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SHORT COMMUNICATION

Pexophagy in Penicillin G Secretion by Penicillium chrysogenum PQ-96

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Abstract

Penicillin G oversecretion by Penicillium chrysogenum PQ-96 is associated with a strictly adjusted cellular organization of the mature and senescent mycelial cells. Abundant vacuolar phagy and extended cellular vacuolization combined with vacuolar budding resulting in the formation of vacuolar vesicles that fuse with the cell membrane are the most important characteristic features of those cells. We suggest as follows: if the peroxisomes are integrated into vacuoles, the penicillin G formed in peroxisomes might be transferred to vacuoles and later secreted out of the cells by an exocytosis process. The peroxisomal cells of the mycelium are privileged in penicillin G secretion.

Key words: Penicillium chrysogenum, penicillin G, secretion

The last two steps in penicillin G biosynthesis are located in the peroxisomes. Secretion of this antibiotic from the peroxisomes across the plasma membrane of Penicillium chrysogenum is poorly understood (Weber et al., 2012). This experimental study was designed to provide details supporting the hypothesis that pexo­ phagy (autophagy of peroxisomes) is involved in the large-scale secretion of penicillin G from the mycelial cell of P. chrysogenum PQ-96.

In this experimental program the high penicillin-producing strain P. chrysogenum PQ-96 was examined. Activity of penicillin G produced by this strain is described in Fig. 1. For comparative ultrastructural analyses the low-penicillin-producing strain P. chrysogenum Q-176 was investigated (Fig. 2). The examined strains were grown in complex media (Kurzątkowski et al., 2014a) and the antibiotic assay was carried out as described previously (García-Estrada et al., 2007). The preparation for transmission electron microscopy and immunoelectron microscopy was performed as described previously (Kurzątkowski et al., 2014a). The ultrathin sections were examined under a transmission electron microscope JEOL, JEM 1220 (Tokyo, Japan).

The lack of clear involvement of any of these ABC transporters (van den Berg, 2001; Patent description number WO 2001/32904) in secretion of penicillin G is intriguing and may indicate that the secretion of this antibiotic in the overproducing strains does not proceed through the classical ABC pumps. At present, new secretion pathways, e.g. the secretion by exocytosis may have been implemented in the high-penicillin producing strains (Martin et al., 2010). Although, fusion of the vacuoles to the plasma membrane by an exocytosis process is possible, there is currently no evidence in the literature to support that this might be a major mecha­ nism of penicillin G secretion (Martin et al., 2010).

In fed-bath cultures the industrial strains secrete 40–55 g of penicillin G per liter of the liquid fermentation medium. The knowledge concerned with the cellular arrangements in penicillin G overproduction is important for further strain improvement, which is of great economical importance. Compartmentalization in penicillin G biosynthesis by P. chrysogenum PQ-96 was described previously (Kurzątkowski et al., 2014a; 2014b). Our studies have proven that overproduction of penicillin G is associated with strongly adjusted mycelial and cellular organization (Fig. 1a–d, Fig. 2i–h, Fig. 3e–h). The productive mycelial cells of the high-yielding strain exhibited numerous large peroxisomes frequently arranged at the periphery of the cytoplasm and around the vacuoles including vacuolar invaginations. The surveys of a large number of hyphal sections

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led us to the conclusion that the immuno-gold marker of isopenicillin N synthase is abundantly arranged at polyribosomes surrounding the peroxisomes. Such a cellular accumulation of isopenicillin N synthase may enhance the selective, continuous and sufficient substrate supply in penicillin G biosynthesis. It was recently found that δ-(L-α-aminoadipyl)-L-cysteinyl-D-valine tripeptide is present in the cytoplasm and accumulates in the fermentation medium to concentrations of up to 2 mM (for review see Kurzątkowski et al., 2014a). The affinity of isopenicillin N synthase for this tripeptide is in the sub-mM range. The high concentration of the tripeptide in the fermentation broth might explain our unexpected results concerning the localization of isopenicillin N synthase at the periphery of the cytoplasm and in channel-like structures of the cell wall. This location might be a precisely adopted structural arrangement enabling the withdrawal of the tripeptide from the fermentation broth and from the cytoplasm for the peripherally located isopenicillin N synthase to increase the efficacy and yield in penicillin G biosynthesis. On the contrary, in the mature non-growing Hyphal cells of the low penicillin-producing strain PQ-96, 72 h culture, high-penicillin-producing strain, activity of penicillin G biosynthesis (U/ml); total yield at 72 h of cultivation – 8300 (4.98 g/1000 ml), increase of yield between 48 h and 72 h of fermentation – 4850.

(a–d) Transmission electron microscopy. (a) In the sub-apical non-growing productive cell numerous electron opaque peroxisomes (P) up to 1.0 μm in diameter can be observed. (b) Sub-apical productive cell of the mycelium is visible. Note the degrading organelle located in invaginations of the tonoplast (t) into a vacuole (V). Vacular engulfed pexophagy (arrow heads) is seen. It is a characteristic feature of the hyphal cell at the highest activity of penicillin G secretion. At the cell wall (cw) and the cross wall (cw) vacuolar vesicles packed with organelle debris are visible (arrows). Some peroxisomes (P) and mitochondria (M) are located at the vacuole beginning the process of autophagy (a). (c) Late sub-apical degrading highly vacuolated hyphal cell. In invaginations of the tonoplast (t) into the vacuole (V) the pexophagy (P) is seen. (d) Late-apical cell of the mycelium is seen. In the interior of an extended vacuole (V) the products of organelle-autophagy (a) can be seen (arrows). Scale bar = 1 μm.

The results of our experiments exhibit that the abundant vacuolar pexophagy of large peroxisomes combined with vacuolar budding and the presence of numerous vacuolar vesicles which fuse with the plasma membrane are the most important structural features characterizing the non-growing productive cells as well as the late-apical degrading highly vacuolated cells of the tested industrial strain. This structural arrangement is closely combined with the period of large-scale secretion of penicillin G. Such a cellular organization was not visible in the mature cells of the low-penicillin-producing strain Q-176.

We suggest that the abundant pexophagy of large peroxisomes as well as the vacuolar budding observed in the productive and senescent cells of *P. chrysogenum* PQ-96 might be directly involved in large-scale secretion of penicillin G. In these cellular arrangements the penicillin G formed in peroxisomes might be transferred to vacuoles and late secreted out of the cells by an exocytosis process. The vacuolar pH of about 5 is suitable for the stability of penicillin G. Our discoveries are consistent with the reported positive cor-
The novelty of this experimental program is the discovery of essential cellular features associated with the large scale secretion of penicillin G from the mycelium of *P. chrysogenum* PQ-96 to the fermentation relation between the number of large peroxisomes and penicillin G secretion (Meijer *et al.*, 2010), as well as between the extended vacuolization and antibiotic secretion (Sakai *et al.*, 2006).

The novelty of this experimental program is the discovery of essential cellular features associated with the large scale secretion of penicillin G from the mycelium of *P. chrysogenum* PQ-96 to the fermentation
medium, i.e.: abundant vacuolar pexophagy of large peroxisomes, intensive cellular vacuolization, budding of vacuoles, fusion of vacuolar vesicles with the plasma membrane. We have come to the conclusion that in large-scale secretion of penicillin G the pexophagy phenomenon and exocytosis should be currently considered as a putative alternative for active secretion by the ABC transporters.

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Literature


