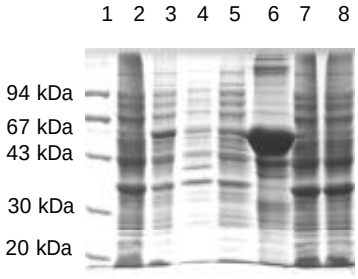

Figure 2 SDS-PAGE analysis of catalase recombinant protein expressed in BL21 (DE3). Lane 1: Molecular weight marker (20, 30, 43, 67, 94)×10^3; Lane 2: BL21 (pET-CAT) cells before induction; Lane 3: BL21 (pET-CAT) cells after 3 h induction with IPTG; Lane 4: BL21 (pET-CAT) cells periplasm protein after 3 h induction with IPTG; Lane 5: sonicate supernatant of BL21 (pET-CAT) cells after 3 h induction with IPTG; Lane 6: inclusion body of BL21 (pET-CAT) cells after 3 h induction with IPTG; Lane 7: control strain BL21 (pET) before induction; Lane 8: control strain BL21 (pET) after 3 h induction with IPTG.

Optimization of expression

An important factor that might affect the expression is the concentration of the inducer. Since IPTG is a rather costly component we decided to investigate the dependency of the expression system on the IPTG concentration. We found that in the range of 0.1-1 mm final concentration of IPTG, the expression level was independent of the inducer concentration, and the catalase activity of the whole cells was from 633.6 U/mg cells (dry weight) to 660.1 U/mg cells (dry weight). The data are shown in Table 1.

Table 1 Induction of glycolate oxidase expression at different IPTG concentrations

IPTG concentration (mm)	0.1	0.2	0.4	0.6	0.8	1.0
Activity (U/mg cells (dry weight))	638.5	653.6	660.1	642.3	646.7	631.2

In order to further optimize the conditions for expression we induced the bacterial cultures at various cell densities. The expression was the highest at OD_{600}=0.6, and at the same time the activity was also optimal at OD_{600}=0.6, (Table 2).

Table 2 Induction of *H.pylori* catalase expression at different cell densities

Induction at OD_{600}	Activity (U/mg cells (dry weight))
0.4	603.2
0.6	661.2
1.0	584.1

DISCUSSION

For a long time, people had presumed that transferring poisonous oxygen metabolite into nonpoisonous water,

sustaining self-metabolite equilibrium and protecting *H.pylori* from the killing of neutrophilic leukocyte were the main functions of *H.pylori* catalase[43]. However, Bauerfeind *et al.* found that *H.pylori* catalase accounts only for 1.5 %, while urease accounts for 10 % of thallus' s gross protein at pH 6 or 7. The activity of urease could not be detected at pH 5. On the other hand, the activity of catalase is still sustained at pH 3. This finding indicated that catalase may play a more important role than urease when *H.pylori* survives in an acid environment[44]. In addition, recent studies found that catalase may play an important role in preventing *H.pylori* from bacillus to coccus[45-47].

Most interestingly, Radcliff *et al.* have suggested that the protective rate of natural catalase can reach 80 %, while that of recombinant catalase can reach 90 %, based on their animal experiments, which indicated that catalase is a new antigen for the preparation of *H.pylori* vaccine[42].

Overall, further studies on catalase are essential, in order to understand the effect of catalase on *H.pylori* mechanism of causing disease, as well as immune prevention and treatment. In this study, *H.pylori* catalase gene was cloned and inserted into fused cloning strain, which was demonstrated by catalase activity. An important experimental basis for further studies on mechanism of catalase causing disease, and immune protective effects has been laid.

Plasmid pET-22b(+) was a secretion vector and the foreign protein was transported into periplasm directed by the signal peptide Pel B. Soluble and functional expression of catalase was achieved by secreting the recombinant protein to periplasm, which provided an environment favorable for the folding of this protein. These may explain why the cloning strain has the high activity. The high activity of the cloning strain may provide better measures to treat diseases caused by free radicals, such as sequela radiotherapy on neoplasm, all kinds of phlogosis, several kinds of dermathosis, second perfusion in open cardiac operation and so on.

REFERENCES

1 **Michetti P**, Kreiss C, Kotloff K, Porta N, Blanco JL, Bachmann D, Herranz M, Saldinger P, Corthesy-Theulaz I, Losonsky G, Nichols R, Simon J, Stolte M, Ackerman S, Monath TP, Blum AL. Oral immunization with urease and Escherichia *coli* heat-labile enterotoxin is safe and immunogenic in *Helicobacter pylori*-infected adults. *Gastroenterology* 1999; **116**: 804-812

2 **Xu CD**, Chen SN, Jiang SH, Xu JY. Seroepidemiology of *Helicobacter pylori* infection among asymptomatic Chinese children. *World J Gastroenterol* 2000; **6**: 759-761

3 **Xiao SD**, Liu WZ. Current statua in treatment of *Hp* infection. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 3-4

4 **Jiang Z**, Tao XH, Huang AL, Wang PL. A study of recombinant protective *H. pylori* antigens. *World J Gastroenterol* 2002; **8**: 308-311

5 **Meyer JM**, Silliman NP, Dixon CA, Siepman NY, Sugg JE, Hopkins RJ. *Helicobacter pylori* and early duodenal ulcer status post-treatment: a review. *Helicobacter* 2001; **6**: 84-92

6 **Xia HX**, Fan XG, Talley Nicholas J. Clarithromycin resistance in *Helicobacter pylori* and its clinical relevance. *World J Gastroenterol* 1999; **5**: 263-265

7 **Peng ZS**, Liang ZC, Liu MC, Ouang NT. Studies on gastric epithelial cell proliferation and apoptosis in *Hp* associated gastric ulcer. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 218-219

8 **Vandenplas Y**. *Helicobacter pylori* infection. *World J Gastroenterol* 2000; **6**: 20-31

9 **Casella G**, Buda CA, Maisano R, Schiavo M, Perego D, Baldini V. Complete regression of primary gastric MALT-lymphoma after double eradication *Helicobacter pylori* therapy: role and importance of endoscopic ultrasonography. *Anticancer Res* 2001; **21**: 1499-1502

10 **Kate V**, Ananthakrishnan N, Badrinath S. Effect of *Helicobacter pylori* eradication on the ulcer recurrence rate aftersimple closure of perforated duodenal ulcer: retrospective and prospective randomized controlled studies. *Br J Surg* 2001; **88**: 1054-1058

11 **Hobsley M**, Tovey FI. *Helicobacter pylori*: the primary cause of duodenal ulceration or a secondary infection? *World J Gastroenterol* 2001; **7**: 149-151

12 **Hou P**, Tu ZX, Xu GM, Gong YF, Ji XH, Li ZS. *Helicobacter pylori* vacA genotypes and cagA status and their relationship to associated diseases. *World J Gastroenterol* 2000; **6**: 605-607

13 **Zhang H**, Jiang SL, Yao XX. Study of T-lymphocyte subsets, nitric oxide, hexosamine and *Helicobacter pylori* infection in patients with chronic gastric diseases. *World J Gastroenterol* 2000; **6**: 601-604

14 **Guo CQ**, Wang YP, Ma SW, Ding GY, Li LC. Study on Helicobacter pylori infection and P53, c-erbB-2 gene expression in carcinogenesis of gastric mucosa. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 313-315

15 **Hiyama T**, Haruma K, Kitadai Y, Masuda H, Miyamoto M, Ito M, Kamada T, Tanaka S, Uemura N, Yoshihara M, Sumii K, Shimamoto F, Chayama K. Clinicopathological features of gastric mucosa-associated lymphoid tissue lymphoma: a comparison with diffuse large B-cell lymphoma without a mucosa-associated lymphoid tissue lymphoma component.*J Gastroenterol Hepatol* 2001; **16**: 734-739

16 **Quan J**, Fan XG. Progress in experimental research of *Helicobacter pylori* infection and gastric cancinoma. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 1068-1069

17 **Delchier JC**, Lamarque D, Levy M, Tkoub EM, Copie-Bergman C, Deforges L, Chaumette MT, Haioun C. *Helicobacter pylori* and gastric lymphoma: high seroprevalence of CagA in diffuse large B-cell lymphoma but not in low-grade lymphoma of mucosa-associated lymphoid tissue type. *Am J Gastroenterol* 2001; **96**: 2324-2328

18 **Hua JS**. Effect of *Hp*: cell proliferation and apoptosis on stomach cancer. *Shijie Huaren Xiaohua Zazhi* 1999; **9**: 647-648

19 **Hu PJ**. *Hp* and gastric cancer: challege in the research. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 1-2

20 **Gao HJ**, Yu LZ, Bai JF, Peng YS, Sun G, Zhao HL, Miu K, Lü XZ, Zhang XY, Zhao ZQ. Multiple genetic alterations and behavior of cellular biology in gastric cancer and other gastric mucosal lesions: *H. pylori* infection, histological types and staging. *World J Gastroenterol* 2000; **6**: 848-854

21 **Yao YL**, Zhang WD. Relation between *Helicobacter* and gastric cancer. *Shijie Huaren Xiaohua Zazhi* 2001; **9**: 1045-1049

22 **Nakamura S**, Matsumoto T, Suekane H, Takeshita M, Hizawa K, Kawasaki M, Yao T, Tsuneyoshi M, Iida M, Fujishima M. Predictive value of endoscopic ultrasonography for regression of gastric low grade and high grade MALT lymphomas after eradication of *Helicobacter pylori*. *Gut* 2001; **48**: 454-460

23 **Uemura N**, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med* 2001; **345**: 784-789

24 **Morgner A**, Miehlke S, Fischbach W, Schmitt W, Muller-Hermelink H, Greiner A, Thiede C, Schetelig J, Neubauer A, Stolte M, Ehninger G, Bayerdorffer E. Complete remission of primary high-grade B-cell gastric lymphoma after cure of *Helicobacter pylori* infection. *J Clin Oncol* 2001; **19**: 2041-2048

25 **Zhuang XQ**, Lin SR. Progress in research on the relationsh between *Hp* and stomach cancer. *Shijie Huaren Xiaohua Zazhi* 2000; **8**: 206-207

26 **Wang RT**, Wang T, Chen K, Wang JY, Zhang JP, Lin SR, Zhu YM, Zhang WM, Cao YX, Zhu CW, Yu H, Cong YJ, Zheng S, Wu BQ. *Helicobacter pylori* infection and gastric cancer: evidence from a retrospective cohort study and nested case-control study in China. *World J Gastroenterol* 2002; **8**: 1103-1107

27 **Yao YL**, Xu B, Song YG, Zhang WD. Overexpression of cyclin E in Mongolian gerbil with *Helicobacter pylori*-induced gastric precancerosis. *World J Gastroenterol* 2002; **8**: 60-63

28 **Morgner A**, Miehlke S, Stolte M, Neubauer A, Alpen B, Thiede C, Klann H, Hierlmeier FX, Ell C, Ehninger G, Bayerdorffer E. Development of early gastric cancer 4 and 5 years after complete remission of *Helicobacter pylori* associated gastric low grade marginal zone B cell lymphoma of MALT type. *World J Gastroenterol* 2001; **7**: 248-253

29 **Miehlke S**, Kirsch C, Dragosics B, Gschwantler M, Oberhuber G, Antos D, Dite P, Lauter J, Labenz J, Leodolter A, Malfertheiner P, Neubauer A, Ehninger G, Stolte M, Bayerdorffer E. *Helicobacter pylori* and gastric cancer:current status of the Austrain Czech

German gastric cancer prevention trial (PRISMA Study). *World J Gastroenterol* 2001; **7**: 243-247

30 **Zhuang XQ**, Lin SR. Research of *Helicobacter pylori* infection in precancerous gastric lesions. *World J Gastroenterol* 2000; **6**: 428-429

31 **Cai L**, Yu SZ, Zhang ZF. *Helicobacter pylori* infection and risk of gastric cancer in Changle County, Fujian Province, China. *World J Gastroenterol* 2000; **6**: 374-376

32 **Liu WZ**, Zheng X, Shi Y, Dong QJ, Xiao SD. Effect of *Helicobacter pylori* infection on gastric epithelial proliferation in progression from normal mucosa to gastriccarcinoma. *World J Gastroenterol* 1998; **4**: 246-248

33 **Suganuma M**, Kurusu M, Okabe S, Sueoka N, Yoshida M, Wakatsuki Y, Fujiki H. *Helicobacter pylori* membrane protein 1: a new carcinogenic factor of *Helicobacter pylori*. *Cancer Res* 2001; **61**: 6356-6359

34 **Goto T**, Nishizono A, Fujioka T, Ikewaki J, Mifune K, Nasu M. Local secretory immunoglobulin A and postimmunization gastritis correlated with protection against *Helicobacter pylori* infection after oral vaccination of mice. *Infect Immun* 1999; **67**: 2531-2539

35 **Watanabe T**, Tada M, Nagai H, Sasaki S, Nakao M. *Helicobacter pylori* infection induces gastric cancer in Mongolian Gerbils. *Gastroenterology* 1998; **115**: 642-648

36 **Honda S**, Fujioka T, Tokieda M, Satoh R, Nishizono A, Nasu M. Development of *Helicobacter pylori*-induced gastric carcinoma in Mongolian Gerbils. *Cancer Res* 1998; **58**: 4255-4259

37 **Bulajic M**, Stimec B, Milicevic M, Loehr M, Mueller P, Boricic I, Kovacevic N, Bulajic M. Modalities of testing *Helicobacter pylori* in patients with nonmalignant bile duct diseases. *World J Gastroenterol* 2002; **8**: 301-304

38 **Pena A**. Genetic factors determining the host response to *Helicobacter pylori*. *World J Gastroenterol* 2000; **6**: 624-625

39 **Gocyk W**, Niklinski T, Olechnowicz H. *Helicobacter pylori*, gastrin and cyclooxygenase-2 in lung cancer. *Med Sci Monit* 2000; **6**: 1085-1092

40 **Tsai CJ**, Huang TY. Relation of *Helicobacter pylori* infection and angiographically demonstrated coronary artery disease. *Dig Dis Sci* 2000; **45**: 1227-1232

41 **Dauden E**, Jimenez A I, Garcia D A. *Helicobacter pylori* and idiopathic chronic urticaria. *Int J Dermatol* 2000; **39**: 446-452

42 **Radcliff FJ**, Hazell SL, Kolesnikow T. Catalase,a novel antigen for *Helicobacter pylori* vaccination. *Infect Immun* 1997; 65: 4668-4674

43 **Nilius M**, Malfertheiner P. *Helicobacter pylori* enzymes. *Aliment Pharmacol Ther* 1996; **10**: 65-71

44 **Bauerfeind P**, Garner R, Dunn BE. Synthesis and activity of *Helicobacter pylori* urease and catalase at low Ph. *Gut* 1997; **40**: 25-30

45 **She FF**, Su DH, Lin JY, Zhou LY. Virulence and potential pathogenicity of coccoid *Helicobacter pylori* induced by antibiotics. *World J Gastroenterol* 2001; **7**: 254-258

46 **Khin MM**, Hua JS, Ng HC, Wadstrom T, Bow H. Agglutination of *Helicobacter pylori* coccoids by lectins. *World J Gastroenterol* 2000; **6**: 202-209

47 **Nakamura A**, Park A, Nagata K. Oxidative cellular damage associated with transformation of *Helicobacter pylori* from a bacillary to a coccoid form. *Free Radic Biol Med* 2000; **28**: 1611-1618

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