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REPORT

A new insect cell line from the longicorn beetle *Plagionotus christophi* (Coleoptera: Cerambycidae)

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Abstract We have established a continuous cell line from the fat body tissue of the longicorn beetle *Plagionotus christophi*. The cells have been serially subcultured in MGM450 medium, and the line has been designated as PC-1. The cells were grown in suspension and comprise largely flattened spindle- or oval-shaped cells morphologically related to blood cells of longicorn beetles. The chromosome number ranged from 19 to 36, with a mode of 20 (diploid). The growth curve was determined at the 100th passage, and the population doubling time was calculated to be 3.79 d. Isozyme analysis of malic enzyme, phosphoglucose isomerase, and phosphoglucomutase revealed that PC-1 cells were enzymatically distinct from coleopteran XP-1, AnCu-35, dipteran AeAl-2, and lepidopteran GaMe-LF1 cell lines.

Keywords Cell line · Coleoptera · *Plagionotus christophi*

Insect cell lines have contributed to the progress in physiological studies on the species from which they are derived. They have also proved useful for the production of recombinant proteins and for propagation of specific pathogens of interest. However, a lack of understanding of the fundamental mechanisms controlling the efficient generation of insect cell lines has limited the number of lines that have been generated to date. In fact, a majority of the over 500 insect cell lines reported so far are derived from lepidopteran and dipteran insects (Lynn 2001). In contrast, only 22 cell lines have been established from eight species of coleopteran insects belonging to the families Scarabedidae (Crawford 1982; Mitsuhashi 1989; Fernon et al. 1996; Mitsuhashi 2003), Chrysomelidae (Lynn and Stoppleworth 1984; Dübendorfer and Liebig 1992; Lynn 1995; Charpentier et al. 2002; Long et al. 2002), Curculionidae (Barcenas et al. 1989; Stiles et al. 1992), and Cerambycidae (Iwabuchi 1999). Taking into consideration the higher degree of species divergence within the order Coleoptera (Hunt et al. 2007), more coleopteran cell lines should be established to enable study of insects in this order. Longicorn beetles, which belong to the family Cerambycidae, are a major phytophagous coleopteran insects and include many common arboreal pests. XP-1 is the sole existing cell line established from a longicorn beetle, *Xylotrechus pyrrhoderus*. XP-1 cells respond to two major insect hormones, ecdysone and juvenile hormone, which is likely related to its blood cell origin (Iwabuchi 1999; Hoshino et al. 2004). Therefore, the generation of an additional established cell line from a longicorn beetle should contribute to both insect physiology and biological control by providing a new model system for both basic studies and assays to identify new pathological agents. Here, we report the establishment of a novel cell line derived from the fat body tissue of the longicorn beetle *Plagionotus christophi*.

P. christophi occurs sporadically and causes damage to logs used for the cultivation of shiitake mushrooms. We collected *P. christophi* larvae from under the bark of the trunk of a fallen oak tree in Yamanashi prefecture, Japan. The final instar larva weighed approximately 300 mg and was used for primary culture. The insect was surface-sterilized by immersing three times in 70% ethanol for 3 min and was subsequently dried on sterile filter paper for a few minutes. A piece of the fat body tissue was isolated from the dorsally dissected larva. The tissue was washed three times with MGM-450 medium containing 10% fetal

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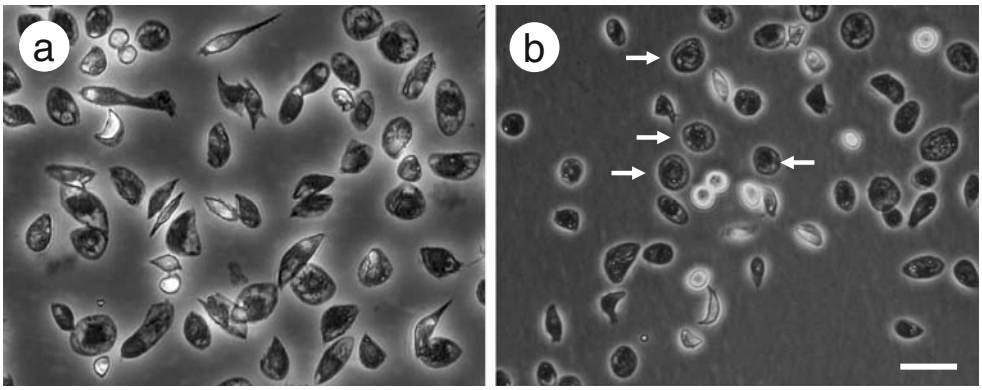

Figure 1. Phase-contrast micrographs. (*a*) PC-1 cells and (*b*) blood cells of the longicorn beetle *X. pyrrhoderus*, both grown in MGM 450 medium. *Arrows* indicate cells with a morphology similar to that of PC-1 cells. Both micrographs are at the same magnification. *Bar*=50 μm.

bovine serum (Mitsuhashi and Inoue 1988) and then transferred to 2 ml of the culture medium in a 35-mm plastic culture petri dish (3801, Falcon, Oxnard, CA). The culture was sealed with Parafilm (American National Can, Greenwich, CT) and maintained at 25°C. We observed both migration and division of cells soon after the initiation of the primary culture, which reached confluency within 2 wk. We subcultured the cells 20 d after initiation of the primary culture by transferal of the cells to a 12.5 cm^2 culture flask (3018, Falcon). Thereafter, cells were subcultured at intervals of approximately 2 wk with a split ratio of approximately 1:2 to 1:3. This cell line has been stably maintained without any appearance of a crisis period and has now been subcultured for more than 130 passages over a period of 5 yr. Moreover, we have been able to store this cell line in 10% glycerol (WAKO, Osaka, Japan) or 12% dimethylsulphoxide (Sigma-Aldrich, St. Louis, MO) in MGM-450 medium for at least 3 yr at −80°C. This cell line has been designated PC-1 after the species of origin *P. christophi*.

We examined the morphology of the PC-1 cells by phase-contrast microscopy (Leica DM IRB). A majority of the cells exhibited a flattened spindle or oval shape, but did not adhere to the culture surface and as such were reminiscent of the blood cells of longicorn beetles (Fig. 1). As in the case of XP-1 cell line, which is derived from fat body tissue and has the character of blood cells, PC-1 will be identified as blood cell line from fat body tissue. Additionally, PC-1 cells responded to the insect hormones, 20-hydroxyecdysone and juvenile hormone, by assuming a floating, elongated spindle shape and causing a progressive fragmentation, respectively (Hoshino and Iwabuchi, unpublished). The response of the PC-1 cells to these hormones probably reflect the response of coleopteran blood cells to specific hormones during metamorphosis, as judged by analysis of intact blood cells (Hoshino et al. 2004). We next performed a chromosome analysis of the cells at the 34th passage as described previously (Iwabuchi 2000). The chromosome number ranged from 19 to 36, with a mode of 20 (Fig. 2*a*). Thus, the cells are predicted to be diploid, since the chromosome number is consistent with that of diploid cells in the closely related species *X. pyrrhoderus* (Iwabuchi 1999). Accordingly, PC-1 cells appear to largely comprise diploid cells, since the chromosome number ranges between 19 and 21 in 86.7% of the

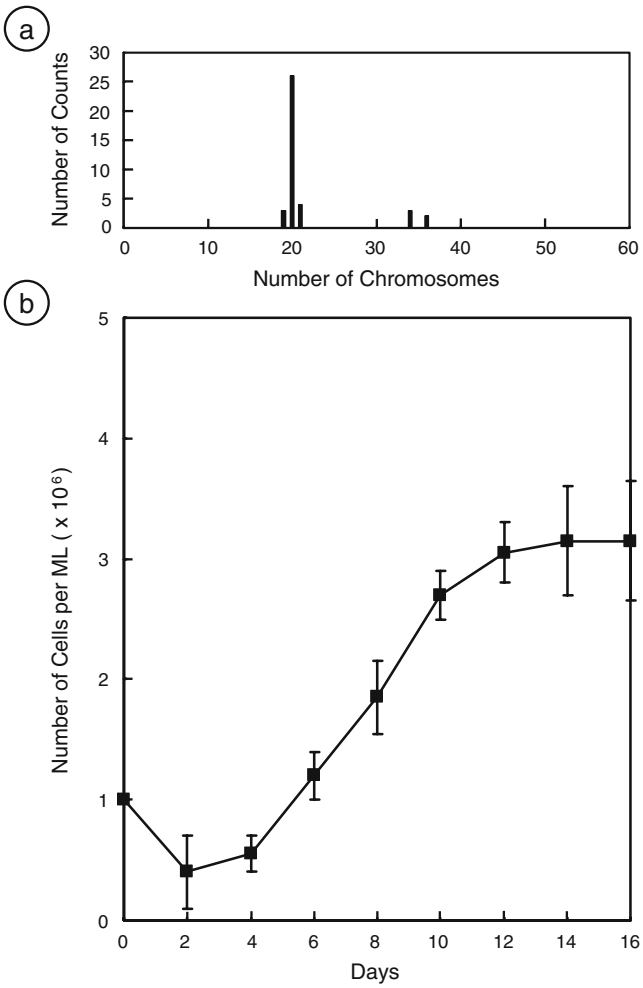

Figure 2. (*a*) Histogram of chromosome numbers in PC-1 cells at the 34th passage. (*b*) Growth curve of PC-1 cells at the 100th passage. Each *point* is the mean of three replicate cultures. *Bars* represent one standard deviation.

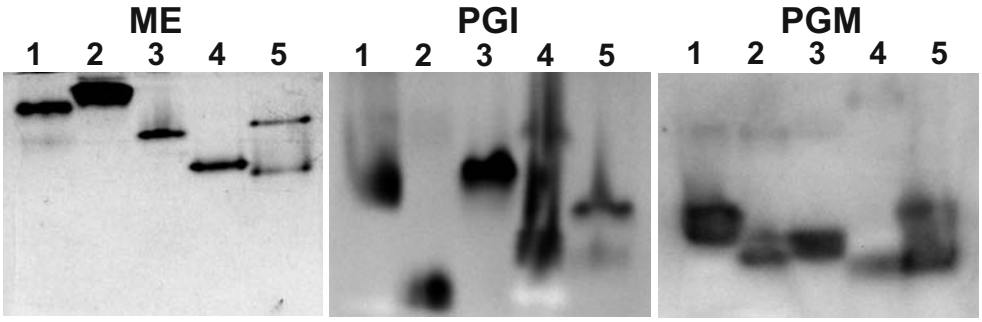

Figure 3. Images from developed electropherograms of three isozymes, malic enzyme (*ME*), phosphoglucose isomerase (*PGI*), and phosphoglucomutase (*PGM*), present in cell lysates prepared from five cell lines: *lane 1* PC-1 cells; *lane 2* XP-1 cells derived from the fat bodies of *X. pyrrhoderus*; *lane 3* AnCu-35 cells derived from embryos of *Anomala cuprea*; *lane 4* AeAl-2 cells derived from neonate larvae of *A. albopictus*; and *lane 5* GaMe-LF1 cells derived from the fat bodies of *G. mellonella*.

cells. The growth curve of PC-1 cells at the 100th passage was subsequently calculated from cell density data measured at 2 d intervals. The cell density was initially set at 1×10^6 cells/ml and reached 3.15×10^6 cells/ml at the plateau phase. The growth of PC-1 cells was slow exhibiting a distinct lag phase and a stable plateau phase (Fig. 2*b*). The population doubling time was calculated to be 3.79 d during the exponential phase. These results suggest that PC-1 cells possess growth characteristics similar to those of normal rather than transformed cells. Thus, taken together, we conclude that PC-1 cells exhibit morphological and physiological characteristics of blood cells.

An isozyme analysis was conducted to provide further support for the idea that PC-1 cells represent a novel cell line. Cell lysates prepared from samples of PC-1 cells and cells from four other cell lines were electrophoresed through a 7.5% polyacrylamide gel using the electrode buffer system described by Steiner and Joslyn (1979). The activity of specific enzymes was subsequently developed in situ in the gel by exposing the gel to chromogenic substrates. We evaluated the electrophoretic patterns of three isozymes, malic enzyme, phosphoglucose isomerase, and phosphoglucomutase, in lysates derived from PC-1 cells and compared these to the patterns of these enzymes in lysates from two coleopteran cell lines (XP-1 from *X. pyrrhoderus*, Iwabuchi 1999; and AnCu-35 from *Anomala cuprea*, Mitsuhashi 2003), a dipteran cell line (AeAl-2 from *Aedes albopictus*, Mitsuhashi 1981), and a lepidopteran cell line (GaMe-LF1 from *Galleria mellonella*, Eguchi and Iwabuchi 2006). This analysis revealed that PC-1 cells were enzymatically distinct from the other cell lines examined, including XP-1 cells derived from the closely related species, *X. pyrrhoderus* (Fig. 3).

In conclusion, we have established a new insect cell line, PC-1, from the coleopteran insect *P. christophi*. The morphology of these cells, which is similar to that of coleopteran blood cells, is different from the morphology of dipteran and lepidopteran blood cell lines, mbn-2 and EA-1174, respectively (Gateff 1997; Wittwer et al. 1997). In addition, PC-1 cells appear to be continuous cell line but also exhibit characteristics associated with normal cells, suggesting that PC-1 cells may be suitable for both physiological and pathological studies as a model of coleopteran blood cells. Further analysis of the insect hormone responsiveness and immune reactivity in PC-1 cells should contribute to our understanding of the physiology of coleopteran insects.

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