

## GC% of DNA of Pathogenic *Cryptococcus* and Varieties

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**Abstract:** The guanine and cytosine content of some pathogenic species and varieties of *Cryptococcus* was reported here as determined by a thermal denaturation technique. The results range from 47.82 to 61.98, which are similar to those in the literature. This study indicated that different species of *Cryptococcus* and different varieties of *C. neoformans* seem to be reflected in their G+C mol% content values. Especially the differentiation of GC% of both *C. neoformans* var. *Shanghaiensis* and *C. neoformans* var. *gatti* were over the range of variation that permitted in this species (1-4%). The determination of G+C mol% content can raise the level of taxonomy of *Cryptococcus* from morphology to nucleotide molecule, and the value is not affected by environment and etc. It is a standard of heredity for the classification and identification of fungi.

**Key words:** cryptococcus; cryptococcus neoformans variety; DNA G+Cmol%

The measurement of G+C% of DNA about *Cryptococcus* is an valuable index of taxonomy, could improved the level of taxonomy from morphology to nucleic acid, meanwhile, little effected by other growth factors, including environment. We studied extraction and purification of DNA of *Cryptococcus*, measured GC% by a thermal denaturation technique, a satisfied result we obtained.

### MATERIALS AND METHODS

#### 1. Source of isolates

All of isolates were reserved in our lab, meanwhile, re-identification was taken by auto microbiology system that produced by Mac Donnell Kouglass Cop, USA. It included *C.laurentii* RV30994, *C. neoformans* B2641, *C. neoformans* var. *Shanghaiensis* S8012, *C. neoformans* var. *gatti* B2643. *C. neoformans* var. *gatti* NIH444 that provided by NIH as control group, target *Cryptococcus* B-G45971 provided by microbiology of department of Jinan university, Gangzhou.

#### 2. Extraction DNA of isolates

##### 2.1 Reagent

(1) glusulase: produced by biology institute of Chinese Academy of Sciences. (2) D-glucitol. (3) 20% SLS solution. (4) 0.1M Tris-HCl buffer, pH9.3. (5) 0.5M EDTA solution, pH 9.0. (6) 10mM citric acid-dibasic sodium phosphate buffer, pH 5.8. (7) SE solution: 0.15M NaCl+0.1 M EDTA, pH8.0. (8) NaCl -citrate sodium (SSC): 0.15MNaCl+0.015M trisodium citrate, pH7.0.

##### 2.2 Extraction

First fungus were pretreated with compound, and then wet fungi/g+5ml citric acid-orthophosphoric acid buffer + 0.91g D-glucitol + 30mg glusulase, 37 °C water bath, shaking repeatedly, collected protoplasm after 60 min. Protoplasm was scrubbed with SE solution until become clear. Protoplasm/g+5ml SE solution+0.5ml SLS, water bathing 55 °C, keep temperature

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Wait 10 min and then cold it to room temperature. Using 5M perchloric acid dilute to the final concentration, 1M, added saturation phenol with equal volume, as well as 1/2 volume chloroform isoamyl alcohol, shaking 30min, 5000r/min centrifugate 10min, collected supernatant. And further made deproteinization, and remove RNA, precipitation of DNA with isopropanol, solute in SSC solution, keep 5? .

The principle of GC% of measurement of thermal denaturation and calculation and Tm of DNA thermal denaturation: see reference<sup>[1]</sup>.

## RESULTS

Purity of DNA: both samples of DNA and natural purifying one were measured during 260nm, 230nm, 280nm respectively to analyze ratio of absorbance. Table 1

Thermal analysis of samples DNA: primitive data were in table 2, and draw curve of thermal denaturation (see Fig. 1, *C. neoformans* var. *Shanghaiensis* as representative), calculate the Tm, empirical formula; calculate the GC%, Table 1.

## DISCUSSION

G+Cmol% is one of important heredity marks that reflect similarity of DNA sequence between two species indirectly. Along with development of molecular heredity and biochemistry, especially in nucleic acid research and taxonomy and identification of microbiology, the differentiation of GC% of DNA has already become the regular index about bacterial taxonomy, however, because fungi own cell wall, it was hard to extract DNA, so it faced challenge about measurement of GC%<sup>[2]</sup>. In China, it just has some reports about *Candida* and *T. rubidum*<sup>[3-6]</sup>. Using liquid nitrogen method to extract DNA of *Cryptococcus* and measured the data of GC% that had reported that outside of China in 1960s<sup>[7]</sup>.

Using glucanase to digest cell wall of *Cryptococcus*, it showed the total of consume less, work time shorter. SLS could split protoplast, make protein denaturated and precipitated, meanwhile, it also inhibit DNA enzyme. It could make DNA released from component of other cells when heating and shaking after add phenol and chloroform. Using our method, it got DNA above 600<sup>μ</sup>g/g, according to formula, 1.0OD(A260) double-stranded (ds) nucleic acid=50<sup>μ</sup>g/m<sup>[8]</sup>. The capsule of *Cryptococcus* contains plenty of polyose, it is difficult to purify DNA. OD (A230) was obviously higher than OD (A260) in the DNA solution that contains polyose. Using SE liquor to scrub protoplast reputedly, it is not until solution become clear; it was match with isopropanol and showed an effect effectively.

The standard strains from NIH as the control group; we found that its result was in concord with one of related documents that have been reported<sup>[4]</sup>. The range of variation about GC% of genus *Cryptococcus* was also very close to that (49-65%) have reported in the past<sup>[7]</sup>. The differentiation of GC% of both *C. neoformans* var. *Shanghaiensis* and *C. neoformans* var. *gatti* were above range of variation that permitted in this species (1-4%). The data of GC% that released from targeted *Cryptococcus* in our study was close to one of *C. neoformans* var. *gatti*. So our study showed that data of GC% were different among different species of genus *Cryptococcus* and different *C. neoformans* var. *neoformans*, this suggested that measurement of GC% of *Cryptococcus* owned significance in taxonomy. Using this thermal denaturation technique to measure GC% of DNA of fungi, it has evidenced that this method could be better worked repeatedly, as well as handle easily.

## REFERENCES

1. Lin WM. Measurement of GC% of DNA of Bacterium by thermal temperature. *Micobiology Correspondence*. 1981, 8: 245.
2. Bicanic T, Wood R, Bekker LG, et al. Antiretroviral roll-out, antifungal roll-back: access to treatment for cryptococcal meningitis. *Lancet Infect Dis*. 2005, 5(9): 530-531.
3. Yao Z, Liao W, Chen R. Management of cryptococcosis in non-HIV-related patients. *Med Mycol*. 2005, 43(3): 245-251.

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4. Aulakh HS. Genetic relatedness of *filobasidiella neoformans* (*Cryptococcus neoformans*) and *filobasidiella bacillispora* (*Cryptococcus bacillisporus*) as determined by deoxyribonucleic acid base composition and sequence homology studies. *Int J Syst Bact.* 1981, 31: 97.
5. Lin H. DNA G+C% of seven strains of *Candida* species. *Chinese Journal of Dermatology.* 1988, 21: 299.
6. Teng CY. Measurement of GC% of DNA of *T. rubum* by thermal temperature. *Academic Journal of Chinese Medical Academy.* 1989, 11: 313.
7. Storck R. Nucleotide composition of deoxyribonucleic acid of some species of *cryptococcus*, *rhodotorula*, and *sporobolomyces*. *J Bact.* 1969, 98: 1069.
8. Davis. *Bacterial genetics.* Tianjin Science and Technology Press. 1984: 180.

**Table 1 Characteristics of DNA of different samples of *Cryptococcus***

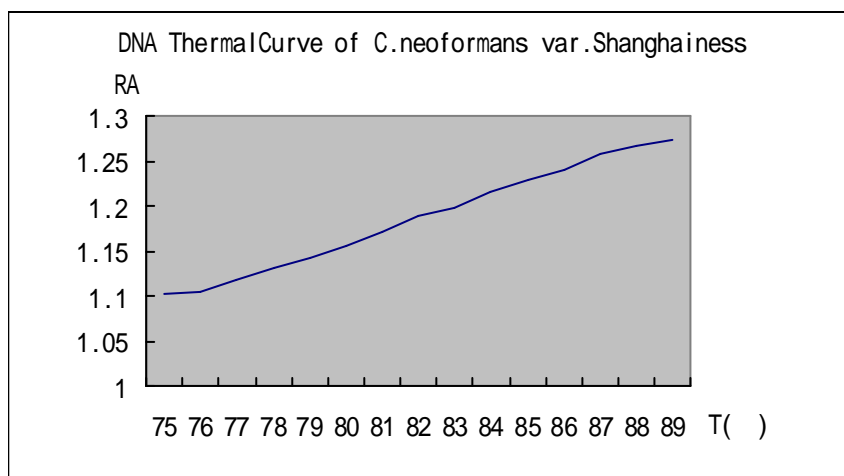
| Sample of DNA   | OD260:230:280   | Tm   | GC%     |
|---|-----------------|------|---------|
| Natural DNA   | 1.0:0.450:0.515 |      |         |
| <i>C. laurentii</i>                                       | 1.0:0.454:0.452 | 81.0 | 47.82   |
| <i>C. neoformans</i>                                      | 1.0:0.509:0.516 | 86.0 | 61.98   |
| <i>C. neoformans</i> var. <i>Shanghaiensis</i>            | 1.0:0.508:0.516 | 82.0 | 50.26   |
| <i>C. neoformans</i> var. <i>gatti</i>                    | 1.0:0.505:0.529 | 84.1 | 55.39   |
| target <i>Cryptococcus</i>                                | 1.0:0.466:0.483 | 84.4 | 56.12   |
| <i>C. neoformans</i> var. <i>gatti</i> NIH <sub>444</sub> | 1.0:0.449:0.478 | 84.9 | 57.34 * |

\* Data of document:  $57.2 \pm 0.2$  [2]

**Table 2 Ratio of thermal absorbance of *C. neoformans* var. *Shanghaiensis* under the condition of 260nm**

| Temperature( ) | Ratio of absorbance | Rectified ratio of absorbance * | Relative ratio of absorbance * * |
|----------------|---------------------|---------------------------------|----------------------------------|
| 25             | 0.376               | 0.376                           | 1.000                            |
| 75             | 0.405               | 0.414                           | 1.102                            |
| 76             | 0.406               | 0.416                           | 1.105                            |
| 77             | 0.411               | 0.421                           | 1.119                            |
| 78             | 0.415               | 0.425                           | 1.131                            |
| 79             | 0.419               | 0.430                           | 1.143                            |
| 80             | 0.424               | 0.435                           | 1.157                            |
| 81             | 0.429               | 0.440                           | 1.171                            |
| 82             | 0.435               | 0.447                           | 1.188                            |
| 83             | 0.438               | 0.450                           | 1.198                            |
| 84             | 0.444               | 0.457                           | 1.215                            |
| 85             | 0.449               | 0.462                           | 1.229                            |
| 86             | 0.453               | 0.467                           | 1.241                            |
| 87             | 0.459               | 0.473                           | 1.258                            |
| 88             | 0.462               | 0.477                           | 1.267                            |
| 89             | 0.464               | 0.479                           | 1.274                            |

\* Rectified ratio of absorbance (CA)= Ratio of absorbance  $\times$  relative volume ( $V_t/V_{25}$ ), \* \* relative Ratio of absorbance (RA) =  $CA_t/CA_{25}$



**Fig. 1 Thermal analysis of DNA of *C. neoformans* var. *Shanghaiensis***

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