Production of Natural Penicillins by Strains of *Penicillium chrysogenum*

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New strains with higher penicillin G and V production were isolated using mutagenic factors in their mutual combination. The penicillin production by these strains was dependent upon the carbon source in medium. Individual strains reacted differently on the change of the carbon source. In the absence of precursors the strains produced a different number of natural penicillins. A direct relationship between increasing production of penicillins and the concentration of 6-aminopenicillanic acid in the filtrate was not established.

Penicillin production by strains of *Penicillium chrysogenum* depends on the sort and concentration of added carbon source. A good antibiotic production occurs in a lactose medium (one-shot addition of lactose). Similar production is obtained in the media containing saccharose or glucose when these sugars are added gradually into medium [1—3]. The formation of individual types of penicillins is influenced by the added precursor. In the media without precursor, the types of synthesized penicillins as well as the amounts of produced 6-aminopenicillanic acid (6-APA) and other compounds reacting with phenyl acetyl chloride, are dependent upon the used strain [4—6].

The purpose of the present work was to compare the results obtained with our new strains with those achieved in other laboratories.

Experimental

Materials and methods

Production strains

Strains of *Penicillium chrysogenum* with higher antibiotic production were isolated after exposing the spores of selected strains to UV-light and simultaneously to the action of *N*-methyl(dichlorodiethyl)amine hydrochloride. The latter mutagenic factor was added to agar medium on which the affected spores were further cultivated. The agar medium was supplemented with 10—25% (v/v) of the filtrate obtained after the *Penicillium chrysogenum* fermentation. Compounds present in the filtrate promoted the simultaneous adaption of strains to new growing conditions in manufacture [7]. Thus attained strains were maintained according to *Backus* and *Stauffer* [8].

Fermentation

The production medium had following composition: lactose 5.8 g, glucose 0.5 g, corn steep (50% dry weight) 1 g, peanut meal 2 g, calcium carbonate 0.65 g, sodium thiosulfate 0.24 g, phenylacetic acid 0.35 g — all dissolved in tap water and made up to 100 ml. pH of the medium before sterilization was 6.4. When saccharose was used as carbon source, glucose and lactose were omitted. Saccharose was added into medium by portions each 24 hours (0.6 g per 60 ml). In experiments in which the formation of 6-APA was followed a synthetic medium without precursor and native nitrogen sources was used [3].

Conditions of cultivation

The microorganism was grown in 500 ml flasks containing 60 ml of the medium on a rotary shaker (220 rev./min., $r = 5.5$ cm).

Bio-assay of penicillins

Penicillin G and V were estimated by diffusion plate method using *Bacillus subtilis SDPC* (1 : 220) as a testing microorganism [9]. 6-Aminopenicillanic acid and compounds reacting with phenyl acetyl chloride were determined by the same method after forgoing transformation into benzylpenicillin [10].

Chromatography of penicillins

Paper chromatograms were developed in water saturated ether. Chromatography chamber was saturated with the system for 4 hours at 24°C before development. A bioautographic method on agar plates seeded with *Bacillus subtilis* was used for localization of antibiotics on chromatograms.

Results and Discussion

The producing abilities of the improved strains of *Penicillium chrysogenum* No. 2/170 and No. 9/138 [7] were verified by cultivation in liquid media. The results obtained with these new strains were compared with those attained using the original strain. The strains grown under the same conditions showed a different overall penicillin G and V producing abilities. In the lactose medium, the penicillin G and V production by the strains No. 2/170 and No. 9/138 was found to be 10%, resp. 35% higher than the production by the strain No. 165 (Fig. 1, curves *1*, *2* and *3*). In the presence of saccharose, the mutual relationship of penicillin G and V production of tested strains was altered. The penicillin V production of the strain No. 2/170 represented 136% and that of the strain No. 9/138 171% against the 100% production of the strain No. 165. The time, at which maximum concentration of penicillin V appeared in media containing saccharose or lactose, varied with all three strains. The replacement of lactose with saccharose did not influence the penicillin V production by the strain No. 165. This was expressively altered, however, in the case of the strain No. 2/170 and No. 9/138 (Fig. 1). Based upon above findings, we can suppose that the producing ability in the course of strain improvement should be evaluated using media which contain the same carbon source as will be used in production medium in manufacture.

The production of above strains was also examined in a synthetic medium without precursor [3]. Concentration of natural penicillins was determined by plate diffusion method [9] and the overall antibiotic activity was expressed in benzylpenicillin

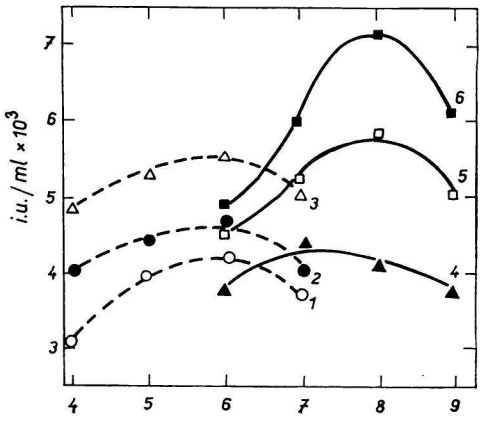

Fig. 1. Production of penicillin V by strains of *Penicillium chrysogenum* in lactose medium: *1.* strain No. 165; *2.* strain No. 2/170; *3.* strain No. 9/138 and in saccharose medium: *4.* strain No. 165; *5.* strain No. 2/170; *6.* strain No. 9/138.

units. 6-APA was reacted with phenyl acetyl chloride to give benzylpenicillin which was estimated together with penicillins in the second test by the same method. The difference between those two determinations represented the content of 6-APA in the filtrate. A direct relationship between penicillin and 6-APA has not been observed. The penicillin V production of both strains No. 2/170 and No. 9/138 increased. The production of 6-APA of the former strain increased too, but that of the latter one was diminished. These findings might indicate that 6-APA is not a precursor of penicillin [11, 12]. Contrary to our results (Tabelle 1), *Ostrouchov* and *Kuznecov* [13] reported a direct relationship between the ratio of penicillins in the media with or without precursor and the overall producing ability of a strain.

Table 1

Production of penicillin G and 6-aminopenicillanic acid by strains of *Penicillium chrysogenum* in lactose medium

Strain	Penicillin G production i.u./ml		Ratio $b/a \cdot 100$	Production of 6-APA in medium without precursor i.u./ml[c]
	medium with precursor[a]	medium without precursor[b]		
165	4200 ± 140	470 ± 110	11.2	380 ± 140
2/170	4600 ± 160	420 ± 160	9.15	260 ± 100
9/138	5600 ± 180	510 ± 170	9.12	480 ± 160

a) Production of penicillin G.
b) Production of natural penicillins expressed as penicillin G.
c) Production of 6-APA expressed as penicillin G.

After reaching the maximum antibiotic activity in the synthetic medium without precursor, the natural penicillins present in the filtrate were examined by paper chromatography. The number of penicillins appeared to be different for each tested strain and to be smaller for strains with enhanced production of penicillin in the media with precursor (Fig. 2).

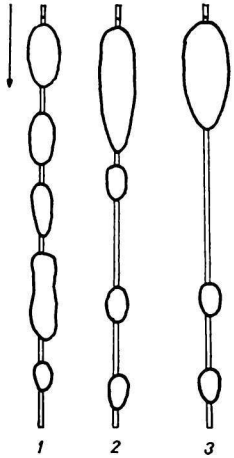

Fig. 2. Paper chromatography of natural penicillins by strains of *Penicillium chrysogenum*:
1. strain No. 165; *2.* strain No. 2/170; *3.* strain No. 9/138.

The obtained results indicate that there is no direct relationship between overall production of penicillins G and V by individual strains and the used carbon source. Producing ability of new strains in course of their further improvement should be evaluated therefore on the same carbon source which will be used in manufacture.

The strain which exhibited an enhanced antibiotic activity in the media with precursor, formed a less number of natural penicillins in the media without precursor. The fact, that the increasing production of penicillins was found to be unrelated to the production of 6-APA, supports the opinion that 6-APA is not a direct intermediate in the biosynthesis of the penicillin molecule.

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