RESEARCHING THE POSSIBILITIES OF THE PECTOLYTIC ENSYME BIOSYNTHESIS WITH THE *ASPERGILLUS SPECIES* OF MICROSCOPIC FUNGI

KIRO MOJSOV,

Technological-Technical Faculty
University "Goce Delcev", Macedonia

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Annotation: For the purpose of acquiring a highly active producer of pectolytic enzymes, there was a probe of isolating 140 species of *Aspergillus's fungi* from different substrates (soils, grape malts, grapes, apples and sugar beet shreds). The isolated kinds of fungi were kept on a slant agar surface according to Chapek, with 2% of pectin. Testing was performed on the isolated layers of fungi as to the production of pectinolytic enzymes. The nourishing base used was the synthetic Chapek base with 2% pectin, 2% lactose and 0.7 % $(NH_4)_2HPO_4$, and a natural base of 1% refuse apple pulp. Within 48 hours after cultivating the fungi, the filtrates were tested by the viscozimetric method to determine their entire pectolytic activity. The acquired results showed that a fungus was gained with a high pectolytic activity on a natural refuse apple pulp. It is a good employment effect. The selected sort *Aspergillus sp.MK-15* as a highly active producer of pectolytic enzymes could, with additional testing, be used for the industrial production of microbe enzymes with pectolytic activity (enzyme preparations).

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Introduction

During the elaboration of methods for the production of microbe enzymes, it is very important to make the correct choice of a very active sort of microorganism. The selection of a good sort presents the first step of the industrial production of microbe enzymes and it comprises examining a large number of microbe species and sorts from natural sources in terms of their capacity for producing the desired enzyme.

The basic requirement for the source is a simple adequate procedure which provides examining a large number of sorts in a relatively short time (Jong at al., 1983).

Aspergillus niger has been recognized as an industrially important fungus since the U.S. Food and Drug Administration (FDA) approved the GRAS status for many substances which were produced with it. These fungi produce several commercial enzymes, amongst which is also the pectinasis, its commercial value being about 20% of a USD billion annual sales of all industrial enzymes (Kashyap et al., 2001).

The pectolytic enzymes of *Aspergillus niger* are used in the fruit processing industry, the wine production, the textile industry, the production of paper etc.

Hence, the aim of this research is to isolate a very active sort of the fungus *Aspergillus niger* from natural sources for the purpose of further laboratory testing of the optimal parameters of the fermentation process for the maximum production of pectolytic enzymes, and later for the industrial production of microbe enzymes upon the refuse apple pulp in the fruit juice producing factories.

Material and method of working

For obtaining a highly active producer of pectolytic enzymes there was a probe of isolating 140 sorts of the *Aspergillus's fungi* species from different substrates (soils, grape malts, grapes, apples and sugar beet shreds).

To isolate the micro-organisms, the agar nourishing base was used, according to Chapek, with 2% of pectin (Bilaj, 1972). The composition of the base for isolation is given in Table 1. The isolated fungi cultures were kept on a slant agarian base according to Chapek, with 2% of pectin.

TABLE 1. CONTENT OF THE BASE AFTER CHAPEK WITH 2% PECTIN

Pectin	20 g.
$NaNO_3$	2 g.
KH_2PO_4	1 g.
$MgSO_4.7H_2O$	0.5 g.
KCl	0.5 g.
$FeSO_4$	0.01 g.
Agar-agar	15-20 g.
Distilled water	1 l.

Testing of different sorts of fungi for the production of pectolytic enzymes was performed in 500 ml Erlenmeyer flasks (with 100 ml nourishing base) on a laboratory rotational shaker with rotational shaking (200 rev/min), at a temperature of 30^0C within 48 hours. The base was previously sterilized in an autoclave at 1 atmosphere for 30 minutes.

The nourishing base used was the synthetic Chapek base with 2% pectin, 2% lactose and 0.7% $(NH_4)_2HPO_4$ (Verbina, 1972) the content of which is shown in Table 2, and a natural base of 1% refuse apple pulp (Table 3). For the acidity of the nourishing base (up to pH=5), H_2SO_4 was used. For inoculation a suspension of spores was used gained with a 72 hour extraction of a culture raised on a slant agarian surface according to Chapek, with 2% of pectin. After the completed cultivation, the content of the Erlenmeyer flasks was filtrated through filters with the help of a vacuum water pump. The filtrates were transferred into 100ml flasks and were used (Moiseenko, 1965) to determine the entire pectolytic activity (PcAc), which evaluates the activity of the respective fungi in the production of pectinolitic enzymes.

TABLE 2. CONTENT OF THE SYNTHETIC BASE AFTER CHAPEK WITH 2% PECTIN, 2% LACTOSE AND 0.7% $(NH_4)_2HPO_4$, % (WEIGHT /VOLUME)

Pectin	2.0
Lactose	2.0
$(NH_4)_2HPO_4$	0.7
KH_2PO_4	0.1
$MgSO_4.7H_2O$	0.05
KCl	0.05
$FeSO_4$	0.001

TABLE 3. CONTENT OF THE NATURAL BASE WITH 1% REFUSE APPLE PULP, % (WEIGHT /VOLUME)

Apple pulp	1
Corn flour	0.5
$(NH_4)_2HPO_4$	0.7
KH_2PO_4	0.1
$MgSO_4.7H_2O$	0.05
KCl	0.05

The refuse apple pulp had the following content: moisture 10-12 %, ashes 3-5%, proteins 6-6.2%, and pectin 9-10%. The ground apple pulp particles were with the diameter under 0.315mm.

TABLE 4. SURVEY OF ISOLATED CULTURES OF THE *ASPERGILLUS'S SPECIES* FUNGI

Sort of fungi	Origin of fungi	Selective base for isolating	Number of isolated fungi
MK-1 to MK-70	soil	Chapek with 2% pectin	70
MK-71 to MK-90	grape malt		20
MK-91 to MK-110	grapes		20
MK-111 to MK-120	apples		10
MK-121 to MK-140	sugar beet		20

TABLE 5 PECTOLYTIC ACTIVITY OF THE FILTRATES *

Sort of fungi	Origin of fungi	Cultivation on synthetic base (I)		Cultivation on natural base (II)	
		pH final	(PcAc) (I.E./ml)	pH final	(PcAc) (I.E./ml)
MK-14	soil	3.68	2.20	2.99	4.87
MK-15	soil	3.65	2.57	2.93	13.23
MK-16	soil	3.70	2.44	3.04	6.82
MK-18	soil	3.69	2.32	3.12	5.75
MK-20	soil	3.65	2.20	2.97	4.77
MK-40	soil	3.64	2.20	2.99	6.62
MK-45	soil	3.68	2.28	3.06	10.23
MK-46	soil	3.55	2.30	3.10	10.42
MK-49	soil	3.40	2.30	2.89	2.43
MK-53	soil	3.37	2.19	2.97	5.84
MK-55	soil	3.65	2.55	2.87	6.82
MK-61	soil	3.69	2.37	3.02	4.77
MK-77	grape malt	4.11	2.18	3.15	5.36
MK-106	grapes	3.40	2.39	3.06	9.35

Note: *Obtained with the cultivation of fungi on: I - Synthetic base after Chapek with 2% pectin, 2% lactose и 0.7% $(NH_4)_2HPO_4$; II - natural base with 1% refuse apple pulp. pH starting on the bases = 5; inoculums - suspension of spores up to three days old; duration of cultivation - 48 hours.

The analytic method

The entire pectolytic activity (PcAc) is determined by the viscozimetric method. The sample taken for analysis is 20 ml 0.35% pectin solution to which a certain amount of enzyme solution (filtrate) is added.

This solution and the control probe (without enzyme) are placed in an ultra-thermostat for analyzing at a temperature of 40^0C for one hour. After this period the enzyme reaction is cut down by a 2-3 minute boiling. The solution is cooled at room temperature and is filtrated through filter paper. With these solutions (the tested and the control) the viscosity is determined by means of the Oswald viscozimetre at 20^0C.

The degree of degraded peptin under the influence of enzymes is determined with the formula:

$$A = t_1 - t / t_1 - t_0 ,$$

where: A - is the degree of degraded pectin; t_1 - is the time in which the control solution of pectin flows out per sec.; t_0 - is the time in which the water solution of enzyme flows out per sec.; t - is the time in which the pectolizate flows out per sec.

The determined percentage of the degraded pectin (A) with the known amount of filtrate (enzyme solution) enables the calculation of the entire pectolytic activity (PcAc) under the terms of international measurements (I.E.).

One conditional measurement unit is the amount of enzyme which catalyses hydrolyse per 1g of pectin in one hour at 40^0C.

One international measurement unit is the amount of enzyme which catalyses hydrolyse per 1 μmol of pectin in 1 minute under given conditions.

1 conditional unit= $1x10^6/176$ x60 = 94.696 I.E., where: 10^6 - represents the transfer of grams into micrograms; 176 - stands for the conditional meaning of the pectin molecular mass (anhydrogalacturon acid); 60 - represents the calculation of the enzyme activity per min.

Results and discussion

In Table 4 it can be seen that the largest number of the isolated fungi (70 fungi) originate from the soils, and the remaining (70 fungi) are from other natural substrates. The usage of this base with pectin as a unique source of carbohydrate provides a very fast growth (2-3 days), especially of the *Aspergillus's* species of fungi which most easily degrade pectin

In Table 5 it can be seen that the largest number of fungi that are highly active producers of pectolytic enzymes, originate from soils and only one from the grape malt and one from grapes.

With the cultivation of fungi on a synthetic base after Chapek with 2% pectin, 2% lactose and 0.7% $(NH_4)_2HPO_4$, the pectolytic activity of the filtrates varies in very close boundaries (from 2.18 to 2.57 I.E./ml) so that it is impossible to determine which of these fungi is the best producer of pectolytic enzymes.

With the cultivation of fungi on a natural base of 1% apple pulp, the pectolytic activity of the filtrates varies in wide boundaries (from 2.43 to 12.23 I.E./ml).

From these tests (Table 5) it can be seen that the filtrate of fungus *Aspergillus sp.MK-15* cultivated on a natural base of 1% apple pulp has the greatest pectolytic activity of all other fungi filtrates.

Conclusion

On account of the performed experiments and the gained results the following conclusions can be drawn:

a. The usage of a base with the pectin as the unique source of carbohydrate provides a very fast growth (2-3 days) especially of the *Aspergillus's* species of fungi which most easily degrade pectin.
b. There are great possibilities for the biosynthesis of the pectolytic enzymes with fungi of the *Aspergillus* species on a base of refuse apple pulp and for its economical and more efficient usage.
c. The biosynthesis of the pectolytic enzymes with fungi of the *Aspergillus* species is far more intense on a base of 1% refuse apple pulp than on a synthetic base after Chapek with 2% pectin, 2% lactose and 0.7 % $(NH_4)_2HPO_4$.
d. The isolated fungus *Aspergillus sp.MK-15* is a highly active producer of pectinolytic enzymes cultivated on refuse apple pulp gained from the fruit juice production factories.
e. With this fungus the research can be continued on the various influences of biosynthesis upon pectolytic enzymes.

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