AN INVESTIGATION ON POLYMORPHISM IN _STREPTOMYCES AMBOFACIENS_ ATCC 15154

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ABSTRACT
The polymorphism in _Streptomyces ambofaciens_ ATCC 15154 has been investigated on 21 nutrient media. The greatest degree of dissociation has been observed on oatmeal agar No1 with the strain having formed six morphological types of colonies, characterized by identical mean rate of radial growth. The percentages and the morpho-cultural traits of the types obtained within the microbial population have been determined. The study dealing with their biosynthetic activity has provided evidence about the second and the third types of colonies to be the most productive ones. Of these two: the third type – proved to be morphologically more stable after a five-fold inoculation and cultivation on oatmeal agar No1, is more likely to be chosen for selection of active varieties, that might later on be used as initial bioobjects for obtaining new spiramycin producers of a greater activity.

Keywords: _Streptomyces ambofaciens_ ATCC 15154, polymorphism, stability

Introduction
_Streptomyces ambofaciens_ ATCC 15154 produces spiramycin – one of the essential representatives of the macrolide antibiotic group, having broad application in medical practice. That makes the interest in it and the attempts for obtaining new strains of increased biosynthetic activity so natural.

The present investigation is the first to deal with polymorphism of _S. ambofaciens_ ATCC 15154 with the aim of discovering more active and more stable morphological types of colonies within the population that are to be used as initial bioobjects in the course of attaining new high-producing strains.

Materials and Methods

Spiramycin producer
_Streptomyces ambofaciens_ ATCC 15154

Polymorphism
Polymorphism has been studied by means of a method proposed by Kuznetsov (4) on 21 nutrient media: mineral agar No1 (in two varieties containing soluble and insoluble starch, respectively), oatmeal agar No1, oatmeal agar No3 (ISP), inorganic salts-starch agar (ISP No4), starch-ammonia agar, Krassilnikov agar with glucose, Czapek agar with glucose, glucose-asparagine agar, starch agar, soya agar, Waksman organic agar, Waksman synthetic agar, glucose-peptone agar, Czapek agar with glucose and maize extract, glucose-yeast agar, potato agar, Raistrick agar, Sotton agar, Sabouraud agar and Czapek-Dox agar with glucose.

After spore suspension inoculation and cultivation for a 14-day period the developed solitary colonies have been united into defined types according to their morpho-cultural peculiarities.

**Mean rate of radial growth Vr (mm/h)**
It is calculated by the formula $V_r = \frac{dR}{dt} = \frac{(R-R_0)}{(t-t_0)}$, where $R_0$ and $R$ stand for the colonies’ radius values, measured at a defined time – $t_0$ and $t$, respectively (2, 5).

**Colour characteristics**
The colour of aerial mycelium has been determined by Tresner-Backus (6), while that of the substrate mycelium – by Bondartsev scale (1).

**Biosynthetic activity**
The isolated solitary colonies’ biosynthetic activity has been determined through measuring the diameter $\varnothing$ of inhibited growth zones around them with the test microorganism being _Bacillus subtilis_ ATCC 6633 (3).

**Stability**
The stability of the morphological types of colonies has been studied once they were subjected to five-fold inoculation and cultivation on oatmeal agar No1.

Results and Discussion
The experimental research has proved the greatest degree of _S. ambofaciens_ ATCC 15154 dissociation to occur on oatmeal agar No 1. Six morphological types of colonies having uneven distribution in the population, tend to be formed on it. Their quantitative presentation is determined as a result of triple experimental testing. The mean values of the three experimental data are given in Table 1.
The different colony types (Fig. 2) are characterized by the following morpho-cultural traits.

Type I: Round colonies with rough edges ranging from 9 to 12 mm in diameter; having a bulged ring in the central part. Aerial mycelium is unevenly developed in both the centre and periphery, white in the centre and ash-like in the other parts. Substrate mycelium color is gray to pale sandy.

Type II: Round colonies with rough edges ranging from 10 to 13 mm in diameter and unevenly clustered aerial mycelium in the centre. The aerial mycelium is white in the centre, gray in the intermediate part and oyster white at the periphery. Substrate mycelium color is gray to pale sandy.

Type III: Round colonies with rough edges ranging from 11 to 13 mm in diameter; having slightly concave central part and a ring surrounding it. Aerial mycelium is well developed, ash-like in the centre and at the periphery, white in the rest of the colonies. Substrate mycelium color is gray to pale sandy.

Type IV: Round colonies with rough edges ranging from 10 to 13 mm in diameter; having two rings at the central part. Aerial mycelium is white, less developed in the intermediate...
part and well developed in the rest of the colonies. Substrate mycelium color is pale yellow.

Type V: Round colonies with rough edges ranging from 10 to 13 mm in diameter; having concave central part and radially furrowed surface. Aerial mycelium is well developed, white and unevenly covering the periphery. Substrate mycelium color is gray to pale sandy.

Type VI: Round colonies with rough edges ranging from 11 to 13 mm in diameter; having a slightly bulged ring in the centre. Aerial mycelium is well developed, gray in the intermediate part and ash-like in the rest of the colonies. Substrate mycelium color is gray to pale sandy.

The results of the essay on the biosynthetic activity of the defined morphological colony types are presented in Table 3.

<table>
<thead>
<tr>
<th>Morphological type</th>
<th>n</th>
<th>Ø (mm)</th>
<th>X (mm)</th>
<th>Relative biosynthetic activity (%)</th>
<th>S (mm)</th>
<th>VC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>460</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>II</td>
<td>340</td>
<td>8.0 – 18.5</td>
<td>10.7</td>
<td>109.2</td>
<td>3.0</td>
<td>28.0</td>
</tr>
<tr>
<td>III</td>
<td>160</td>
<td>8.0 – 21.5</td>
<td>10.5</td>
<td>107.1</td>
<td>5.3</td>
<td>50.4</td>
</tr>
<tr>
<td>IV</td>
<td>459</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>V</td>
<td>152</td>
<td>8.3 – 17.0</td>
<td>9.8</td>
<td>100.0</td>
<td>1.8</td>
<td>18.5</td>
</tr>
<tr>
<td>VI</td>
<td>110</td>
<td>8.3 – 16.5</td>
<td>9.8</td>
<td>100.0</td>
<td>2.0</td>
<td>20.7</td>
</tr>
</tbody>
</table>

Where: n is the number of solitary colonies studied; X – mean values of the diameters Ø of inhibited growth zones; S – mean square fluctuation and VC – variation coefficient.

<table>
<thead>
<tr>
<th>Original morphological type</th>
<th>Morphological types observed after the five-fold inoculation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>I 97.4            II –            III –            IV 2.6          V –            VI –</td>
</tr>
<tr>
<td>II</td>
<td>I 32.9            II –            III 29.0        IV –            V 33.3          VI 4.8</td>
</tr>
<tr>
<td>III</td>
<td>I 31.0            II 3.1           III 57.6        IV –            V –            VI –</td>
</tr>
<tr>
<td>IV</td>
<td>I –               II –             III 0.8          IV 99.2        V –            VI –</td>
</tr>
<tr>
<td>V</td>
<td>I –               II 19.8          III –            IV –            V 80.2          VI –</td>
</tr>
<tr>
<td>VI</td>
<td>I 10.6            II 7.6           III 45.9         IV 21.6        V –            VI 14.3</td>
</tr>
</tbody>
</table>

The test microorganism growth inhibition zones around colonies of the first and the fourth types tend to be very poorly displayed and practically cannot be measured. Statistical analysis of the experimental data shows, that the varieties of the fifth and the sixth morphological types have equal mean values of their inhibited growth zone diameters (Ø). The latter have lower biosynthetic activity and are considered basic (100%). The most productive are the second and the third morphological types, that tend to be more variable in activity as well.

Once the morphological types have been subjected to a five-fold inoculation and cultivation on oatmeal agar No1, the first and the fourth have been defined as the most stable and unlikely to undergo any dissociation. The rest tend to dissociate to different extent (Table 4), while the more active third type is actually the best preserved one (57.6%).

Conclusions

It is for the first time that S. ambofaciens ATCC 15154 has its polymorphism studied. The experimental results show the greatest colony type diversity to occur in the population on oatmeal agar No1. The six types of colonies, identified and characterized according to their morpho-cultural traits, have identical mean rate and similar dynamic profiles of radial growth. Of the latter, the second and the third ones have proved to be of highest and almost equal biosynthetic activity. The third type, both more variable in terms of activity and morphologically more stable while subjected to a multi-fold inoculation and cultivation, should be used as an initial biobject for obtaining high-spiramycin-producing strains.

REFERENCES