



EFFECT OF THE MAGNETIC FIELD ON THE BIOLOGICAL CLOCK IN *PENICILLIUM CLAVIFORME*

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Cultures of *Penicillium claviforme* were grown in a magnetic field (60–70 mT) for 12 days under constant light or constant darkness. In light, the magnetic field affected the length of the rhythm period, which was at first somewhat shortened and later prolonged (24 h → 24 h → 36 h → 36 h). In darkness, activation of the biological clock was manifested in the rhythm of the formation of coremia rings. The period of induced rhythm was different from that induced in light, representing a multiple of the 24 h period (96 h → 96 h → 96 h). The magnetic field distinctly affected the morphology of the newly formed coremia, which were identical in light and in darkness. In the applied conditions the magnetic field replaced the inductive effect of light, activating the expression of the biological clock in darkness. This is the first time that a magnetic field has been used to replace the inductive effect of light.

Key words: *Penicillium claviforme*, magnetic field (static), biological clock.

INTRODUCTION

Among the earth's complex of conditions affecting the life and development of populations is the sphere of electromagnetic interactions extending from the magnetosphere to the centrosphere. Living organisms have electrical and magnetic characteristics, but it is difficult to present them in biochemical terms. Electromagnetic interactions influence living organisms, which may modify their structures and processes under the influence of electromagnetic waves. Recent years have brought increased interest in the action of the electromagnetic field upon living organisms, especially animals as well as man (e.g., Ramchand et al., 2001). Only a few studies deal with the influence of the magnetic field on plants or microorganisms (Pittman, 1963; Pittman and Ormrod, 1970; Celestino et al., 1998; Garcia Reina and Pascual, 2001; Zhang et al., 2002). The biological clock, a characteristic feature of all living organisms, both eukaryotes and prokaryotes (Bün-

ning, 1953; Aschoff, 1965; Sweeney, 1969; Feldman and Dunlap, 1983; Tan-Chi-Huang et al., 1990; Jerebzoﬀ and Jerebzoﬀ-Quintin, 1992; Piskorz-Bińczycka, 1995), is an interesting subject for study of the operation of the magnetic or electromagnetic field. Investigations of the physiology and functioning of the biological clock in *Penicillium claviforme* (Bińczycka-Piskorz, 1987) suggest that it may be a good model for this kind of investigation.

The functioning of the biological clock as expressed in endogenous rhythmic sporulation by some representatives of the section *Penicillium clavigerum* has been described (Piskorz-Bińczycka, 1995). *P. claviforme* belongs to the same section. The endogenous rhythm is induced by light and manifested in the formation of coremia rings. The rhythm is non-circadian, has a period of 30 h, and does not function in darkness.

The action of electromagnetism is a complex phenomenon to study. It is convenient to start with

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one of its components, magnetism. The present investigation initiates a series of studies on the functioning of the biological clock under the action of a magnetic field. We examined the effect of a static magnetic field on the biological clock in *Penicillium claviforme*.

MATERIALS AND METHODS

The investigations used a strain of *Penicillium claviforme* Bainier No. 126–28 obtained from the Centraalbureau voor Schimmelcultures in Baarn, Holland.

The fungi were grown on Piskorz's medium (Piskorz, 1967) modified for investigation of rhythm (Piskorz-Bińczycka and Jerebzoﬀ, 1987). The source of carbon was 2% glucose (w/v). Ammonium nitrate was the source of mineral nitrogen. The pH (6.5) was adjusted with Sorensen's buffer (96.7 mmole $\text{Na}_2\text{HPO}_4 \text{ dm}^{-3}$). The medium was autoclaved for 30 min under 0.1 MPa pressure at 120°C. The sterile medium was poured into 12 cm dia. Petri dishes, 14 ml medium per dish.

The dishes with medium were inoculated using discs of 36 h old mycelia, 4 mm dia., placed in the center of the dishes (Bińczycka, and Jerebzoﬀ 1987).

The cultures were placed in darkness for 24 h, then grown in light or dark thermostats at constant 22°C. Continuous white light was supplied by fluorescent lamps (25 W, $1.5 \text{ W}\cdot\text{m}^{-2}$). Irradiance was measured with an LI-1800 spectroradiometer (LICOR, U.S.A.).

A static magnetic field (60–70 mT) was generated with permanent plate magnets; their arrangement is shown in Figure 1. The controls were cultured under identical conditions, under light and in darkness, without the magnetic field.

ANALYSIS OF RHYTHM

The period of the sporulation rhythm was calculated as in earlier investigations (Piskorz-Bińczycka, and Jerebzoﬀ 1987), applying the formula:

$$\tau = \frac{24 \cdot b}{v}$$

where: v – growth rate (mm/24 h); b – distance between successive zonations; τ – length of rhythm period in hours.

The results presented in the tables are means of three replicates. For morphology measurements, 10 coremia were cut from each of ten dishes. Meas-

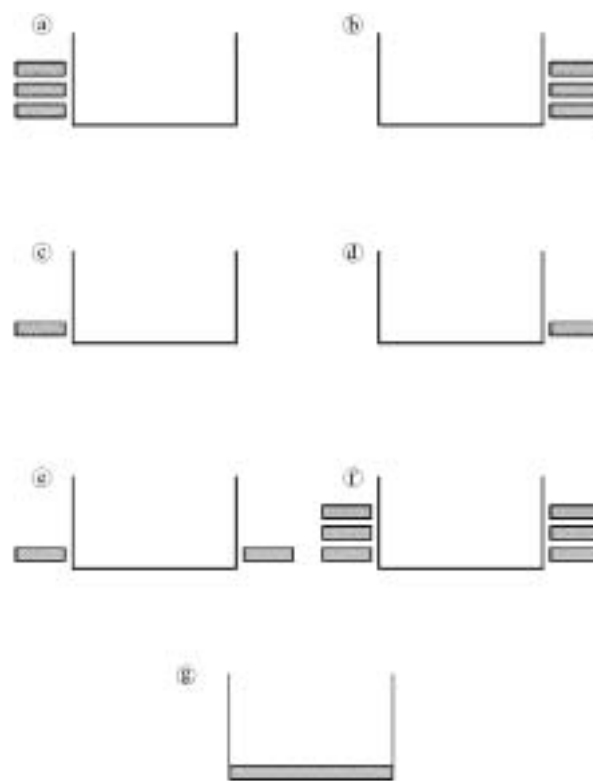


Fig. 1. Scheme of magnet arrangement. Hatched areas show the positions of the plate magnets.

urements of mycelium diameter are means of ten dishes in three replicates.

RESULTS

Side placement of the magnets did not induce any observable changes of the biological clock. The only visible effect was on coremia morphology. The presented results are from treatments with the magnets placed below the dishes (Figs. 1–4).

Table 1 shows data on the effects of the magnetic field on the functioning of the biological clock and the growth rate of mycelia in cultures grown under light. The static magnetic field accelerated growth slightly, mainly in the first phase of growth. The biological clock functioned as in the controls, but the rhythm period lengthened gradually from 30 h to 40 h, a few hours longer than in the control under light with no magnetic field.

Table 2 shows the results on the effect on the magnetic field on cultures grown in darkness. Under continuous darkness, the magnetic field affected the endogenous rhythm as expressed in the formation of

TABLE 1. Effect of static magnetic field (60–70 mT) on growth and sporulation rhythm in *Penicillium claviforme* in light

	Diameter of mycelium [mm]	Growth rate v [mm/24 h]	No. of zonations	Distance between zonations b [mm]						Length of period τ [h]
				b_1	b_2	b_3	b_5	b_{10}	b_{11}	
Control	56	2.0	12	2.5	2.5	3.0	3.0	3.0	3.0	30
Magnetic field	64	2.2	13	2.5	2.5	3.0	3.5	2.0	5.5	24–36

Results are the means of three replicates.

TABLE 2. Effect of static magnetic field (60–70 mT) on growth and rhythm expression of *Penicillium claviforme* in darkness

	Diameter of mycelium [mm]	Growth rate v [mm/24 h]	No. of zonations	Distance between zonations b [mm]			Length of period τ [h]
				b_1	b_2	b_3	
Control	50	2.3	absence of zonations	–	–	–	0
Magnetic field	80	2.4	2–3	5.0	10.0	10.0	70–96

Results are the means of three replicates.

coremia rings. The rhythm period was long, 96 h, and thus a multiple of the circadian rhythm (4×24 h). This rhythm was retained for 2 to 3 periods in our experimental conditions (Tab. 2; Fig. 2).

The action of the magnetic field on coremia morphology is shown in Table 3 for mycelia grown under light and in Table 4 for cultures grown in darkness.

Figure 3 diagrams the morphology of coremia exposed to the action of the magnetic field. It induced certain changes in coremia morphology irrespective of the presence or absence of light. The height of the coremia and the shape of the coremium head were affected. Coremia from cultures grown in a magnetic

field in darkness were distinctly higher than in the control cultured in darkness without the magnetic field.

The coremium head in fungi grown in darkness differed in shape. Under control conditions the coremium head was nearly round; under the magnetic treatment it was elongated and shaped like the coremium head from the control cultured under light.

Certain differences in the morphology of coremia cultured under the magnetic treatment and under light were observed; the coremia were slightly lower, and the more rounded shape of the head resembled the head of coremia from darkness.

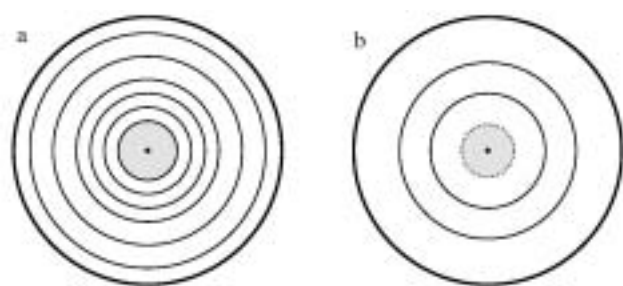


Fig. 2. Scheme of functioning of the clock in a magnetic field (a) Culture grown under light, (b) Culture grown in darkness. Length of rhythm period: light (white, continuous) – 20 h → 24 h → 24 h → 36 h → 36 h → 40 h; darkness – 70 h → 96 h → 96 h → 96 h.

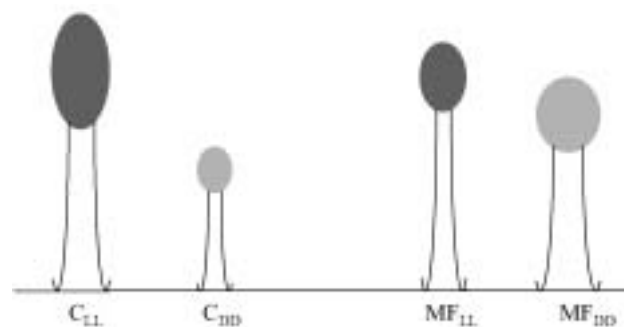


Fig. 3. Scheme of coremium morphology. C_{LL} – coremium from control (continuous light); C_{DD} – coremium from control (darkness); MF_{LL} – coremium from magnetic field under light; MF_{DD} – coremium from magnetic field in darkness. (Drawing based on data in Tables 3 and 4).

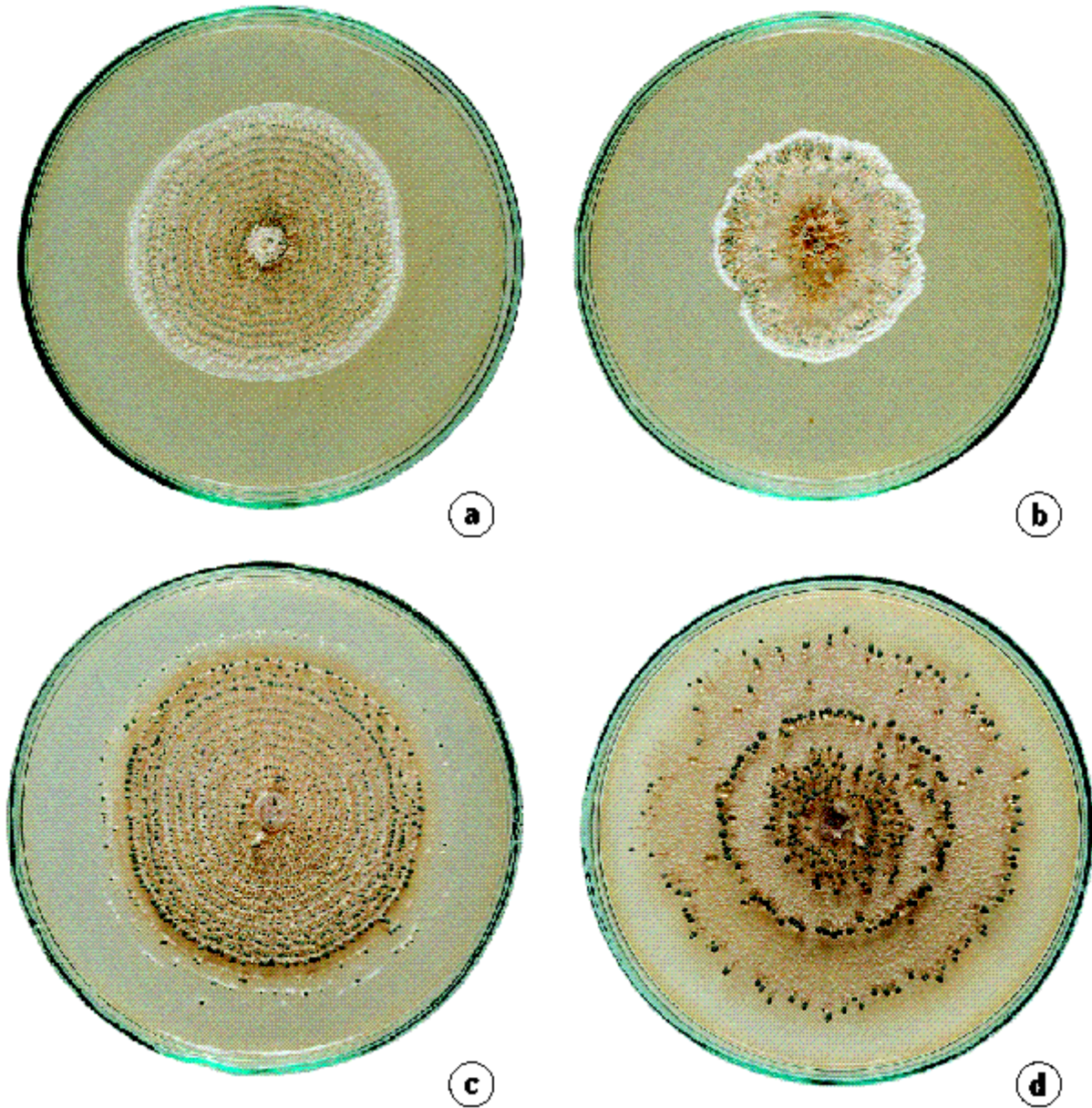


Fig. 4. Cultures of *Penicillium claviforme*. Functioning of the biological clock. (a–b) Control cultures: from light (a), coremial zonations visible, indicating the functioning of the biological clock; from darkness (b), absence of zonations; (c–d) Cultures in static magnetic field (60–70 mT) under light (c) and in darkness (d); coremial zonations are visible in both cases, indicating the functioning of the biological clock.

DISCUSSION

The results suggest that the action of the magnetic field may be an important environmental factor affecting the functioning of the biological clock as well as the morphology of the examined microorganisms. Growth of mycelia was slightly accelerated in

the examined *Penicillium claviforme* exposed to a static magnetic field, and the biological clock was distinctly affected. The rhythm period was a few hours longer in cultures grown under light with the magnetic field. In cultures grown in darkness but exposed to the magnetic field, an endogenous rhythm was expressed.

TABLE 3. Effect of static magnetic field (60–70 mT) on the morphology of coremia in *Penicillium claviforme* in light

	Height of coremium [mm]	Head		Leg	
		Length [mm]	Height [mm]	Length [mm]	Height [mm]
Control	12.5	5.5	3.5	7.0	3.5
Magnetic field	10.9	3.6	3.2	7.3	3.0

Results are means of three replicates.

TABLE 4. Effect of magnetic field (60–70 mT) on morphology of coremia in *Penicillium claviforme* in darkness

	Height of coremium [mm]	Head		Leg	
		Length [mm]	Height [mm]	Length [mm]	Height [mm]
Control	6.5	2.8	3.0	3.8	2.9
Magnetic field	10.4	3.6	3.0	6.8	3.0

Results are means of three replicates.

The inductive effect of light on activation of the biological clock (Sweeney, 1969; Piskorz-Bińczycka, et al., 1989; Jerebzoﬀ and Jerebzoﬀ-Quintin, 1992) was in some way imitated by the magnetic field. This is the first observation of the action of the magnetic field on expression of the biological clock.

The influence of an electromagnetic or magnetic field on biological activity has aroused great interest, especially in medicine (Picazo et al., 1996; Dobson et al., 2000; Ramchand et al., 2001; Zhang et al., 2002), microbiology and biotechnology (Gusakov et al., 1996; Al-Qodah, 2000; Al-Qodah and Lafi, 2001; Seong and Park, 2001).

There are many scientific reports on the influence of magnetic or electromagnetic fields on living organisms. Many physiological responses to electromagnetic field pulses have been studied, but not much of this work has addressed growth and morphogenesis (Celestino et al., 1998).

Magnetic field-induced acceleration of growth, especially of young seedlings, has been observed (Pittman, 1963; Rochalska, 1997). A number of researchers have studied the action of a static magnetic field on seed germination or other physiological processes in green plants (Pittman, 1963, 1970; Alexander and Doijode, 1995; Garcia Reina and Arza-Pascual, 2001). In our studies of *Penicillium claviforme* we observed better and slightly accelerated (10–20 h faster) germination of spores (unpubl. data).

It has been demonstrated experimentally (Lednev, 1991) that the application of a low-frequency, weak magnetic field, both static and time-varying, induces considerable changes in the metabolism characteristic of tested organisms. These changes are manifested primarily in altered ion flow through

cell membranes and in the motion of cells. Such results indicate the role of resonance. Swedish researchers found that a magnetic field of extremely low frequency affected the oscillation of cellular calcium content (Lindström et al., 1995): when cells were exposed to a magnetic field, Ca²⁺ content increased; thus the magnetic field acted as a physiological stimulator. A magnetic field has also been observed to influence calcium signal transduction (Yost et al., 1992).

Other investigated effects of magnetic fields include those on the activity of ion channels (Galt et al., 1993) and on ion transport in cells (Garcia-Sancho and Jawier, 1994).

There are few studies of the influence of magnetic fields on growth and morphogenesis in fungi. In *Aspergillus giganteus* mut. alba, a 150 mT horizontal magnetic field reduced the increment of mycelium mass, especially in cultures in darkness (Fiema and Filek, 1998). In the last decade there has been more interest in the influence of magnetic fields on living organisms, evidenced in the growing number of publications. The next step of our investigations will be a study of the influence of a static magnetic field on elements of the biological clock, that is, on the enzymes aspartate aminotransferase (AspAT) and alanine aminotransferase (AlaAT).

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