

Development of a Free-living Nematode *Panagrellus redivivus* in *Saccharomyces cerevisiae* with *cip* Genes

YOU Juan¹ HUANG Jian-lin² CAO Li³ HAN Ri-chou^{3*}

(1 Department of Biochemistry & Molecular Biology, Guangdong Pharmaceutical University, Guangzhou 510006, China)

(2 Guangzhou Institute of Metrology & Testing Technology, Guangzhou 510030, China)

(3 Guangdong Entomological Institute, Guangzhou 510260, China)

Abstract CipA and CipB are two types of intracellular crystalline inclusion proteins produced by *Photobacterium luminescens* bacteria, which are symbionts of entomopathogenic *Heterorhabditis* nematodes. To understand the biological function of these proteins for free living *Panagrellus redivivus* nematodes, recombinant *Saccharomyces cerevisiae* expression system of Cip proteins were constructed and the resulting yeast cells were used to feed the sterile J1 juveniles of *P. redivivus*. In recombinant yeast cells with CipA and/or CipB, the nematodes developed about 24 h faster than those in the yeast cells without Cip proteins. This promotion was reflected in two aspects: to short the cycle time and to enhance the reproductive ability of *P. redivivus* nematode. It means that the nutrient sources from entomopathogenic nematodes are acceptable by this free-living nematode.

Key words Crystalline inclusion protein *Saccharomyces cerevisiae* *Panagrellus redivivus*

Panagrellus redivivus is a small, free-living (non-pathogenic) nematode in soil. As a suitable food source for first-feeding fish and crustacean, *P. redivivus* has been recommended to replace brine shrimp *Artemia salina*, the most commonly used living organisms. Due to its small size and elongated shape, *P. redivivus* can serve for small-mouthed fish larvae and larval penaeid shrimps^[1]. The nutritional value of *P. redivivus* is high and comparable to that of *A. salina*. Nematodes can survive for over 72 h in sea water and never grow too large to be consumed by shrimp larvae^[2-3]. How to get enough *P. redivivus* nematodes? A variety of microorganisms can be preyed by *P. redivivus*, such as *Escherichia coli*, *Saccharomyces*

cerevisiae. As *Saccharomyces cerevisiae* is recognized as being non-harmful and is available in commercial formulations, this yeast was regarded to be suitable as a food source for the mass production of *P. redivivus*^[4]. Large-scale production processes have been developed for *P. redivivus* both in liquid culture^[5] and in solid culture^[6]. However, no commercial products are available in the markets.

Phase I cells of *Photobacterium luminescens* bacterium, symbiotic with entomopathogenic *Heterorhabditis* nematode, produce two types of intracellular crystalline inclusion proteins designated CipA and CipB. These two crystal proteins, whose amino acid composition and content is pretty match the nutrient requirement of entomopathogenic nematodes, are believed to be involved in development and reproduction of their nematode symbiont^[7-8] and have

significant influence on the completion of *Steinernema* nematode’s lifecycle in liquid culture system [9].

In order to explore whether the Cip proteins can be used by *P. redivivus* nematode as nutrients, the *cipA* and *cipB* genes encoding CipA and CipB proteins from *P. luminescens* H06 were respectively expressed in *S. cerevisiae* and these recombinant cells were used to feed the J1 juveniles of *P. redivivus* nematodes. The present work is

expected to base a new technique for the mass multiplication of *P. redivivus* as promising live food for aquaculture in the further market.

1 Materials and methods

1.1 Strains and culture conditions

Strains and plasmids used in this study are described in Table 1.

Table 1 Strains and plasmids used in this study

Strain or plasmid	Relevant properties	Source
Bacterial strains		
<i>Escherichia coli</i>		
TOP10F’	Cloning strain	Invitrogen
OP50	General strain	
TOP10F’ / pYES2.1/V5-His-TOPO-cipA	TOP10F’ with pYES2.1/V5-His-TOPO-cipA; Amp ^r	This study
TOP10F’ / pYES2.1/V5-His-TOPO-cipB	TOP10 with pYES2.1/V5-His-TOPO-cipB; Amp ^r	This study
<i>Photorhabdus luminescens</i> H06	Strain H06, phase I variant	China
<i>Saccharomyces cerevisiae</i> INVSc1	Typical yeast host strain	
<i>Saccharomyces cerevisiae</i> INVSc1/pYES2.1/V5-His-TOPO-cipA	INVSc1 with pYES2.1/V5-His-TOPO-cipA	This study
<i>Saccharomyces cerevisiae</i> INVSc1/pYES2.1/V5-His-TOPO-cipB	INVSc1 with pYES2.1/V5-His-TOPO-cipB	This study
Plasmid vectors		
pCR4-TOPO-cipA	pCR TM 4-TOPO with 333bp insert containing <i>cipA</i> ; Amp ^r , Kan ^r	[9]
pCR4-TOPO-cipB	pCR TM 4-TOPO with 321bp insert containing <i>cipB</i> ; Amp ^r , Kan ^r	[9]
pYES2.1/V5-His-TOPO [®]	Shuttle and expression vector; Amp ^r	Invitrogen
pYES2.1/V5-His-TOPO-cipA	pYES2.1/V5-His-TOPO [®] with 315bp insert containing <i>cipA</i> ; Amp ^r	This study
pYES2.1/V5-His-TOPO-cipB	pYES2.1/V5-His-TOPO [®] with 303bp insert containing <i>cipB</i> ; Amp ^r	This study
Nematode strain		
<i>Panagrellus redivivus</i>	Free-living, non-pathogenic nematode	China

Bacterial colonies of *P. luminescens* H06 were obtained from the haemolymph of *Galleria mellonella* larvae 48 h after infection with *Heterorhabditis bacteriophora* H06. The primary form (phase I) of these bacterial isolates was obtained by selecting green or blue-green colonies on NBTA medium [10]. The bacteria were cultured in peptone-water broth (1% peptone, 0.5% NaCl) (PW broth) at 25°C on a shaker. Stock cultures were maintained in 15% glycerol (vol/vol) in PW broth at -80°C.

Escherichia coli TOP10F’ (Invitrogen Co., Carlsbad, USA) and OP50 strains were grown at 37°C in Luria-Bertani (LB) broth or on LB agar (1.5% Bacto-agar). 100 µg of ampicillin per ml (Sigma chemical Co., St. Louis, USA) were added to the media as required. *Saccharomyces cerevisiae* INVSc1 strain was provided kindly by General Hospital of Guangzhou Army District and inoculated in Yeast Extract Peptone Dextrose medium (1% yeast extract, 2% peptone, 2% D-glucose) (YPD

medium). All medium components used in this study were purchased from Oxoid Company (Basingstoke, UK).

Panagrellus redivivus was provided kindly by Institute of Biological Control, the Chinese Academy of Sciences. Feeding on *E. coli* OP50, this nematode grew and multiplied on Nematode Grow Medium [0.3% NaCl, 0.25% peptone, 1.7% agar, 1mmol/L CaCl₂, 2µg/ml Uracil, 5µg/ml Cholesterol, 25mmol/L KPO₄ buffer (pH 6.0), 1mmol/L MgSO₄] (NGM) plates at room temperature.

1.2 Construction and expression of recombinant Cip proteins

Restriction enzymes (Promega) were used according to the manufacturer’s instructions. Plasmids were extracted from *E. coli* with QIAprep Miniprep kit (Qiagen Co., Hilden, German). When required, DNA fragments were extracted and purified from agarose gels using SpinPrep Gel DNA kit (Novagen, San Diego, USA).

The *cipA* and *cipB* genes of *P. luminescens* H06 were amplified respectively by PCR from the cloning vectors pCR4-TOPO-*cipA* and pCR4-TOPO-*cipB* constructed previously^[9]. The products were purified and ligated into pYES2.1/V5-His-TOPO[®] vector (Invitrogen) to yield pYES2.1/V5-His-TOPO-*cipA* and pYES2.1/V5-His-TOPO-*cipB*, and transformed into *E. coli* strain TOP10F', respectively. The positive clones were analyzed by PCR with the appropriate primers and restrict digestion with *Bam*H I and *Xba* I. All the primers used in this study were listed in table 2. The recombinant plasmids with the correct inserts were purified and transformed into *S. cerevisiae* INVSc1. The transformants were selected on SC-U (SC minimal media lacking uracil) selective plates and maintained in SC-U medium containing 2% glucose (Amresco Inc., Solon, USA).

The yeast recombinants were inoculated in SC-U medium (2% glucose), induced in SC-U medium (2% galactose) according to the protocols in the manual of pYES2.1 TOPO[®] TA expression kit (Invitrogen). The occurrence and accumulation of recombinant Cip proteins during culturing were examined with Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunodetection with polyclonal CipA or CipB antiserum prepared by You et al^[9].

Table 2 Amplification primers used in this study

Primer	Sequence
<i>cipA</i> -for	5'-CCT CATATGATGATTAACGACATGCATC-3'
<i>cipA</i> -rev	5'-GTC GGATCCTTACATAGAGATTTCAC-3'
<i>cipB</i> -for	5'-CGC CATATGATGATTATTAAGAAAGACA-3'
<i>cipB</i> -rev	5'-CTA GGATCCTTAAATTTCAACACCTAC-3'
GAL1 for	5'-AATATACCTCTATACTTTAACGTC-3'
V5C-term rev	5'-ACCGAGGAGAGGGTTAGGGAT-3'

1.3 Gel electrophoresis and Western blot analyses

SDS-PAGE was performed to analyze the concentrated cell lysate with the upright electrophoresis system (Liuyi Co. Beijing, China) according to Laemmli et al^[11]. For electrophoresis assay, yeast cell lysates were prepared using acid-washed glass beads. In brief, frozen cells were washed and suspended in breaking buffer (10mmol/L NaCl, 20mmol/L Tris, 1mmol/L EDTA, 0.5% SDS, pH8.0) to obtain an *OD*₆₀₀ (Optical Density) value of 100 and mixed with an equal volume of acid-washed glass

beads. The mixture was vortexed for 30 sec, followed by 30 sec on ice. Cells would be lysated mostly after repeating this six times. After centrifuged at maximum speed, the pellet was resuspended in 50 ~ 100 μl deionized distilled H₂O and mixed with the same volume of gel-loading buffer. The samples were boiled for 3 min before running 10 μl per lane on SDS-15% PAGE. The gels were stained with Coomassie Brilliant Blue R-250. 10 μl of protein molecular weight markers (Tiangen Co., Beijing, China) per lane were used.

Western blotting was performed by electrophoretically transferring proteins from polyacrylamide gels to 0.2 μm-pore-size polyvinylidene fluoride (PVDF) membranes (Immobilon-P[®], Millipore, Bedford, USA) with a tank blotter (Liuyi). The immunodetection procedure was performed as previously described^[9].

1.4 Bioassay of the nematode development

To assay the nematode development on different yeast cultures in liquid cultures, axenic *P. redivivus* J1 juveniles were prepared with a modified method according to Lunau et al^[12]. Briefly, the gravid female were collected and rinsed in sterile M9 buffer (0.6% Na₂HPO₄, 0.3% KH₂PO₄, 0.5% NaCl, 0.025% MgSO₄ · 7H₂O). After surface-sterilized for 30 min, the body of the female was dissected to collect the eggs. After rinsing twice in sterile M9 buffer, these eggs were then placed on 24-well cell plates (Corning, New York, USA) in sterile NGM medium and checked for their axenicity after two days. If no contaminants were present in the cell plates, the J1 larvae were used for the following biological assay.

To exclude any influences from the extracellular products, the yeast cells were obtained by centrifugation, washed and resuspended in sterile NGM medium. As the indicator for the population density, the *OD*₆₀₀ value of *S. cerevisiae* cells suspended in sterile NGM medium was measured. Before applied to biological assay, the suspensions of different yeast cells were diluted to the *OD*₆₀₀ value of 2.5 with the sterile NGM medium to make the same cell density.

For biological assay in liquid media, 250μl yeast cell suspension of INVSc1/pYES2.1/V5-His-TOPO-*cipA*, INVSc1/pYES2.1/V5-His-TOPO-*cipB* and INVSc1

strains (see Table 1) were added into 24-well cell plates , respectively. And 20 ~ 40 axenic J1 nematodes were placed in each well. Sterile NGM medium was used as the control. Three replicates were established for each recombination. The cell plates were shaken slowly at room temperature. Growth and reproduction of nematodes in the cell plate wells was observed every day.

1.5 Statistical analysis

Data presented as percentages were normalized using arcsine square root transformation and analyzed by ANOVA.

2 Results and analysis

2.1 Construction of recombinant yeasts with *cip*

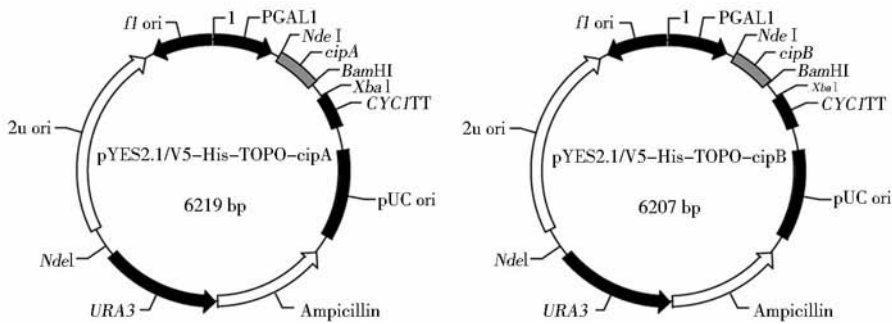


Fig. 1 Physical map of pYES2.1/V5-His-TOPO-cip vectors

For the foreign inserts ligate into the T vector in two directions, the probability to obtain positive clones is 50% . The transformants were prescreened by colony PCR, before made a tube culture to extract recombinant plasmids. digest analysis and PCR with multiple primer pairs further confirmed that *cip* genes had inserted into pYES2.1/V5-His-TOPO vector in the correct direction. (Fig.2 and Fig.3) Then, the confirmed plasmids were transformed into INVSc1 cells and screened on Uracil atrophic selection plates. Colony PCR analysis identified that pYES2.1/V5-His-TOPO-cipA (B) plasmids had accepted by the host INVSc1 cell, respectively. (Fig.4)

2.2 Expression of Cip proteins in recombinant INVSc1 cells

Induced in SC-U (2% galactose) over 4 h , recombinant cells can express CipA or CipB protein visualized in the map of SDS-15% PAGE. A prominent band of about 11 kDa was visualized in the induced cell

genes

Before amplified by PCR , the *cipA* and *cipB* fragments in the vectors pCR4-TOPO-cipA and pCR4-TOPO-cipB have been verified by DNA sequence determination [8] .

pYES2.1/V5-His-TOPO[®] plasmid used in this study is an episomal *E. coli* -*S. cerevisiae* shuttle plasmid. The *cip* sequences, ligated with linearized pYES2.1/V5-His-TOPO[®] vector, included an ATG initiation codon and the native stop codon (Fig.1) . So the native-size Cip proteins would be expressed in *S. cerevisiae*. The resulting vectors, pYES2.1/V5-His-TOPO-cipA and pYES2.1/V5-His-TOPO-cipB, were transformed into *E. coli* cells to multiply, respectively.

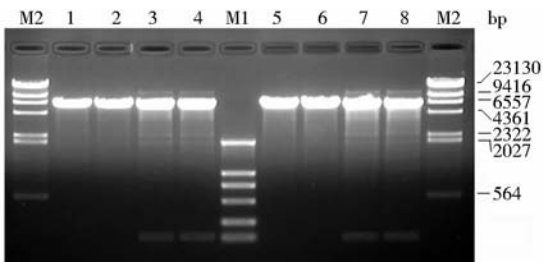


Fig. 2 Restriction endonuclease analysis of recombinant shuttle plasmid pYES2.1/V5-His-TOPO-cip

M1;DL 2000 DNA Marker M2. λ /Hind III digest DNA marker;1,2; pYES2.1/V5-His-TOPO-cipA digested with *Bam*H I;3, 4;pYES2.1/V5-His-TOPO-cipA digested with *Bam*H I + *Xba* I;5, 6;pYES2.1/V5-His-TOPO-cipB digested with *Bam*H I;7, 8;pYES2.1/V5-His-TOPO-cipB digested with *Bam*H I + *Xba* I

lysates, but not found in uninduced cultures (Fig. 5 A). The molecular weight size of the expressed proteins matched with those estimated. Western blot analyses of *P. luminescens* H06 and different INVSc1 cell lysates probed

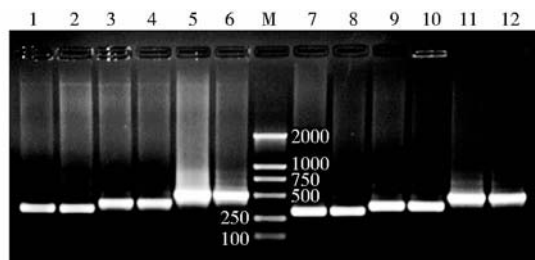


Fig. 3 PCR analysis of recombinant shuttle plasmid pYES2.1/V5-His-TOPO-cip

M:DL 2000 DNA Marker(bp); 1~6:PCR analysis of recombinant plasmid pYES2.1/V5-His-TOPO-cipA; 1, 2: Amplified fragments with the primers of cipA-for/rev (333bp); 3, 4: Amplified fragment with the primers of cipA-for/V5C-term rev (393bp); 5, 6: Amplified fragment with the primers of *GAL1* for/V5C-term rev (492bp); 7~12: PCR analysis of recombinant plasmid pYES2.1/V5-His-TOPO-cipB; 7, 8: Amplified fragment with the primers of cipB-for/rev (321bp); 9, 10: Amplified fragment with the primers of cipB-for/V5C-term rev (381bp); 11, 12: Amplified fragment with the primers of *GAL1* for/V5C-term rev (480bp)

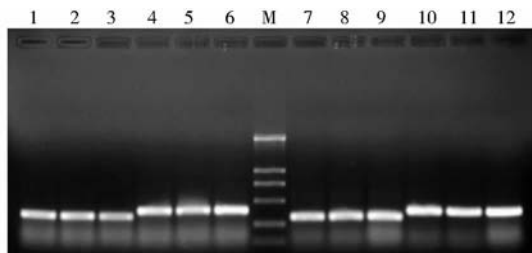


Fig. 4 Identification of INVSc1 recombinant colonies

1~6:PCR analysis of colonies with pYES2.1/V5-His-TOPO-cipA; 1, 2, 3: Amplified fragments with the cipA-for/rev primers (333bp); 4, 5, 6: Amplified fragments with the cipA-for/V5C-term rev primers (393bp); 7~12: PCR analysis of colonies with pYES2.1/V5-His-TOPO-cipB; 7, 8, 9: Amplified fragments with the cipB-for/rev primers (321bp); 10, 11, 12: Amplified fragments with the cipB-for/V5C-term rev primers (381bp)

with CipA or CipB antiserum showed strong combination with the target protein (Fig. 5 B). Induced for 8 h, two kinds of Cip proteins achieved the highest level of expression in INVSc1 cells. And to prolong induction time made not more production. So when required, the recombinant INVSc1 cells induced for 8 h were used in biological assay.

2.3 Biological assay

Preliminary experiments showed that sterile NGM

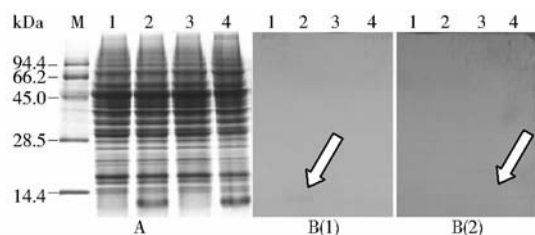


Fig. 5 SDS-PAGE and Western blot analysis of the induced expression products

A:SDS-PAGE analyses (15% gel) of whole-cell lysates; B: Western blot analyses of the same samples with either CipA (B1) or CipB (B2) polyclonal antiserum. Lanes are the same as in the panel A. M: low molecular weight protein standards; 1: INVSc1/pYES2.1/V5-His-TOPO-cipA uninduced; 2: INVSc1/pYES2.1/V5-His-TOPO-cipA induced with galactose; 3: INVSc1/pYES2.1/V5-His-TOPO-cipB uninduced; 4: INVSc1/pYES2.1/V5-His-TOPO-cipB induced with galactose

liquid culture can supply a comfort circumstance but necessary nutrition for *P. redivivus* nematode and *S. cerevisiae* cells. That is, J1 nematodes and yeast cells can live but not develop or multiply in this culture. The yeast cells suspended in NGM made a stable density, only in the case of the feeding from nematodes. For these reasons, sterile NGM culture was used as the background and the control in the following biological assay.

The mixtures of recombinant and native yeast cells in different proportions were performed to determine the effects of expressed Cip proteins on the development of these free-living nematodes. *P. redivivus* nematodes fed the recombinant and native yeast cells non-selectively. After several days, all the cells in the cultures were consumed up. It was observed that only supplied with the yeast cells expressing CipA and/or CipB proteins, the sterile J1 nematodes couldn't complete their lifecycle. After one fifth of the recombinant yeast cells were replaced by the same density of the native yeast cells, the nematodes feeding the mixed yeast cells showed better growth than those feeding the native yeast cells in all the aspects.

In the NGM liquid with different *S. cerevisiae* cells, all J1 juveniles of *P. redivivus* nematodes finally developed into next productive adults and completed their lifecycle. But the develop rate & state of nematodes made obvious

differences between culture combinations.

In biological experiment, the four major stages of nematode development were paid more attention. Those are Juvenile Stage, Adult Stage, Gravid Stage and the Next J1 Stage.

Feeding on yeast cells in several nematode-yeast culture combinations, the target J1 nematodes developed through the above four stages at different rates. The culture time when 5% and 80% of nematodes in each combination reached at some stage are considered as the start point and peak point of this stage in this combination, respectively. The two key points reflected the development velocity of target nematodes in each culture combination.

In the blank control of sterile NGM liquid, the target nematodes kept their sizes and J1 stage to die off after three days.

From the beginning, it seemed that all the J1 nematodes in different yeast cells developed at equal pace to enter Juvenile 2 ~ 4 Stage. After that, significant differences appeared between nematode-yeast culture combinations. In Comb. 1, 3 and 5, with yeast cells expressed CipA and/or CipB proteins, almost all of the target nematodes developed into the adults for a 72-h culture. After about 24 h, the number of the gravid females with 65 ~ 85 eggs reached the peak, and next generation of J1 juveniles was crawling out from the large females after a day. (Fig. 6 and Fig. 7)

However, in Comb. 2, 4, 6 and 7, in the yeast cells without CipA or CipB protein, a few of gravid females with 47 ~ 55 eggs began to appear for 96-h culture (Fig. 6 and Fig. 11). To feed on recombinant yeast cells without Cip proteins expressed (Comb. 2, 4, 6), or on native yeast cells (Comb. 7), the *P. redivivus* nematodes showed no distinct difference of development, and delayed for about 24 h than those feeding on yeast cells with Cip proteins expressed at each stage. (Fig. 6 and Fig. 7)

More than 90% of the nematodes could develop to the adult stage in the combinations with Cip proteins expressed. While in those combinations without Cip, 70% ~ 75% of the total nematodes reached this stage. Except of those in the sterile NGM, there were a very small population that failed to grow up. (Fig. 8)

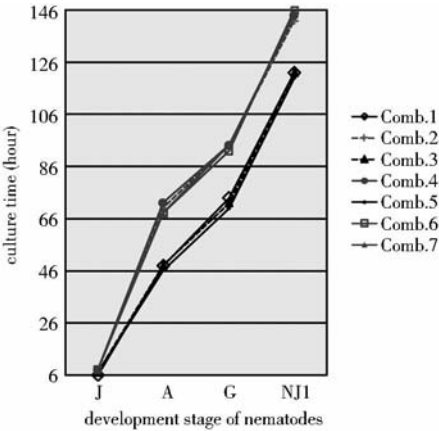


Fig. 6 Occurrence time of each nematode stage in different *P. redivivus*-*S. cerevisiae* culture combinations

Abbreviations; Comb; combination; J; juvenile (stage 2 ~ 4); J1; juvenile stage 1; A; adult; G; gravid; NJ1; the next juvenile stage 1 1; *P. redivivus* J1 + INVSc1/pYES2. 1/V5-His-TOPO-cipA cells induced; 2; *P. redivivus* J1 + INVSc1/pYES2. 1/V5-His-TOPO-cipA cells uninduced; 3; *P. redivivus* J1 + INVSc1/pYES2. 1/V5-His-TOPO-cipB cells induced; 4; *P. redivivus* J1 + INVSc1/pYES2. 1/V5-His-TOPO-cipB cells uninduced; 5; *P. redivivus* J1 + the mixture of INVSc1/pYES2. 1/V5-His-TOPO-cipA cells induced and INVSc1/pYES2. 1/V5-His-TOPO-cipB cells induced; 6; *P. redivivus* J1 + the mixture of INVSc1/pYES2. 1/V5-His-TOPO-cipA cells uninduced and INVSc1/pYES2. 1/V5-His-TOPO-cipB cells uninduced; 7; *P. redivivus* J1 + INVSc1 cells

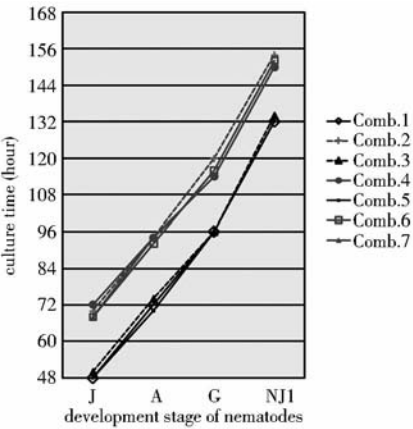


Fig. 7 Peak time of each nematode stage in different *P. redivivus*-*S. cerevisiae* culture combinations

Abbreviations & numbers represent the same as in Fig. 6

A few nematodes died in all the culture combinations. Low mortalities of the adult were kept in the test

combinations with active adults. In the yeast cells producing CipA, only 5.6% of the adult couldn't go through this stage. And the most death came from Comb. 7, with native INVSc1 cells, not more than 16.6% (Fig. 9).

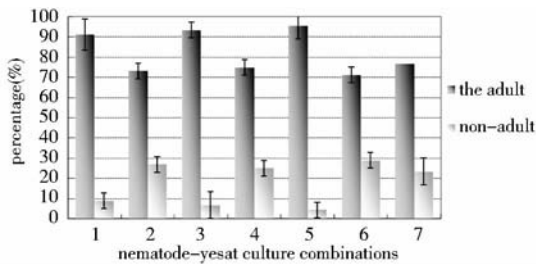


Fig. 8 Development of the J1 nematodes in different *P. redivivus*-*S. cerevisiae* culture combinations

Numbers represent the same as in Fig. 6

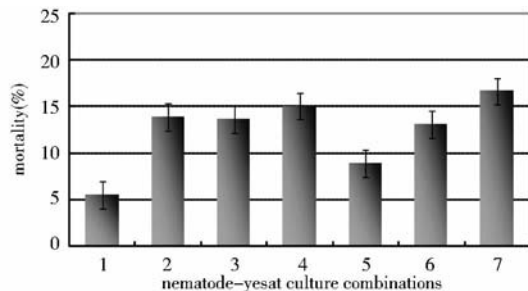


Fig. 9 Mortalities of adult nematodes in different *P. redivivus*-*S. cerevisiae* culture combinations

Numbers represent the same as in Fig. 6

In general, above 50% of the female could be gravid with average more than 40 eggs. Either feeding on recombinant or native yeast cells, the female made a high gravid rate of over 55%. But distinct difference appeared between combinations with and without Cip proteins. In Combination 1, 3 and 5, with yeast cells expressed CipA and/or CipB proteins, over 75% of the female had conceived with 65 ~ 90 eggs inside (Fig. 10 and Fig. 11). To contrast clearly, the gravid rates in the combinations without Cip proteins were less than 65%. (Fig. 10)

Besides the gravid rate, the amount of eggs carried by the gravid also reflects the reproductive capacity of the target nematodes. In the combinations with Cip proteins, every gravid nematode made over 65 eggs. Especially in the combination with CipB, there were about 85 eggs in one gravid female. However, in the cultures without Cip

proteins, the average of eggs fell to 45 ~ 55. (Fig. 11)

According to the gravid female and eggs in different *P. redivivus*-*S. cerevisiae* culture combinations, Cip proteins made a better reproductive ability of nematodes.

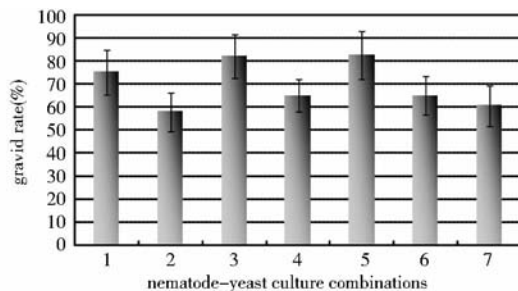


Fig. 10 Gravid rate of the female nematodes in different *P. redivivus*-*S. cerevisiae* culture combinations

Numbers represent the same as in Fig. 6

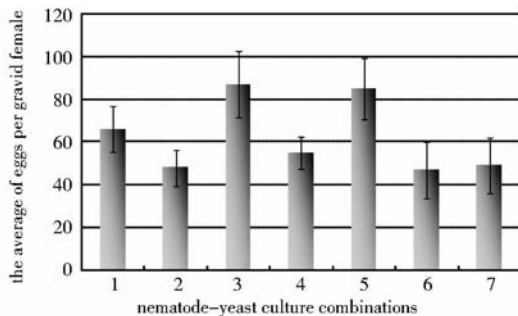


Fig. 11 Quantity of eggs inside the gravid in different *P. redivivus*-*S. cerevisiae* culture combinations

Numbers represent the same as in Fig. 6

3 Discussion

The huge increases in world aquaculture industry require not only the development of specific culture techniques, but also in most cases the production and use of live organisms as feed for the developing larvae [6].

Entomopathogenic *Steinernema* and *Heterorhabditis* nematodes depend on the presence of their symbiotic bacteria. As the specific production of the bacteria, crystalline inclusion proteins which represent about 40% of the total cell proteins play an important role in the development of the nematodes [7].

As a suitable food source for first-feeding fish, free-living nematode *P. redivivus* requires rich and specific nutrition according to the specific needs of the fish species.

The development of a technology for the mass production and formulation of *P. redivivus* is a big step towards a cheap, standardized and permanently available live food product and thereby for the further development of sustainable aquaculture. To explore the possible nutrient functions of Cip proteins for this free-living nematode, recombinant *S. cerevisiae* expression systems of CipA and CipB were utilized for *P. redivivus* nematodes.

To exclude the interruption from other metabolites of yeast and make a general survey of the whole development, we harvested the cells cultured and induced when required, washed and resuspended them in sterile NGM liquid before applied them in biological assay. To J1 nematodes and yeast cells through the whole experiment, NGM made a suitable liquid circumstance but no food. It's an ideal background and control. During the nematode-yeast co-culture, we noticed a very few deaths of the adult. The low mortalities of the adult nematodes in all the culture combinations may be caused by the background liquid of sterile NGM. Even confirmed to be harmless, NGM can't supply the same comfort surroundings to nematodes as the cell inoculums.

Surprisingly, it is difficult to culture sterile J1 of *P. redivivus* nematodes only by the yeast cells expressed Cip proteins. It seems that the intracellular metabolites from the recombinant yeast cells are incomplete nutrients for the development of J1 nematodes. However, when the recombinant yeast cells are combined with the native yeast cells, the nutrients are better than those only from the native yeast cells. This promotion was reflected in two aspects: to short the cycle time and to enhance the reproductive ability of *P. redivivus* nematode. It seems that although some growth factors are absent from the recombinant yeast cells, these cells are much richer in some important growth factors than the native cells. As a unique product of bacterium symbiotic with Entomopathogenic nematode, Cip proteins supply especial nutrition not only for the symbiotic nematodes, but also for other nematode strains, such as the free-living nematode, *Panagrellus redivivus*.

Yeast cells serve as a kind of safe and high-quality food for *P. redivivus* nematodes. Carried by the yeast

cells, Cip proteins are also acceptable by this free-living nematode. The present study indicated the feasibility of utilizing other sources and improving the food quality of this nematode by genetic manipulation.

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携带 *cip* 基因的酿酒酵母对自由生活线虫 *Panagrellus redivivus* 生长繁殖的影响

游 娟¹ 黄建林² 曹 莉³ 韩日畴^{3*}

(1 广东药学院生物化学与分子生物学系 广州 510006 2 广州市计量检测技术研究院 广州 510030)

(3 广东省昆虫研究所 广州 510260)

摘要 *Photorhabdus luminescens* 细菌与昆虫病原异小杆属 *Heterorhabditis* 线虫专性共生。初生型共生细菌产生两种胞内晶体蛋白 CipA and CipB, 为共生线虫提供营养。为探索 Cip 蛋白是否对自由生活的全齿复活线虫 *Panagrellus redivivus* 具有类似的营养功能, 建立了 Cip 蛋白的重组酿酒酵母表达体系, 并用于饲喂无菌的 *P. redivivus* 线虫 J1 幼虫。重组酿酒酵母表达的 Cip 蛋白能为线虫所利用, 表现为营养支持作用, 体现为线虫生长发育速度的加快以及繁殖能力的提高, 说明 Cip 蛋白能为此种自由生活线虫提供营养来源。

关键词 胞内晶体蛋白 酿酒酵母 全齿复活线虫

中图分类号 Q819