

PATENT SPECIFICATION

934,528

DRAWINGS ATTACHED.

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934,528

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COMPLETE SPECIFICATION.

Improvements in or relating to the Production of Antibiotics.

We, GLAXO GROUP LIMITED, formerly GLAXO LABORATORIES LIMITED, a British Company, of Greenford, Middlesex, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention is concerned with improvements in or relating to the production of the antibiotic griseofulvin.

Griseofulvin, which is an important antifungal antibiotic, may be produced by the culture of various species of the genus *Penicillium*, and in British Patent No. 784,618 are described methods of conducting such cultures under submerged aerobic conditions more suited to commercial production than the previously described surface culture techniques.

As generally practised hitherto, the submerged aerobic culture of griseofulvin producing organisms to yield griseofulvin has been carried out in media wherein the nitrogen requirements of the organism have been provided by so-called complex organic sources of nitrogen, e.g. various vegetable materials such as corn steep liquor, oatmeal, cottonseed meal etc. or nitrogenous milk products such as whey, the material commonly used being corn steep liquor.

In studies upon the production of griseofulvin by submerged aerobic fermentation, we have found it considerably advantageous to replace part of the complex organic source of nitrogen by ammonia or an ammonium salt. Such replacement apart from substantially improving the fermentation from an economic point of view enables a substantial

improvement in titre to be achieved. It thus appears that the inclusion in the medium of ammonia or ammonium salts which are quickly assimilable, as compared with the organic nitrogen sources, can result in a stimulation of growth of the organism in the early stages of the fermentation, and that such stimulation is very beneficial.

According to the invention, therefore, we provide a process for the production of griseofulvin by the submerged aerobic culture of a griseofulvin producing organism in a nutrient medium therefor, the assimilable nitrogen in said medium being provided by at least one complex source of nitrogen and ammonia or an ammonium salt.

The complex organic nitrogenous material may for example be of the type commonly used in fermentation technology, suitable materials being corn steep liquor, cottonseed meal, soya bean meal, penicillin felt, ground nut meal, maize gluten meal, distillers solubles, animal nitrogenous materials such as meat extracts, animal tankage (feathermeal), nitrogen-containing milk products, e.g. butter milk, whey powder, liquid whey etc. In general we find that corn steep liquor is the preferred complex source of nitrogen, although particularly advantageous results are obtained by using a mixture of corn steep liquor and distillers solubles. Mixtures wherein the ratio on a nitrogen basis of corn steep liquor to distillers solubles is between 1:4 and 4:1 by weight are satisfactory, mixtures giving equal amounts of nitrogen by weight from both sources being particularly advantageous.

Where an ammonium salt is used it should of course be one, the anion of which is non-

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toxic to the griseofulvin producing organism. Suitable salts are for example ammonium chloride, ammonium carbonate, ammonium nitrate, ammonium acetate etc. Ammonium sulphate is especially beneficial. It will of course be appreciated that in place of ammonia or an ammonium salt one can use substances giving rise to the presence of ammonia or an ammonium salt in the medium; such substances are for example urea and calcium cyanamide which are converted at least in part to ammonia upon sterilisation of the medium. We have particularly found it convenient to use a mixture of ammonia or an ammonium salt together with urea.

The relative proportions of the two types of nitrogen sources can vary widely; in general we prefer that of the total available nitrogen 5% by weight as N and preferably at least 10% by weight should be constituted by ammonia or an ammonium salt. In general up to some 20% by weight of the total nitrogen (as N) may with advantage be constituted by ammonia or an ammonium salt nitrogen source, but up to 40% by weight and even more may be used. The complex nitrogen source preferably comprises a major proportion (in terms of N) of the total nitrogenous nutrient.

In conducting the fermentation, the medium may be formulated to contain all of the assimilable nitrogen at the beginning of the fermentation, or alternatively some of the nitrogenous material may be added at a later stage. In particular a whole or part of the complex nitrogenous source may be added at a later stage, for example, after the growth of the organism is well developed, e.g. after some 90 hours.

In the obtaining of good results there are, of course, various other factors to which attention must be paid in conducting the production of griseofulvin by submerged fermentation, as discussed generally in our Patent No. 784,618.

In general, however, we prefer to conduct the fermentation in accordance with the procedures described in our Application No. 7197/59 (Serial No. 868,958) according to which a nutrient carbohydrate is repeatedly added (continuously or batchwise) to the fermentation as it proceeds, in such quantities and at such times that the pH of the medium follows a controlled pH curve as defined in the said Specification. In general it is preferable that the pH of the medium is maintained between 6.6 and 7.6, and preferably 6.8 and 7.2 for the greater part of the fermentation. Suitable carbohydrates include glucose, lactose, sucrose, hydrolysed starch, invert sugar, molasses, invert molasses and starch, of which glucose is preferred.

As discussed in Specification No. 784,618, the total level of assimilable nitrogen used

in the medium is important. According to the said Specification the total assimilable nitrogen should be between 0.04% and 0.3% (of N) and preferably be between 0.075 and 0.25% N. In our Application No. 15819/60 (Serial No. 934,527) we have, however, shown that provided certain other conditions are also employed, it is possible with advantage to use levels of nitrogen in excess of 0.3% N. In accordance with the present invention it is desirable that the total nitrogen present (as N), i.e. the nitrogen represented by both the complex nitrogenous source and the ammonia or ammonium salt (and urea if used) should lie within the range stated in Patent No. 784,618, unless the conditions described in our said Application No. 15819/60 (Serial No. 934,527) are also employed, when total levels of nitrogen above 0.3% can with advantage be used.

We have found it advantageous in the present process to include in the medium an assimilable source of sulphur, for which purpose sulphates and thiosulphates, etc. may be used, preferred substances being alkali metal, e.g. sodium and potassium sulphates and thiosulphates, as well as sulphuric acid itself. Where ammonium sulphate is used as a source of nitrogen, this substance will, of course, also supply sulphate.

In general the source of sulphur should be included in the medium to provide a level of sulphur (as S) between 0.01% and 0.05% by weight, preferably 0.02—0.04%.

The medium should further desirably contain a water-soluble phosphate, preferably one of an alkali metal, sodium or potassium dihydrogen phosphate being, for example, satisfactory. The phosphate may be present in the medium in an amount, for example, between 0.4 and 0.8% by weight. The medium should further contain a soluble chloride, the amount thereof being at least sufficient to provide the whole of the chlorine in the resulting griseofulvin. Suitable chlorides are the alkali metal chlorides such as sodium and potassium chloride, which are in general preferably used at a level of at least 0.2%, and preferably at least 0.3% by weight. Ammonium chloride is also a useful source of chloride which also of course, acts as a source of nitrogen.

It is also advantageous to have present a methyl donor as described in our co-pending Application No. 14265/60 (Serial No. 934,526) a suitable substance being choline.

It will be understood that the precise proportions in which the various components of the medium are employed to some extent depend on the particular combination of nutrients employed and upon other conditions, e.g. aeration and the design of the fermenter vessel employed.

Various griseofulvin producing species of the genus *Penicillium* may be used in the

process according to the present invention, and in particular those referred to in Patent No. 784,618. The preferred organism is, however, *Penicillium patulum* CMI 39809 : 5 NRRL 989. Apart from the conditions referred to herein, the fermentation is conveniently carried out in accordance with general fermentation practice. Thus the organism is preferably introduced into the 10 production medium as a vegetative inoculum, some 7.5—10% of inoculum being preferably used. The rate of stirring and the rate of aeration in the medium for optimum results vary according to the shape of the fermentation vessel used, and must be determined for 15 the particular vessel in question. Generally it is desirable to initiate the fermentation at lower rates of airflow, which are increased as the fermentation proceeds. Antifoam agents, 20 e.g. of the white mineral oil type may be used to control undue foaming action in the medium.

For the better understanding of the invention, the following Examples are given by 25 way of illustration only :—

EXAMPLE 1.

The experiments were carried out in 100 ml. conical flasks containing 30 ml. of medium, shaken on a rotary shaker at 30 200—220 r.p.m. The standard medium was the corn steep liquor (C.S.L.) medium given below. To the experimental flasks ammonium sulphate was added to give 0.1% nitrogen.

35	C.S.L. Medium.	% w./v.
	Corn Steep Liquor	
	Nitrogen	0.15
	Lactose	7.0
40	KH ₂ PO ₄	0.4
	KCl	0.25
	Limestone	0.80

80	Organic (NH ₄) ₂ SO ₄	N(%)	N(%)	S(%)
	Control	0.15	0	0.026
		0.15	0.025	0.027

The medium was adjusted to pH 5.0 and sterilised at a steam pressure of 15 p.s.i.g. for 20 minutes. The inoculum was developed 45 by seeding spores obtained from a culture of *P. patulum* grown on Czapek-Dox agar into a medium consisting of :—

	% w./v.	
Corn Steep Liquor		
Nitrogen	0.30	50
Sucrose	2.0	
Chalk	1.0	

Medium adjusted to pH 6.5 pre-sterilisation.

1.5 ml. of a 48 hour culture was inoculated 55 into the production medium which gave the following titres.

Treatment	Griseofulvin mgm./L. at days	
(NH ₄) ₂ SO ₄ as %		60
Nitrogen	7 12	
0.0 control	2370 4450	
0.1	3270 4790	

EXAMPLE 2.

The stimulatory effect of (NH₄)₂SO₄, 65 during the early part of the fermentation, in a C.S.L. : Distillers solubles medium fermented under the same conditions as above, is shown below :—

	% w./v.	70
Corn steep liquor		
nitrogen	0.075	
Dried distillers solubles		
nitrogen	0.075	
Lactose	8.00	75
KH ₂ PO ₄	0.40	
KCl	0.15	
Limestone	0.80	

Griseofulvin mgm./L. at day		
9 day	9 day	13 day
3220	5780	8200
3850	6610	8580

The control flask sulphur was supplied as 0.1% Na₂S₂O₃.

EXAMPLE 3.

The Substitution of Corn steep liquor by Ammonium Sulphate or Supplementation of media containing the former with the latter in 1000 gallon stirred tanks.

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Medium.

	C.S.L. Nitrogen	..	As table and text
	Ammon. sulphate	..	As table and text
10	Nitrogen	..	text
	KH ₂ PO ₄	..	0.40% w./v.
	KCl	..	0.10% w./v.
	CaCO ₃	..	0.40% w./v.
	H ₂ SO ₄	..	0.0125% v./v.
15	Pre-inoculation Volume	..	800 gal.
	Fermentation	..	
	Temperature	..	25° C.
	Inoculum Volume	..	10%
	"Mobilpar S"	..	
20	(Registered Trade Mark—proprietary anti-foam)	..	0.0275% w./v.
	White mineral oil	..	0.0275% w./v.

The experiments were carried out on the 1,000 gallon scale. 3 impellers 1' 6" diameter

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at 220 r.p.m. were employed. The aeration rates were 0—5 hr. 40 c.f.m., 5—10 hr., 80 c.f.m. and after 10 hr., 125 c.f.m. Pre-inoculation volume 800 gal. The inoculum was grown for about 40 hr. on the following medium :—

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Corn steep liquor	Nitrogen	0.30% w./v.
Brown sugar		2.0% w./v.
Chalk		1.0% w./v.
Maize Oil		1.0% w./v.
Hodag MF Antifoam oil		0.033% v./v.

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This was inoculated with a spore suspension of *Penicillium patulum* (1 litre containing $3-5 \times 10^7$ spores/lm.) and grown at 25°, in an unbaffled 100 gallon tank. It was transferred at 40 hr. and when the mycelial volume after spinning 10 min. at 3,000 r.p.m. was over 25%.

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The fermentation was conducted as near to the "ideal" pH curve as possible by addition of crude glucose (50%) containing chloride ($\equiv 1.5\%$ KCl). The drawing accompanying the Provisional Specification shows the ideal pH curve as well as the actual pH curve and rate of glucose addition (for experiment 5).

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Exp.	% Nitrogen w/v.		g/l griseofulvin at hr.			
	As CSL	As ammon. sulphate	100	150	200	250
55	5	0.35	0.05	3.4	7.0	10.7
	6	0.40	0.00	2.8	4.8	8.1
	3	0.30	0.025	3.0	6.9	8.9
	4	0.30	0.05	2.1	6.2	9.5
	1	0.25	0.0	2.4	4.9	6.0
	2	0.25	0.025	2.6	4.8	8.0

Comparison of Experiments 1 and 2 shows the beneficial effect of ammonium sulphate additional to low levels of C.S.L.

Comparison of Experiments 2 and 3 shows the advantage of raising corn steep liquor further.

Comparison of Experiments 3 and 4 shows the advantage of increasing the ammonium sulphate from 0.025 to 0.05 at a basic level of 0.30% C.S.L. nitrogen.

Comparison of Experiments 5 and 6 shows the benefit of substituting ammonium sulphate for C.S.L. nitrogen at the 0.40% C.S.L. level.

EXAMPLE 4.

A medium was devised which consisted of optimal levels of organic nitrogen, ammonium sulphate and urea, sulphur ions, choline chloride, sugar, limestone, potassium phos-

phate and potassium chloride, as follows :—

	w/v.
Distillers solubles	0.075% N.
C.S.L.	0.075% N.
(NH ₄) ₂ SO ₄	0.025% N.
Lactose	10.0%
Choline Chloride	0.1%
Limestone	0.8%
KH ₂ PO ₄	0.4%
KCl	0.3%

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The inoculum development and production stage fermentation techniques used were identical with those used in Example 1. Varying amounts of the corn steep liquor nitrogen in this medium was replaced by urea without any loss in griseofulvin yield, as shown below :—

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	(NH ₄) ₂ SO ₄	% Nitrogen as		Urea	Griseofulvin mgm/L at day		
		Dist. Sols.	C.S.L.		7 day	10 day	13 day
5	0.025	0.075	0.075	—	6015	9750	10655
	0.025	0.075	0.05	0.025	6040	9250	9495
	0.025	0.075	0.037	0.037	6000	9597	10750
	0.025	0.075	0.025	0.050	5890	9560	11140
	0.025	0.075	—	0.075	5970	9120	10740

EXAMPLE 6.

- 10 A similar experiment to those described in Example 3 was conducted on the 1,000 gallon scale with the following combinations of nitrogen sources urea, ammonium sulphate and corn steep liquor. Fermentation control was effected in exactly the same way.

15	% N as sources			g/l griseofulvin at hr.			
	C.S.L.	Urea	Ammonium Sulphate	100	150	200	250
20	0.35	0	0.05	3.4	7.0	10.7	14.0
	0.30	0.05	0.05	2.0	2.4	7.8	11.1
	0.20	0.10	0.05	3.3	6.3	9.2	13.4

EXAMPLE 7.

- 25 The stimulatory effect on griseofulvin production of ammonium hydroxide is illustrated in this example. The seed development and incubation conditions were as described in Example 1.

Fermentation medium	% w/v.	30
Lactose	10	
Limestone	0.8	
KH ₂ PO ₄	0.4	
KCl	0.3	
Choline chloride	0.1	

35	% Nitrogen as			NH ₄ OH	Griseofulvin mgm/L at day		
	C.S.L.	Disr. Sols.	(NH ₄) ₂ SO ₄		6 day	9 day	12 day
35	0.075	0.075	0.025	Nil	4690	7830	9790
	0.037	0.075	0.025	0.037	4540	7700	10130
	0.05	0.05	0.025	0.05	4820	7270	10290

- 40 The pH of media containing ammonium hydroxide was adjusted to 5.0 with hydrochloric acid prior to sterilisation at 121° C. for 20 minutes.

EXAMPLE 8.

- 45 The use of two ammonium salts is illustrated in this example. Fermentation conditions were as in the previous example.

50	% Nitrogen as			CH ₃ COONH ₄	Griseofulvin mgm/L at day		
	C.S.L.	Dist. Sols.	(NH ₄) ₂ SO ₄		4 day	10 day	13 day
50	0.075	0.075	0.025	Nil.	2130	7970	11990
	0.05	0.05	0.025	0.05	780	6200	13070
	0.037	0.075	0.025	0.037	780	6650	11500

EXAMPLE 9.

- 55 Improvements in yield of griseofulvin may be brought about by the addition of a complex nitrogen source during the fermentation. Inoculum was developed as in Example 1. The fermentation medium was prepared as follows:—

60	Grams	
	Corn steep liquor nitrogen	0.0625 N.
60	Distillers Solubles nitrogen	0.0625 N.
	(NH ₄) ₂ SO ₄	0.025 N.
	KH ₂ PO ₄	0.40
	KCl	0.30

65	Grams.	
	Chalk	0.80
	Lactose	10.0
	Water	to 100 ml.

- 70 The pH was adjusted to 5.0, 30 ml. of medium placed in each of a series of 100 ml. conical flasks and sterilised by heat. After inoculation the flasks were shaken on a rotary shaker at 200—220 r.p.m. During fermentation they received three additions each of 1.5 ml. of medium 1 at 7 and 9 days and one or two additions of 1.5 ml. medium 2.

	<i>Medium 1.</i>		<i>Medium 2.</i>	
	<i>Grams</i>		<i>Grams</i>	
	Lactose	50	Distillers solubles	Nitrogen 0.5
5	Potassium Chloride	1	Corn Steep Liquor	Nitrogen 0.5
	Water	to 100 ml.	Water	to 100 ml.
10	<i>Treatment</i>	<i>Total Nitrogen</i>	<i>Griseofulvin mg/L (days)</i>	
	<i>Medium 2</i>		7	12
	added at (days)	%		
	0	0.15	5806	11700
	7	0.20	5676	11684
	7, 9	0.25	6089	11628
15	7, 9	0.30	5903	11425

WHAT WE CLAIM IS:—

1. A process for the production of griseofulvin by the submerged aerobic culture of a griseofulvin producing organism in a nutrient medium therefor, the assimilable nitrogen in said medium being provided by at least one complex organic source of nitrogen and ammonia or an ammonium salt. 65
2. A process as claimed in Claim 1 in which the complex organic nitrogenous material is corn steep liquor, cottonseed meal, soya bean meal, penicillin felt, ground nut meal, maize gluten meal, distillers solubles, meat extract, animal tankage or a nitrogen-containing milk product. 70
3. A process as claimed in Claim 1 in which the complex organic nitrogenous material is a mixture of corn steep liquor and distillers solubles. 75
4. A process as claimed in Claim 3 in which the ratio of corn steep liquor to distillers solubles on a nitrogen basis is within the range of from 1 : 4 to 4 : 1 by weight. 80
5. A process as claimed in Claim 3 in which the ratio of corn steep liquor to distillers solubles on a nitrogen basis is 1 : 1 by weight. 85
6. A process as claimed in any of the preceding claims in which the medium includes ammonium sulphate, ammonium chloride, ammonium carbonate, ammonium acetate or ammonium nitrate. 90
7. A process as claimed in any of the preceding claims in which at least 5% by weight of the total available nitrogen (as N) in the medium is constituted by the ammonia or ammonium salt. 95
8. A process as claimed in Claim 7 in which at least 10% by weight of the total available nitrogen (as N) is constituted by the ammonia or ammonium salt. 100
9. A process as claimed in any of the preceding claims in which the complex organic nitrogen source constitutes a major proportion (in terms of nitrogen) of the total nitrogenous nutrient.
10. A process as claimed in any of the preceding claims in which 10—20% by weight of the total nitrogenous source (in terms of nitrogen) is constituted by the ammonia or ammonium salt. 65
11. A process as claimed in any of the preceding claims in which the medium also contains urea.
12. A process as claimed in any of the preceding claims in which the culture is initiated in a medium containing the ammonia or ammonium salt and if desired some of the complex organic source of nitrogen, the complex organic source (or remainder thereof) being added at a later stage. 75
13. A process as claimed in Claim 12 in which the complex organic nitrogen source is added after the growth of the organism is well developed. 80
14. A process as claimed in any of the preceding claims in which nutrient carbohydrate is repeatedly added (continuously or batchwise) to the fermentation as it proceeds in such quantities and at such times that the pH of the medium follows a controlled pH curve as herein defined. 85
15. A process as claimed in Claim 14 in which the pH of the medium is maintained between 6.6 and 7.6 for the greater part of the fermentation. 90
16. A process as claimed in Claim 14 in which the pH of the medium is maintained between 6.8 and 7.2 for the greater part of the fermentation. 95
17. A process as claimed in any of Claims 14—16 in which the nutrient carbohydrate is glucose, lactose, sucrose, starch, hydrolysed starch, invert sugar, molasses, or invert molasses. 100
18. A process as claimed in any of the preceding claims in which the medium also contains an assimilable source of sulphur.
19. A process as claimed in Claim 18 in which the source of sulphur is a sulphate, thiosulphate or sulphuric acid. 105
20. A process as claimed in either of



	Medium 1.		Medium 2.	
	Grams		Grams	
	Lactose	50	Distillers solubles	Nitrogen 0.5
5	Potassium Chloride	1	Corn Steep Liquor	Nitrogen 0.5
	Water	to 100 ml.	Water	to 100 ml.
	Treatment Medium 2 added at (days)	Total Nitrogen %	Griseofulvin mg/L (days)	
10	0	0.15	7	12
	7	0.20	5806	11700
	7, 9	0.25	5676	11684
	7, 9	0.30	6089	11628
15	7, 9	0.30	5903	11425

WHAT WE CLAIM IS:—

1. A process for the production of griseofulvin by the submerged aerobic culture of a griseofulvin producing organism in a nutrient medium therefor, the assimilable nitrogen in said medium being provided by at least one complex organic source of nitrogen and ammonia or an ammonium salt.

2. A process as claimed in Claim 1 in which the complex organic nitrogenous material is corn steep liquor, cottonseed meal, soya bean meal, penicillin felt, ground nut meal, maize gluten meal, distillers solubles, meat extract, animal tankage or a nitrogen-containing milk product.

3. A process as claimed in Claim 1 in which the complex organic nitrogenous material is a mixture of corn steep liquor and distillers solubles.

4. A process as claimed in Claim 3 in which the ratio of corn steep liquor to distillers solubles on a nitrogen basis is within the range of from 1 : 4 to 4 : 1 by weight.

5. A process as claimed in Claim 3 in which the ratio of corn steep liquor to distillers solubles on a nitrogen basis is 1 : 1 by weight.

6. A process as claimed in any of the preceding claims in which the medium includes ammonium sulphate, ammonium chloride, ammonium carbonate, ammonium acetate or ammonium nitrate.

7. A process as claimed in any of the preceding claims in which at least 5% by weight of the total available nitrogen (as N) in the medium is constituted by the ammonia or ammonium salt.

8. A process as claimed in Claim 7 in which at least 10% by weight of the total available nitrogen (as N) is constituted by the ammonia or ammonium salt.

9. A process as claimed in any of the preceding claims in which the complex organic nitrogen source constitutes a major proportion (in terms of nitrogen) of the total nitrogenous nutrient.

10. A process as claimed in any of the preceding claims in which 10—20% by weight of the total nitrogenous source (in terms of nitrogen) is constituted by the ammonia or ammonium salt.

11. A process as claimed in any of the preceding claims in which the medium also contains urea.

12. A process as claimed in any of the preceding claims in which the culture is initiated in a medium containing the ammonia or ammonium salt and if desired some of the complex organic source of nitrogen, the complex organic source (or remainder thereof) being added at a later stage.

13. A process as claimed in Claim 12 in which the complex organic nitrogen source is added after the growth of the organism is well developed.

14. A process as claimed in any of the preceding claims in which nutrient carbohydrate is repeatedly added (continuously or batchwise) to the fermentation as it proceeds in such quantities and at such times that the pH of the medium follows a controlled pH curve as herein defined.

15. A process as claimed in Claim 14 in which the pH of the medium is maintained between 6.6 and 7.6 for the greater part of the fermentation.

16. A process as claimed in Claim 14 in which the pH of the medium is maintained between 6.8 and 7.2 for the greater part of the fermentation.

17. A process as claimed in any of Claims 14—16 in which the nutrient carbohydrate is glucose, lactose, sucrose, starch, hydrolysed starch, invert sugar, molasses, or invert molasses.

18. A process as claimed in any of the preceding claims in which the medium also contains an assimilable source of sulphur.

19. A process as claimed in Claim 18 in which the source of sulphur is a sulphate, thiosulphate or sulphuric acid.

20. A process as claimed in either of

Claims 18 or 19 in which the source of sulphur is included in the medium in a quantity sufficient to provide a level of sulphur (as S) of from 0.01—0.05% by weight.

5 21. A process as claimed in Claim 20 in which the source of sulphur is included in the medium in a quantity sufficient to provide a level of sulphur (as S) of from 0.02—0.04% by weight.

10 22. A process as claimed in any of the preceding claims in which the medium contains from 0.4 to 0.8% by weight of a water-soluble phosphate.

15 23. A process as claimed in any of the preceding claims in which the medium contains at least 0.2% by weight of soluble chloride.

24. A process as claimed in any of the preceding claims in which the medium also contains a methyl donor.

25. A process as claimed in Claim 24 in which the methyl donor is choline.

26. A process for the production of griseofulvin substantially as herein described with reference to the Examples.

27. Griseofulvin when produced by a process as claimed in any of the preceding claims.

For the Applicants,
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