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SHORT COMMUNICATIONS

A new species of marine yeast *Kluyveromyces penaeid* isolated from the heart of penaeid shrimp *Penaeus chinensis*

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A new species of marine yeast *Kluyveromyces penaeid* (Saccharomycetoideae) was isolated from the heart tissue of a subadult shrimp *Penaeus chinensis* during tissue culture. The yeast grew well in seawater supplemented with 2% shrimp extract, but did not grow in chemically defined media. The vegetative cells reproduced by multilateral budding and formed rudimentary pseudohyphae occasionally. Asci were spheroidal and evanescent containing 2–13 smooth or oval ascospores. The best temperature for the yeast to grow was 20–25°C and 37°C was lethal. The yeast grew well in half to full strength seawater supplemented with shrimp extract, but did not grow in 25% strength seawater. The carbohydrate fermentation test was positive, the diazonium blue B and urea hydrolysis tests were negative.

During tissue culture of a subadult penaeid shrimp *Penaeus chinensis* with the medium for penaeid shrimp (MPS; Tong & Miao, 1996), many unusual yeast cells migrated from heart tissue and formed colonies. The colony was surrounded by a thick capsule, from which numerous spines irradiated (Figure 1A). The colonies expanded rapidly and formed a monolayer on the substrate of the cell culture flask. There were no such yeast cells migrating from the other tissues of the same individual shrimp, such as the lymphoid, ovary and cerebral ganglion tissues. Because of the unusual morphology of the yeast colonies, a series of tests were carried out to determine its taxonomic position.

The media tested for the yeast were as follows:

(1) Natural seawater (SW) and seawater agar (SW agar). The seawater was collected from the coast of Qingdao City, China, and was sterilized at 121°C for 15 min. Seawater agar contained 2% agar in SW.

(2) Shrimp extract seawater (SESW) and SESW agar. SESW was prepared with SW, to which 2% of shrimp extract was added. In preparing shrimp extract, equal parts of fresh shrimp muscle and 2% NaCl solution (W/V) was mixed and sterilized at 121°C for 15 min. SESW agar contained 2% agar in SESW.

(3) Glucose-yeast extract-peptone waters (GYP water) and GYP agar. They were prepared according to the method of Walt & Yarrow (1984), but were supplemented with 2% NaCl to meet the osmolarity requirement of the yeast.

(4) Malt extract water (ME water) and ME agar were prepared according to Walt & Yarrow (1984), and were supplemented with 2% NaCl.

(5) Yeast morphology broth (YM broth) and YM agar (Difco) were supplemented with 2% NaCl.

In SW the yeast cells attached onto the substrate of cell culture flask. The cells were 2–3 μm in diameter and were surrounded by a thick capsule (Figure 1B). The yeast did not form colonies in the solid SW agar medium.

As the SW was replaced by GYP water, the attached cells grew vegetatively into giant, round, and capsulated cells with a diameter of 20–40 μm in 3–5 d at 20°C, and became suspended (Figure 1C). A small part of the cells (<1%) reproduced by multilateral budding with a wide isthmus between maternal and daughter cells (Figure 1D). Rudimentary pseudohypha with 3–5 cells were occasionally observed (Figure 1E). The yeast did not form colonies in the GYP agar.

In the SESW medium, the yeast cells attached onto the substrate of cell culture flask. Most cells were in the medium size range 5–15 μm and underwent active protoplasmic division which resembled to cell fission (Figure 1F). The asci formed were round, evanescent, with 2–13 smooth, spheroidal to ellipsoidal ascospores (Figure 1G). Cell conjugation was not observed. Inoculation of the yeast cells into the solid SESW agar resulted in white, round, butyrous colony formation. Photomicroscopical examination of the cells in the colony did not show essential differences from those in the SESW.

The yeast did not grow in chemically defined media YM broth and YM agar, and hence the sugars and nitrate assimilation tests as well as the response to vitamin absence were not performed.

The fermentation of carbohydrates was carried out in the SESW medium with large Durham tubes (50×6 mm) at 20°C according to the procedure of Walt et al. (1984). The carbohydrates tested were D-glucose, D-galactose, maltose, α-methyl-D-glucoside, sucrose, trehalose, melibose, lactose, cellobiose, melezitose, raffinose, inulin, starch, and D-xylose. The positive fermentation only occurred on D-glucose and D-galactose.

Diazonium blue B (DBB) and urea hydrolysis tests of the yeast were negative according to the methods of Walt et al. (1984) and Barnett et al. (1990).

The best temperature range for the yeast growth was 20–25°C (Figure 2A). The growth rate declined at 30°C and the temperature of 37°C was lethal for the yeast. At 15°C the yeast grew very slowly but could survive for three months.

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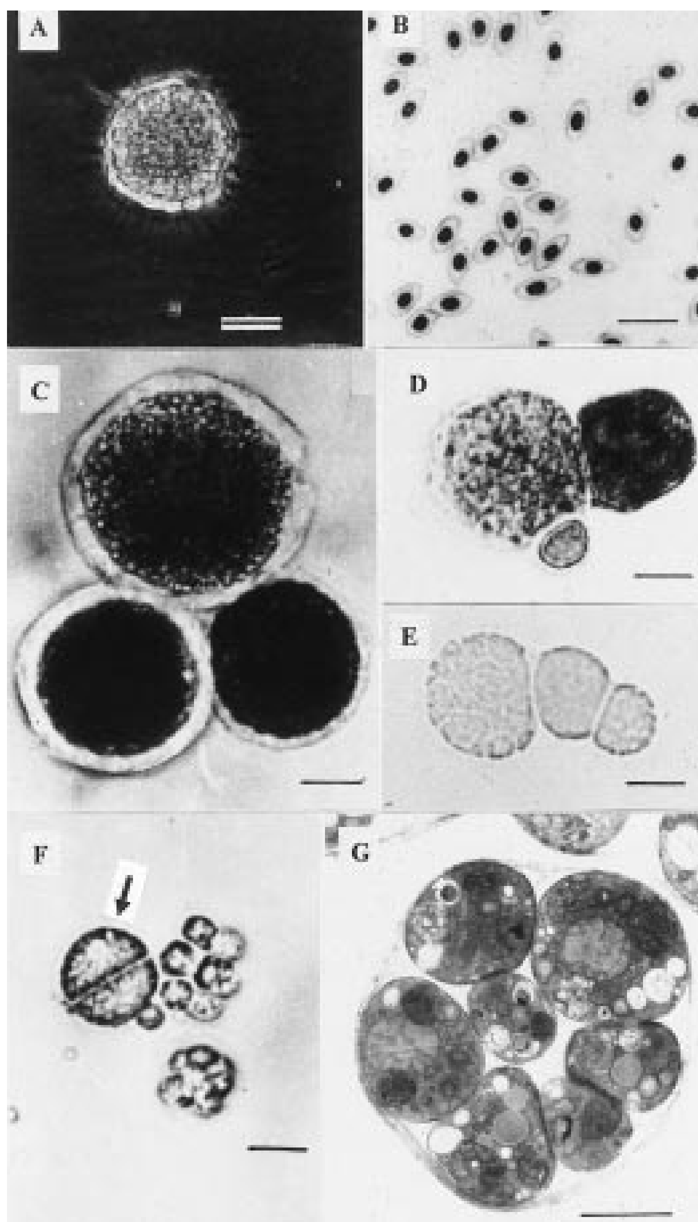

Figure 1. The yeast cells grown in different media exhibited different morphologies and behaviour. (A) Phase contrast micrograph of a yeast colony formed in MPS medium. The colony was surrounded by a thick capsule, from which numerous spines irradiated. (B) Phase contrast micrograph of yeast cells in seawater. The cells attached onto the substrate of the cell culture flask and were surrounded by a thick capsule. (C–E) Phase contrast micrograph of the yeast cells in GYP medium. The cells grew vegetatively into giant cells (20–40 μm) and became suspended (C); cells were budding multilaterally with wide isthmus between maternal and daughter cells (D) and formed pseudohypha with three cells (E). (F) Phase contrast micrograph of the yeast cells in SESW medium. The protoplast of one cell was dividing (arrow) in forming ascospores. (G) Transmission electromicrograph of an ascus which contained seven round to oval, smooth ascospores. Scale bars: A, 80 μm; B, 20 μm; C–F, 10 μm; G, 2 μm.

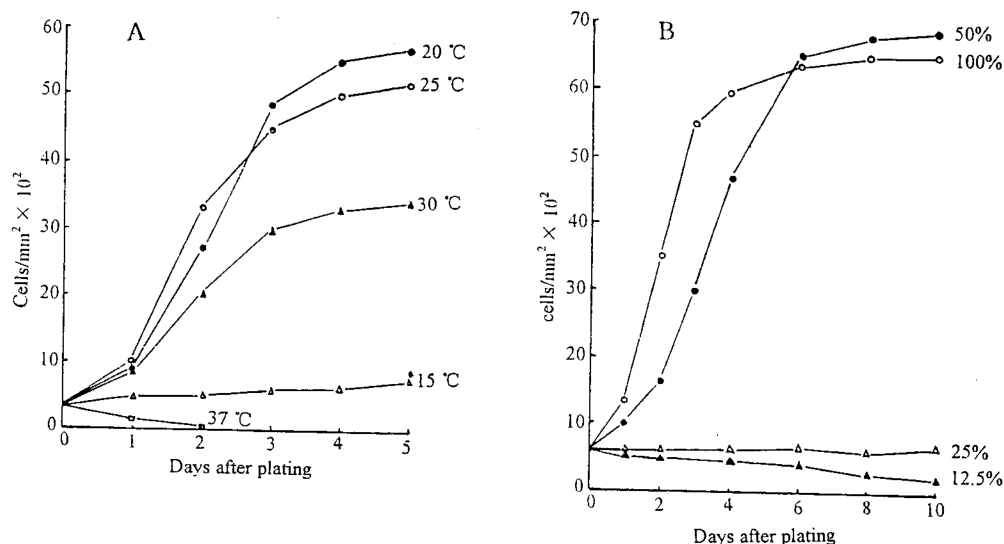

Figure 2. Growth responses of the yeast in SESW medium to selected temperature (A) and salinity at 20°C (B). The salinity was expressed as percentage of SW.

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The yeast grew well in 50–100% strength seawater supplemented with 2% shrimp extract, but did not grow in 25% or less strength seawater (Figure 2B).

The main features of the yeast fit the criteria of the genus *Kluyveromyces*. The general characteristics of the genus were: vegetative cells reproduced by multilateral budding; pseudohyphae existed sometimes; asci were evanescent with 1–60 smooth, round, oval or reniform ascospores; fermentation was positive; nitrate assimilation, urea hydrolysis and DBB tests were negative (Barnett et al., 1990; Kurtzman, 1988). There were 15 species in this genus. Among which 14 species were terrestrial, only one species, *K. aestuarii* was from seawater and other marine substrates (Barnett et al., 1990; van der Walt, 1970). The yeast reported here did not grow in fresh and low salinity brackish water, which suggested that the yeast was obligate or indigenous marine (Kohlmeyer et al., 1979). However, the yeast was different from *K. aestuarii* in several aspects. For instance, *K. aestuarii* contained 1–4 ascospores in each ascus; the isthmus between maternal and daughter cells was narrow; fermentation occurred on D-glucose and sucrose; whereas the yeast reported here contained 2–13 ascospores in each ascus; had a wide isthmus; fermentation occurred on D-glucose and D-galactose. Furthermore, *K. aestuarii* could grow in chemically defined media, but the new species did not. It would be adequate to name the new species as *K. penaeid* in considering its original isolation.

The new species of yeast was more closely related to *K. blattae*, a terrestrial yeast isolated from the intestinal tract of cockroach (*Blatta orientalis*) in regarding to the cell morphology, ascospores number in each ascus, and fermentation response (Barnett et al., 1990).

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