ORIGINAL ARTICLE

Expression, Purification and Activity Study of Amuc_1100

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Abstract: Objective: To construct prokaryotic expression vector of Amuc_1100 gene, the ELP (ELSTIN-LIKE PROTEIN) is used as purification label to express fusion with the target protein and detect the biological activity of recombinant protein at cell level and animal level. Methods: the recombinant plasmid pET28a-Amuc_1100-ELP is designed and synthesized, and transformed into e. coli competent cell bl21 (de3) to induce expression. The recombinant egg was isolated and purified by the reversible phase change cycling (ITC) characteristic of ELP10 white. At the cellular level, PBMCs cells from mice were extracted to detect the expression level of IL-10. At the animal level, obese mice induced by gastric gavage with high fat detected the improvement effect of the protein on mouse metabolism. Results: The recombinant protein with the expected molecular weight of 60 kDa was expressed by E.coli expression system. After circulating through ITC, the recombinant protein with high purity was obtained. The protein increased the expression level of IL-10 in PBMCs cells, and significantly improved the obesity and metabolic disorders induced by high-fat diet in mice. Conclusion: The recombinant protein expression plasmid pET28a-Amuc_1100-ELP was successfully constructed. After expression and purification, the recombinant protein expression plasmid has high purity and has 15 biologically active recombinant protein. This study laid a foundation for the industrial production and clinical application of Amuc_1100.

Keywords: Molecular Biology; Escherichia Coli; Recombinant Protein; ITC cycle; Metabolic Syndrome

Introduction

In recent years, with the continuous improvement of the global economic level, people are more and more keen on high-calorie diet, which makes the incidence rate of metabolic syndrome such as obesity, nonalcoholic fatty liver disease, type 2 diabetes mellitus rise year by year[1-2]. In 2004, Backhed *et al.* research suggested that intestinal microorganisms may be an important influencing factor in the development of obesity and metabolic syndrome, which has aroused heated discussion on intestinal microorganisms[3]. It is estimated that 500~1500 kinds of bacteria[4] live in human intestinal tract. In normal physiological strips.

Under the 45 piece, these intestinal flora have the ability to self-recover and maintain steady state, and through interaction with the body, affect the host's nutrition, immunity, metabolism and other physiological processes[5-7]. In 2012, Vrieze *et al.* reported that the flora from the feces of emaciated people was transplanted into the patients with metabolic syndrome, and found that the obesity and insulin sensitivity of the patients were significantly improved[8], indicating that intestinal flora plays an important role in regulating the metabolic disorders of the body. Microbes on the surface of intestinal mucosa are more closely related to the health of the host, Akkermansia muciniphila is a typical representative of this kind of microbe[9].

A muciniphila is a strictly anaerobic intestinal bacteria isolated and cultured from human feces in recent years. Its abundance accounts for 1%-5% of intestinal flora. It can improve obesity and metabolic syndrome caused by obesity, participate in immune response in vivo, and maintain metabolic balance[10-11]. Clinical studies have found that the

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abundance of the bacterium is greatly reduced[12] in patients with obesity, type 2 diabetes and enteritis. In 2017, Plovier *et al.* Study reported that the weight and insulin resistance of obese mice were significantly improved after intragastric administration of pasteurized A. mucinihila, and the effect was better than that of inactivated A. mucinihila. The outer membrane protein Amuc_1100 of 55 plays a major role[13]. Amuc_1100 is one of the most abundant outer membrane proteins on its surface. It remains active at pasteurization temperature and can independently perform the beneficial bacteria function[14]. Amuc_1100 acts on TLR2 receptor and regulates the expression of inflammatory factors such as IL-10, TNF-α, IL-6, IL-8, etc. It can also enhance intestinal barrier function by regulating the expression of intestinal tight junction protein, thus improving obesity and body metabolism[15]. Amuc_1100 has shown great application value in inhibiting obesity, improving body metabolism, systemic inflammation, etc. A. muciniphila is difficult to cultivate and extremely sensitive to oxygen feeling, so Amuc_1100 has more advantages in clinical application. In this study, the biological activity of Amuc_1100 protein was expressed by Escherichia coli, and a new purification method was used to separate the protein. The recombinant protein was purified and its biological activity was detected. So as to establish the expression and purification process of Amuc_1100 protein and lay a foundation for its mass production.

1. Materials and methods

1.1 Materials

1.1.1 Plasmid and strain

pUC57-Amuc_1100 plasmid was synthesized by Suzhou Jinweizhi Company, pET28a-ELP plasmid was stored in our laboratory, and competent cells DH5α and BL21 (DE3) of Enterobacteriaceae were purchased from Shanghai Bioengineering Company.

1.1.2 Reagent and instrument

Protein ladder, 2×taq master mix and BCA protein quantitative kits were purchased from Thermophilic Technologies (China) Limited Company, plasmid extraction kits, gel recovery kits and SDS-PAGE gel kits were purchased from Bi Yun Company. Isopropyl thiogalactoside (IPTG) and kanamycin were purchased from BBI Life Science Co., Ltd., Quick Ligation Kit was purchased from BioLab Company, 60% high-fat feed was purchased from Shanghai Fanbo Biotechnology Co., Ltd., blood glucose meter and blood glucose test paper were purchased from Sannuo Company.

1.2 Experimental methods

1.2.1 Construction of recombinant vector

Plasmids pUC57-Amuc_1100 and pET28a-ELP are subjected to double enzyme digestion with NdeI and XhoI, respectively. the enzyme digestion product is recovered after electrophoresis with 1% agarose gel. the vector and the target fragment are connected with 1-2 h at room temperature with T4 DNA ligase at 1:3 (molar ratio). The ligation product was transformed into DH5α competent cells and coated on LB solid culture plate (containing 50 μg/mL Kan), 37 ℃. inverted overnight for 14-16 h. Monoclonals were selected for plasmid amplification, plasmids were extracted for NdeI/XhoI double enzyme digestion identification, and positive clones with correct identification were sent to Jin weizhi for sequencing analysis.

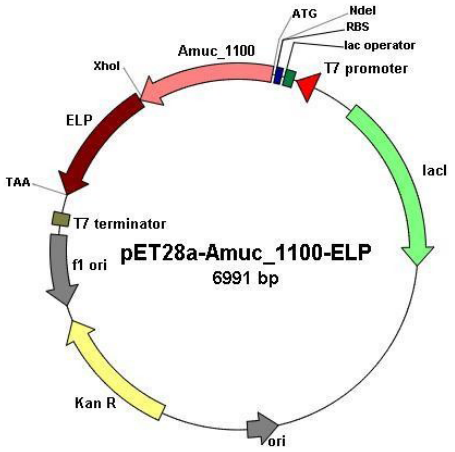

Figure 1. The map of pET28a-Amuc_1100-ELP plasmid.

1.2.2 Induced expression and solubility analysis of 1.2.2 recombinant protein

The plasmid correctly identified by enzyme digestion was transformed into host bacterium BL21 (DE3) and added to 10 mL LB medium (containing 50 μg/mL Kan), 37℃, 220 r/min overnight culture 14-16 h. Inoculate 1% inoculum into LB medium (containing 50 μg/mL Kan and 1% glucose), 37℃, 220 r/min shake bacteria 2-3 h and add IPTG with a final concentration of 1 mM to induce expression when the OD 600 of bacteria liquid grows to 0.4-0.6, and induce 0 h, 2 h, 4 h, 6 h and 8 h at 37℃, 220 r/min. 7000 r/min centrifugation 10 min to collect thalli, high-pressure homogenizer to crush thalli, 12000 r/min centrifugation 10 min, collecting supernatant and precipitate for 12% SDS-PAGE analysis.

2. Result

2.1 Construction of recombinant vector

As shown in Figure 2, the recombinant plasmid was double-enzymatically cleaved by NdeI and XhoI to obtain a band of about 870 bp, which was consistent with the target band size. The plasmid identified by enzyme digestion was sent to sample for sequencing. The sequencing results showed that the gene sequence was correct. The above results showed that the recombinant vector pET28a-Amuc_1100-ELP was successfully constructed.

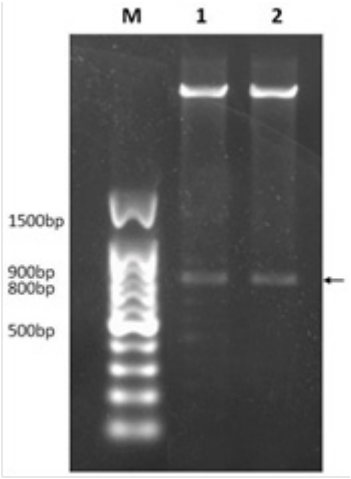

Figure 2. Enzyme digestion identification results of recombinant plasmid.

2.2 Induced expression and solubility analysis

The strain pET28a-Amuc_1100-ELP was induced to express 0 h, 2 h, 4 h, 6 h and 8 h respectively by 1 mM IPTG. After taking samples, the 12% SDS-PAGE analysis was carried out. the gel running results showed (Figure 3a) that there was an obvious band near 60 kDa . No such band was found in Lane 1 without induction by IPTG and the protein expression reached the highest when the induction time was 4 h (Lane 3). After the collected thallus is crushed by a high-pressure homogenizer, the supernatant and precipitate are respectively taken for 12% SDS-PAGE analysis. The

results show (Figure 3b) that the recombinant protein is mainly expressed in soluble form.

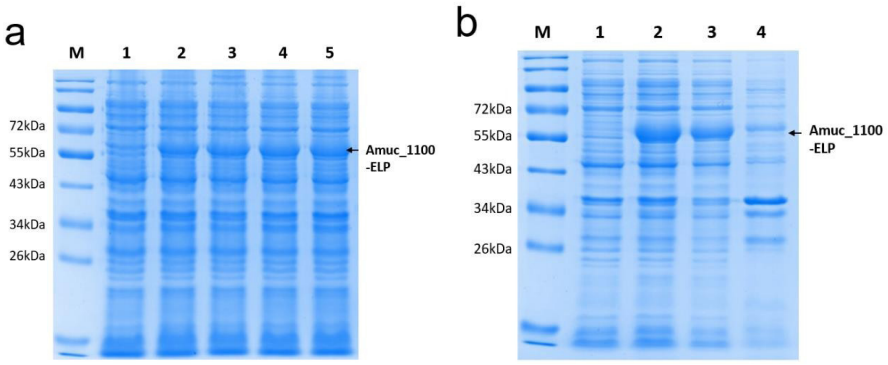

Figure 3. Expression of recombinant protein (a) and Soluble analysis (b). a: (M: Protein Marker; Lane 1-5: Cell lysate induced by IPTG for 0 h , 2 h, 4 h, 6 h and 8 h) b: (M: Protein Marker; Lane 1: Cell lysate induced by IPTG for 0 h; Lane 2: Cell lysate by IPTG for 4 h; Lane 3: Supernatant of Cell lysate; Lane 4: Precipitation of Cell lysate)

3. Discussion

In this study, Escherichia coli expression system was used to produce and express recombinant protein. Escherichia coli is currently the most commonly used epitope system, which has the advantages of easy culture, fast growth speed, low cost and mass production of target protein. the vector selection is more commonly used pET system, and strong promoter T7 is used to start expression[16-17] and finally recombinant protein is successfully expressed (Figure 3a). soluble analysis shows that the protein is mainly expressed in soluble form (Figure 3b). A new non-color spectrum purification label ELP is used for protein purification. ELP belongs to elastin-like protein and has the characteristics of reversible phase change cycle (ITC), that is, when the ambient temperature is lower than the phase change temperature (inverse temperature transition, TT), the ELP is in a dissolved state; When the ambient temperature is higher than Tt, the ELP aggregates to precipitate[18-19]. Moreover, adding NaCl into the solution appropriately can effectively reduce the phase transition temperature, and the target protein still has this property[20] after fusing the ELP label. Therefore, the separation and purification of recombinant protein are carried out by utilizing the circulating characteristic of ITC. Compared with the traditional purification method, the method has the advantages of simple operation, low cost, easy expansion and the like, and the ELP label can also be used as a drug delivery carrier[21] so that excision from the recombinant protein is not required.

The purpose of this study is to produce and express the target protein with biological activity, so in vivo and in vitro activity tests have been carried out respectively. Some studies have shown that Amuc_1100 can regulate the expression of inflammatory factors through interaction with TLR2 receptor to maintain the balance of immune system. TLR2 receptor is an important protein molecule in non-specific immune process. It is widely distributed in the first-line defense of the immune system. It has a high level of expression[22] in PBMCs cells. With the development of obesity in mice, insulin resistance will occur in mice, i.e. the efficiency of insulin intake and glucose utilization will decrease, and the body will compensate and secrete more insulin to maintain blood sugar stability[23]. Fasting blood glucose of mice in HFD group increased and glucose tolerance decreased significantly. Blood glucose level of mice in HFD-Amuc_1100 group was similar to that of ND group, close to normal level.

To sum up, this study explored the expression, purification and biological activity of the protein, and obtained ideal experimental results. Amuc_1100 has potential application value, but the specific regulation mechanism of AMUC _ 1100 to the body is not very clear. Therefore, it needs to be explored through practical tests, so as to more reasonably develop the value of clinical application.

4. Conclusion

In this paper, Amuc_1100 protein was expressed by e. coli expression system, and the recombinant protein was efficiently separated and purified by ELP fusion expression. It was confirmed that the recombinant protein expressed in this study has biological characteristics at cell level and animal level. The activity has laid a foundation for its industrial production.

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