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Effects of *deg*U32(Hy), *deg*Qa and *deg*R Pleiotropic Regulatory Genes on the Growth and Protease Fermentation of *Bacillus Subtilis* Ki-2-132

PAN Xue-Feng[1,2,①]

1. *Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing* 100101, *China;*

2. *Beijing Institute of Technology, Beijing* 100081, *China*

Abstract: Effects of *deg*U32 (Hy), *deg*R genes from *Bacillus subtilis* 168 and *deg*Qa gene from *Bacillus amyloliquefaciens* on *Bacillus subtilis* Ki-2-132 cell growth, sporulation and protease fermentation were investigated by introducing these genes into *B. subtilis* Ki-2-132 chromosome and/or cytoplasm. Although the genes come from different species and strains, they showed pleiotropic effects in *B. subtilis* Ki-2-132. *B. subtilis* Ki-2-132*deg*U32 (Hy) showed increased protease production, and when cooperating with *deg*Qa either in plasmid or in chromosome, further altered cell growth, increased protease production and affected the spore formation in a glucose and dosage dependent manner. By contrast, *deg*R did not significantly affect the protease productivity in *deg*U32 (Hy) mutant, consisting with that DegR was used to stabilise DegU-phosphate, which in *deg*U32 (Hy) strain no longer further amplify the DegU-phosphate effect.

Key words: *deg*U32 (Hy); *deg*Qa; *deg*R; pleiotropic regulatory gene; *Bacillus subtilis*

DegS-DegU two-component regulatory system in *Bacillus subtilis* controls various processes that characterise transition from exponential to stationary growth, including induction of extracellular degradative enzymes, down-regulation of sigma D regulon, expression of late competence genes and expression of genes responsible for salt stress [1-5]. Amongst them, DegS acts as a sensor, a kinase, to phosphorylate DegU, the effectors, which during the regulation of protease transcription needs also at least two other regulatory genes, *deg*R and *deg*Q, encoding polypeptides of 60 and 46 amino acids respectively[6-10]. Phosphorylated form of DegU (DegU-P) is normally stabilised by DegR, while it can also be produced by a *deg*U32 (Hy) mutation, resulting in overproduction of several extracellular degradative enzymes [11]. *deg*R was shown to be transcripted by using sigma D, and the expression was suppressed by ComK and ProB through sigma D [8-10]. The expression of *deg*Q was shown to be decreased in the presence of glucose and increased under following conditions: growth with poor carbon sources, amino acid deprivation, phosphate starvation, or growth in the presence of decoyinine (a specific inhibitor of GMP synthetase), and can also be controlled by DegS-DegU and ComP-ComA two-component systems, which share several target genes in common, such as late competence genes [1].

Regulation of *deg*Q expression by amino acid deprivation was shown to be ComA dependent, and regulations by catabolic repression, phosphate starvation and decoyinine were involving sequences downstream from position −78 and was independent of the two-component systems[1]. The targets for regulation of *deg*Q gene expression by DegS-DegU and ComP-ComA located in positions between −393 and

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① Corresponding author. xuefengpancam@yahoo.com.cn

−186, and −78 and −40, respectively [1].

B.subitilis Ki-2-132 is a novel bacillus strain that was isolated by our laboratory, which has recently been developed into a genetic system for the expression of several proteins[12]. Compared to *B.subtilis* 168, *B.subitilis* Ki-2-132 showed several significant distinctions, such as no phage infection when cultivated in liquid medium, high transformation frequency when compared with that of *B.subtilis* 168, r^-m^- etc[13]. To further develop *B.subitilis* Ki-2-132 genetic engineering system, DNA sequence diversities among *deg*U, *deg*Q and *deg*R genes in *B.amyloliquefaciens, B.subtilis* 168 and *B.subtilis* Ki-2-132 were taken into consideration, of which in this work, have been tested on the improvement on the protease fermentation in *B. subitilis* Ki-2-132. The *deg*U gene in *B. subitilis* Ki-2-132 was substituted by *deg*U32(Hy), and then the *deg*Q and *deg*R genes were replaced by *deg*Qa, a homologous of *deg*Q in *B.amyloliquefaciens*, and the *deg*R from *B.subtilis* 168, respectively. In the meantime, hyperexpression of *deg*Qa and *deg*R were conducted by cloning these genes into a multicopy plasmid pMK4, respectively. The work as reported here showed that introduction of a *deg*U32(Hy) mutation in *B. subitilis* Ki-2-132 increased its protease production, when combined with *deg*Qa, further promoted the protease production in a glucose and gene-dosage dependent manner. While combination of *deg*U32(Hy) mutation with *deg*R mainly affected cell growth and differentiation.

1 Materials and Methods

1.1 Bacteria and plasmids

B.subtilis Ki-2-132 derivatives used in this work has been listed in Table 1. *B.subtilis* Ki-2-132 was the stock of our laboratory. *B.subtilis* 168 *deg*U32(Hy) was obtained from BGSC. *deg*R and *deg*Qa genes from *B.subtilis* 168 and *B. amyloliquefaciens* strain were subcloned into plasmid pJM101 and pJM102 respectively, which were used when integrating the *deg*Qa and *deg*R into *B. subtilis* Ki-2-32 chromosome. Hyper-expression of *deg*Qa and *deg*R were carried out by cloning these genes into plasmid p*MK*4. Plasmid p*K*307 carrying Km resistant gene was isolated and stocked by our laboratory. Plasmid p*KP*1 carrying *apr*E gene and Km resistant gene was constructed by our laboratory. *E. coli* JM109 was used as the host for plasmid pJM101, pJM102 and their derivatives.

1.2 Media and cultivation

LB medium was prepared by following the protocol in Molecular Cloning [14]; LBG medium was LB medium with the supplement of 2% glucose; LBM agar medium, LB containing 1% skimmed milk, was prepared before pouring the plates. Cell cultivations were carried out in LB, LBG-agar plates and/or liquid at 37℃; when cultivation was in liquid medium, shaking was applied.

1.3 Isolation and purification of chromosome DNA and plasmid DNA

Isolation and purification of *B.subtilis* 168 chromosome DNA and plasmid DNA from either *B.subtilis* strains or *E.coli* strains were conducted by strictly following Molecular Cloning [14].

1.4 Measurements of protease activity and cell density

0.2 mL of *B.subtilis* bacteria culture grown at 37℃ with shaking for 24 hours, was taken and placed in a 5 mL eppendorf tube, centrifuged at top speed for two minutes. The supernatant was transferred to a new eppendorf tube and 0.5% of Azocasein reagent (5 mg/mL) was added. Each eppendorf tube was further incubated at 37℃ for 30 minutes. 1 mL of 10% trichloroacetic acid was added to each eppendorf tube to stop the reaction. The reaction was then kept at 4℃ for 30 minutes. 0.75 mL of each sample was transferred to

a new eppendorf tube and centrifuged at top speed for 5 minutes. 1.5 mL of 0.5 mol/L NaOH was added to each eppendorf tube to neutralize the trichloroacetic acid. The solution was then transferred to cuvettes, and measured the absorbance at 440 nm. The corresponding cell density was measured at 600 nm.

1. 5 Construction of *Bacillus subtilis* Ki-2-132 mutants by competent cell transformation

B. subtilis Ki-2-132 competent cells were prepared by using a protocol established by our laboratory [15]. Transformation with either chromosome or plasmid DNA was carried out when introducing *deg*U32(Hy), *deg*Qa, and *deg*R into *B. subtilis* Ki-2-132 chromosome or cytoplasm.

1. 6 Analyses of sporulation and cell morphology

Cell morphological observation and sporulation analysis associated with *B. subtilis* Ki-2-132 and its derivatives were carried out by using a light microscope with oil immersion objective. Staining of the vegetative cells and spores was conducted by using safranin and malachite green, respectively.

2 Results

2. 1 Construction of *B.subtilis* Ki-2-132 strains with *deg*U32(Hy), *deg*Qa and *deg*R

B. subtilis Ki-2-132*deg*U32(Hy) were constructed by co-transforming *B. subtilis* 168 chromosome DNA carrying *deg*U32(Hy) and plasmid p*K*307 into *B. subtilis* Ki-2-132 competent cells. As the transformation frequency of chromosome DNA was much higher than that of plasmid DNA in *B.subtilis* Ki-2-132, it was expected that each cell of *B. subtilis* Ki-2-132 transformed with p*K*307 should also be transformed by chromosome DNA. Transformants that appeared on the selective LB plates containing kanamycin and skim milk and showed increased protease halo sizes were further tested for the decreased transformation frequency and the release from the repression of protease production in the presence of 2% glucose [15]. Genes of *deg*Qa and *deg*R were previously cloned into the *Cla* I and *Eco*R I /*Hin*dIII sites in plasmid pJH101, respectively. pJH101 is a vector that is capable of integrating DNA into *B. subtilis* chromosome by homologous recombination. By using pJH101 and by selecting the chloramphenicol resistant, the *deg*Qa and *deg*R were integrated into the chromosomes in *B. subtilis* Ki-2-132 and *B. subtilis* Ki-2-132*deg*U32(Hy), respectively. Subjected to the homologous recombination mechanism, the integration of *deg*Qa and *deg*R into the Ki-2-132 chromosome will inactivate and replace their homologous genes in *B. subtilis* Ki-2-132, respectively. In the meantime, *deg*Qa and *deg*R genes were also subcloned into plasmid p*MK*4 for higher expression of the genes. p*MK*4 is a replicative, multicopied plasmid in *B. subtilis* Ki-2-132. By means of these constructions, *B. subtilis* strains 2 to 10 were obtained (Table 1, strains 2 to 10).

Table 1 Bacteria strains used in this work

Strain No.	Strains and their genotypes	Resources
1	Ki-2-132	This laboratory
2	Ki-2-132*deg*U32(Hy)	Reference [15]
3	Ki-2-132*deg*U32(Hy) *deg*Qa	This work
4	Ki-2-132 *deg*U32(Hy)*deg*R	This work
5	Ki-2-132*deg*U32(Hy) (p*deg*Qa)	This work
6	Ki-2-132*deg*U32(Hy) (p*deg*R)	This work
7	Ki-2-132*deg*Qa	This work
8	Ki-2-132(p*deg*Qa)	This work
9	Ki-2-132*deg*R	This work
10	Ki-2-132(p*deg*R)	This work
11	Ki-2-132(p*KP*1)	This work
12	Ki-2-132*deg*Qa (p*KP*1)	This work
13	Ki-2-132*deg*U32(Hy) (p*KP*1)	This work

2. 2 Effects on cell growth when combining *deg*U32(Hy), *deg*Qa and *deg*R in *B. subtilis* Ki-2-132

The morphological changes and the protease halos associated with the propagations of strains 2 to 6 (Table 1) were initially compared on LB plates containing 5% skim milk. It was found that, when compared to *B.subtilis* Ki-2-132, all five strains have changed their colony appearance and protease-producing capacity. Ki-2-132*deg*U32(Hy) mutant showed irregular colony morphology and increased protease halo sizes, indicating that *deg*U32(Hy) mutation in *B. subtilis* Ki-2-132 has increased its protease-producing capacity by approximate 7-fold and altered cell morphology[15]; however, the combination of *deg*U32(Hy) mutation with either *deg*Qa or *deg*R in chromosome has shown a significant alteration in colony morphology on LB skim milk plates, irrespective of the *deg*Qa and *deg*R genes were in the chromosome or mulitcopied in the cytoplasm. Ki-2-132*deg*U32(Hy)*deg*R, Ki-2-132*deg*U32(Hy)*deg*Qa, Ki-2-132*deg*U32(Hy)(p*deg*Qa) and Ki-2-132*deg*U32(Hy)(p*deg*R) (The *deg*Qa and *deg*R were in cytoplasm) formed fiberfaced colonies on LB plates, without significant distinctions in protease halo sizes (Fig.1 A, B and C). The whole colonies can be easily peeled off by toothticks, and such desquamated colonies appeared meshy, showing a significant distinction to the colonies formed by Ki-2-132 and Ki-2-132*deg*U32(Hy).

2. 3 Filamentous growth and lack of sporulation

Based on the above observation, the cell morphology and sporulation associated with the strain propagation were further analysed. It was found that strains 2 to 6 all showed lack of spore formation when compared to those of Ki-2-132 controls in the same cultivation conditions and cultivation time (Fig.2). Moreover, the cells also showed filamentous growth to certain extents (Fig.2), suggesting that the alteration in colony appearance as associated with *deg*U32(Hy), *deg*Qa and *deg*R genes and their combinations in *B.subtilis* Ki-2-132 strains may be simply due to cell filamentous growth (Fig. 1A, B, and C).

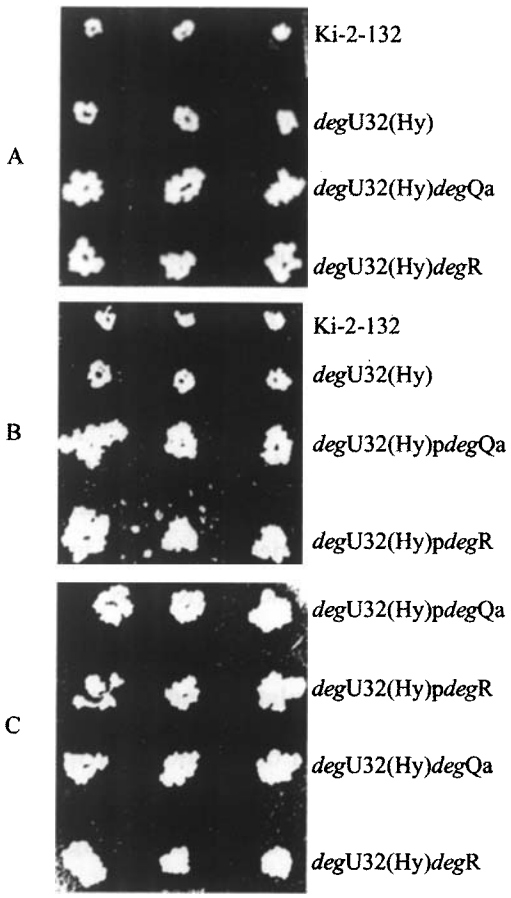

Fig. 1 Effects of *deg*U32(Hy), *deg*Qa and *deg*R in the chromosome and the multicopied *deg*Qa and *deg*R when coordinating with integrated *deg*U32(Hy) on growth and protease production of *B. subtilis* Ki-2-132

A: *deg*U32(Hy), *deg*Qa and *deg*R in the chromosome; B: Multicopied *deg*Qa and *deg*R when coordinating with integrated *deg*U32(Hy); C: Effects of *deg*Qa and *deg*R in the chromosome and in the multicopied plasmids and coordinating with integrated *deg*U32(Hy).

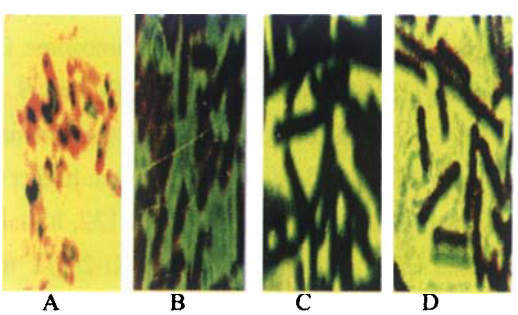

Fig. 2 Cell morphology and spore formation associated with Ki-2-132, *deg*U32(Hy)*deg*Qa, *deg*R and *deg*U32(Hy)(p*deg*Qa), (p*deg*R)

A: Ki-2-132, wild-type, with spore; B: Ki-2-132*deg*U32(Hy), without spore; C: *deg*U32(Hy)*deg*Qa similar to *deg*U32(Hy) *deg*R, without spore; D: *deg*U32(Hy)(p*deg*Qa) similar mutation, *deg*U32(Hy)(p*deg*R), without spore.

2.4 Effects on protease fermentation

The protease fermentation associated with the propagation of Ki-2-132*deg*Qa, Ki-2-132(p*deg*Qa), Ki-2-132*deg*U32(Hy)*deg*R, Ki-2-132*deg*U32(Hy)*deg*Qa, Ki-2-132*deg*U32(Hy)(p*deg*Qa), Ki-2-132*deg*U32(Hy)(p*deg*R), Ki-2-132*deg*U32(Hy) and Ki-2-132 were further investigated by cultivating these strains in LB and LBG liquid media containing 0.5% glucose at 37℃ with shaking. The cell density and protease activity were measured at OD_{600} and OD_{440}, respectively, after 24-hour cultivation. It was found that the cell densities associated with Ki-2-132*deg*U32(Hy)*deg*Qa, *deg*R derivatives grown in LBG were higher than those grown in LB liquid, but slightly lower than the cell density of Ki-2-132*deg*U32(Hy) grown either in LB or in LBG, suggesting an inhibiting effect of *deg*Qa and *deg*R genes on Ki-2-132*deg*U32(Hy) cell growth, while the cell densities associated with Ki-2-132*deg*U32(Hy) *deg*Qa, *deg*R derivatives can be improved by adding 0.5% glucose (Table 2, Group 1). However, the protease–producing capacity associated

Table 2 Effects of *deg*U32(Hy), *deg*Q and *deg*R on protease production and growth of *B. subtilis* Ki-2-132

Strains /Co-ordinating patterns	Antibiotic	Glucose 0.5%	$OD_{600\,nm}$ cell density	$OD_{440\,nm}$ protease	$OD_{440\,nm}/OD_{600\,nm}$
Ki-2-132*deg*U32(Hy)	–	+	13.20	0.699	0.053
Ki-2-132*deg*U32(Hy)	–	–	3.60	1.76	0.490
Ki-2-132*deg*U32(Hy)*deg*R	–	+	3.42	0.87	0.254
Ki-2-132*deg*U32(Hy)*deg*R	–	–	2.70	0.26	0.096
Ki-2-132*deg*U32(Hy)*deg*Qa	–	+	3.47	0.89	0.256
Ki-2-132*deg*U32(Hy)*deg*Qa	–	–	2.40	0.34	0.140
Ki-2-132*deg*U32(Hy)(p*deg*R)	Cm	+	8.55	0.47	0.055
Ki-2-132*deg*U32(Hy)(p*deg*R)	Cm	–	3.90	0.34	0.087
Ki-2-132*deg*U32(Hy)(p*deg*Qa)	Cm	+	10.08	0.61	0.061
Ki-2-132*deg*U32(Hy)(p*deg*Qa)	Cm	–	2.55	0.34	0.133
Ki-2-132	–	+	9.90	0.800	0.081
Ki-2-132	–	–	7.05	0.349	0.050
Ki-2-132*deg*Qa	–	+	6.80	1.117	0.164
Ki-2-132*deg*Qa	–	–	6.15	0.306	0.050
Ki-2-132p*deg*Qa	–	+	5.68	0.977	0.172
Ki-2-132p*deg*Qa	–	–	2.05	0.373	0.182
Ki-2-132(p*KP*1)	–	+	10.35	0.200	0.019
Ki-2-132(p*KP*1)	–	–	2.63	0.232	0.088
Ki-2-132(p*KP*1)	Km	–	5.86	12.62	2.154
Ki-2-132*deg*Qa (p*KP*1)	–	+	7.00	1.003	0.143
Ki-2-132*deg*Qa(p*KP*1)	–	–	2.50	0.221	0.089
Ki-2-132*deg*U32(Hy)(p*KP*1)	Km	–	1.60	6.16	3.850

Notes: "–" and "+" stand for without adding and adding the corresponding components, respectively. "Cm" stands for chloramphenicol and "Km" stands for Kanamycin. (The averaged data were used in the table, due to the filamentous growth; the sizes of colonies can not be distinguished for *deg*U32(Hy) and its *deg*Qa, *deg*R combinations); The data from three measurements including big, middle and small sized colonies were collected and averaged for *deg*Qa and p*deg*Qa strains. The data from at least three independent measurements including big, middle and small sized colonies were collected and averaged for plasmid p*KP*1-containing strains. Ki-2-132 can produce big, middle and small sized colonies during propagation for unknown reasons.

with these strains were similar to that of Ki-2-132 (Table 2), indicating that the combination of the genes promoted the protease production while inhibited the cell density. With regard to the morphological variations associated with *deg*U32(Hy) and its *deg*R and *deg*Qa derivatives in *B. subtilis* Ki-2-132 on LBM plates (Fig. 1 A, B, and C), the alterations of colony growth of *deg*U32(Hy) and *deg*R in *B. subtilis* Ki-2-132 chromosome and in multicopied plasmid were indistinguishable, but *deg*Qa increased the protease production either in chromosome or in multicopied plasmid increased the protease production (Table 2), suggesting that DegQa and DegU32(Hy) may work in different routes in promoting protease production. DegR in *deg*U32(Hy) background cannot play a similar role in most situations in our test, except that it was in the chromosome and when cells were cultivated in the LBG medium (Table 2). As can be seen in Table 2, combination of *deg*U32(Hy) with *deg*R either in chromosome or in multicopied plasmid in cytoplasm affected its protease production in significantly when cultivated in LB medium. The alteration of cell density instead of the protease-producing capacity in Ki-2-132*deg*U32(Hy) *deg*R and Ki-2-132*deg*U32(Hy) (p*deg*R) in LB medium may be suggestive of an epistatic effect between these two genes. Indeed it has been reported that DegU positively stimulates the expression of ComK, and ComK in turn negatively regulates *deg*R expression [8,10]. Therefore, a lower DegR level in Ki-2-132*deg*U32(Hy) can be expected, which in the condition of this work should be compensated by introducing multicopied *deg*R in Ki-2-132*deg*U32(Hy)(p*deg*R). However, as the *deg*U32 (Hy) already produced DegU-phosphate form, it thus became futile for further promotion the protease productivity by creating an elevated DegR through its stabilising the DegU-p[11]. Interestingly, increased protease-producing capacity in *B. subtilis* Ki-2-132*deg*Qa can also be seen when Ki-2-132*deg*Qa was cultivated in LBG medium (with supplement of 0.5% glucose), which was 3.28-fold of that cultivated in LB as indicated by OD_{440}/OD_{600} (Table 2). However, when cultivated in LB medium, the protease productivity of *B.subtilis* Ki-2-132*deg*Qa was similar to that of Ki-2-132 as indicated by the OD_{440}/OD_{600} (Table 2), suggesting that glucose supplement was actually helpful to its protease production. Supplement of glucose during Ki-2-132(p*deg*Qa) propagation did not significantly increase its protease productivity. As indicated by the value of OD_{440}/OD_{600}, the protease-producing capacities associated with the Ki-2-132(p*deg*Qa) were similar to each other, either in LB or in LBG medium, suggesting that the *deg*Qa promotion on protease production was also dependent on DegQa-dosage dependent. Similarly, we have found that a significant increase in protease production (10 to 20-fold) can be associated with Ki-2-132*deg*Qa strain when it was propagating in an industrial medium, in which maize powder served as carbon source, while in the same medium, Ki-2-132(p*deg*Qa) did not show any significant increase in protease production (data not shown).

These findings suggested that the regulation of *deg*Qa gene expression in Ki-2-132 was also carbon source dependent, while the effects of *deg*Qa on protease productivity were dependent on the DegQa dosage. It has been reported that the expression of *deg*Q can be increased under the condition of nitrogen starvation. The glucose dependent enhancement on protease production as seen in Ki-2-132*deg*Qa, however, might be a reflection of the presence of an unbalanced ratio of Nitrogen/Carbon in LBG, which is consistent with observations that DegQ responds to environmental signals such as the limitations of nitrogen, carbon, or phosphate sources, and the stringent factor RelA is also being involved in the protease production[1,16,17].

2. 5 Expression of alkaline protease in *B. subtilis* Ki-2-132*deg*U32(Hy) and *B. subtilis* Ki-2-132*deg*Qa

The effects of *deg*U32(Hy) in *B. subtilis* Ki-2-132 can be further amplified by introducing *B. amyloliquefaciens deg*Qa gene in a carbon source and a gene dosage dependent manner, while *deg*R gene of *B. subtilis* 168 mainly affected the cell growth *B. subtilis* Ki-2-132. Its was known that *deg*U32(Hy), *deg*Qa and *deg*R genes in *B. subtilis* Ki-2-132 worked in the post-exponential growth stage, which if altered either by mutations or by elevated expressions affected cell proliferation (Fig.3), suggesting that transition from the exponential to the

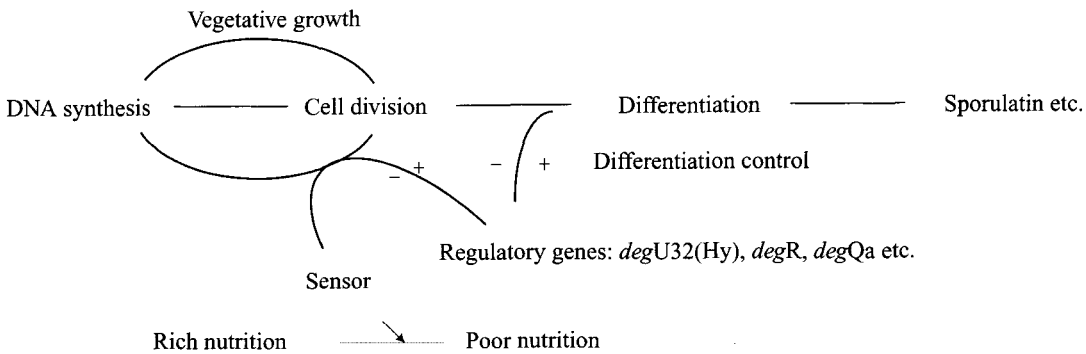

Fig. 3 A possible model of *deg*U32(Hy), *deg*Qa and *deg*R regulatory genes on the growth and diffrentation of *B. subtilis* Ki-2-132

stationary growth phase of *B. subtilis* Ki-2-132 might be interfered by the introduction of *deg*U32(Hy), *deg*R or *deg*Qa, which may be beneficial to the expression of degradative enzymes, such as *apr*E, by an "extended period of time" when compared to wildtype Ki-2-132. This idea was tested by the introduction of plasmid p*KP*1 carrying *apr*E gene into Ki-2-132*deg*Qa, Ki-2-132 *deg*U32(Hy) and Ki-2-132, respectively. The cell density and the protease-producing were measured in these strains after 24-hour cultivation (Table 2). As can be seen in Table 2, the protease production of Ki-2-132*deg*Qa (p*KP*1) was 7.5-fold higher than that of Ki-2-132 (p*KP*1), when they were cultivated in LBG under the same conditions, but no significant difference can be seen between the Ki-2-132*deg*Qa (p*KP*1) and Ki-2-132 (p*KP*1) in LB medium. Similarly, the protease production associated with Ki-2-132*deg*U32 (Hy)(p*KP*1) propagation was 1.789-fold of that associated with Ki-2-132 (p*KP*1) under the same conditions.

3 Discussion

B. subtilis Ki-2-132 is a bacillus strain differed to *B. subtilis* 168 in aspects of higher resistance to phage infection in liquid medium; higher transformation frequency and r^-m^- etc, and showed potentials in industrial application[13]. Indeed this strain has already been used to produce some commercially important proteins, such as EGF etc. However, the molecular genetics of *B. subtilis* Ki-2-132 has not been thoroughly developed when compare to those of *B. subtilis* 168 and other bacillus strains. This work reported here showing that the effects of *deg*U32 (Hy), *deg*R genes from *B. subtilis* 168 and *deg*Qa gene from *B. amyloliquefaciens* on *B. subtilis* Ki-2-132 were also pleiotropic, such as the altered cell growth -filamentous growth, lack of sporulation, and the altered protease fermentation etc., and suggesting that they were also presented in *B. subtilis* Ki-2-132; more interestingly, the results of this research seemed to be suggestive to an idea that the pleitropic effects of abovementioned genes in *B. subtilis* Ki-2-132 may somehow be able to offer a physiological environment for an "extended" expression of degradative enzyme (Fig.3), which in this regard implicates an interfered growth and development in stationary phase in bacillus may be commercially useful in developing genetically engineered system for hypyer-exprssion of some proteins.

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多效调控基因 *deg*U32(Hy)，*deg*Qa 和 *deg*R 对枯草芽孢杆菌 Ki-2-132 生长和蛋白酶发酵的影响

潘学峰 [1,2]

1. 中国科学院遗传与发育生物学研究所，北京 100101；

2. 北京理工大学，北京 100081

摘 要：通过向枯草芽孢杆菌 Ki-2-132 染色体和/或细胞质导入来自枯草杆菌 168 菌株的 *deg*U32 (Hy)和 *deg*R 基因，以及来自芽孢杆菌解淀粉菌株（*Bacillus amyloliquefaciens*）的 *deg*Qa 基因，对上述基因对枯草芽孢杆菌 Ki-2-132 细胞的生长、孢子发生、蛋白酶发酵的影响进行了研究。尽管上述多效调控基因来自不同的芽孢杆菌种和菌株，它们在枯草芽孢杆菌 Ki-2-132 中依然表现多效性。枯草杆菌 Ki-2-132 *deg*U32 (Hy)表现出增高了的蛋白酶产量；当和质粒或染色体上的 *deg*Qa 基因协作，可以进一步依赖葡萄糖的水平和 *deg*Qa 的基因剂量影响细胞生长，增加蛋白酶产量，以及影响孢子的形成。与此不同，*deg*R 在 *deg*U32 (Hy) 突变体中并不显著影响其蛋白酶的产量，这一发现支持 DegR 蛋白通常稳定磷酸化的 DegU，而其在 *deg*U32 (Hy) 菌株中不再进一步放大该突变体内已被磷酸化的 DegU 的调控作用。

关键词：*deg*U32 (Hy); *deg*Qa; *deg*R；多效调控基因；枯草芽孢杆菌

作者简介：潘学峰，男，中国国籍，英国永久居民。微生物学与生物化学学士、遗传学硕士、生物化学博士，研究生导师，研究方向：重大疾病的分子基础。E-mail: xuefengpancam@yahoo.com.cn