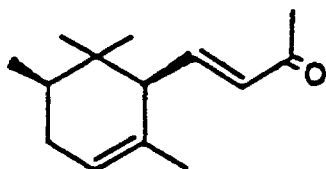


Description

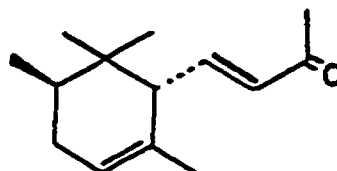
The invention relates to a process for the preparation of γ -irone by bioconversion.

This process comprises treating iris rhizomes, parts of such rhizomes, iris extracts, iris wastes, or plant cell cultures of iris, with bacteria or their active enzyme fractions in the presence of a plant cell culture medium.

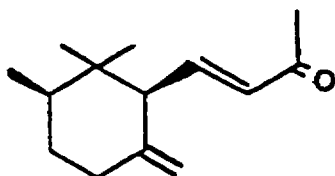
Under γ -irone there is to be understood in particular *cis*- γ -irone as depicted by formula 3 below. This *cis*- γ -irone may be associated in the reaction product with one or more of the irones depicted by formulae 1, 2 and 4.



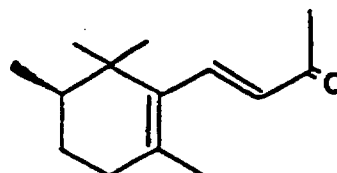
1



2



3



4

The bacterial strains of primary interest are preferably from the genera *Enterobacteriaceae* and *Pseudomonaceae* and which are preferably isolable from iris rhizomes.

Preferred species are the following:

Serratia liquefaciens

Enterobacter cloacae

Escherichia coli

Pseudomonas fluorescens

Pseudomonas maltophilia

As pointed out above, the suitable strains are preferably isolated from iris rhizomes. These rhizomes are preferably first cleaned, peeled decontaminated, reduced to small pieces and placed in a nutrient broth, then incubated in order to allow the bacteria to grow, the grown strains isolated, e.g. by the conventional dilution method, purified and identified by, e.g. biochemical test strips. The suitable nutrient broth for the bacterial growth may contain:

(i) peptone;

(ii) meat extract;

(iii) yeast extract;

(iv) inorganic salts, e.g. NaCl, NH_4NO_3 , etc.

Convenient media for the treatment of the rhizomes with the bacteria and a culture of these bacteria respectively according to the present invention are the conventional, the standard media used for the growth of plant callus and suspension cultures. They thus may contain:

(i) major inorganic nutrients;

(ii) trace elements;

(iii) an iron source;

(iv) organic supplements (vitamins);

(v) a carbon source;

(vi) organic supplements (plant growth regulators)

and may be supplemented by additional nutrient broth such as outlined above for achieving the bacterial growth.

5 These media may be, in particular, semi-solid or liquid, see for example; S.S. Bhojwani and M.K. Razdaw, Plant Tissue culture: Theory and Practice, Elsevier (1983), e.g. 21 seq., and R.A. Dixon, Plant cell culture, a practical approach, e.g. 2 seq., IRL Press, Oxford, Washington DC (1985).

Particularly suitable are thus the following media: Murashige and Skoog, Gamborg modified Heller, modified Skoog.

10 Under the term iris there is to be understood in particular *Iris pallida*, *Iris florentina*, *Iris germanica* or iris of Verona.

The rhizomes, preferably taken from the plant in a vegetative state, may already be used fresh, i.e. shortly after harvest in the form of:

- crushed or powdered rhizomes,

15 - parts or rhizomes,

- iris extracts, e.g. alcoholic, preferably methanolic

- extracts, or in the form of

- iris wastes, i.e. the residues recovered after the industrial extraction of the iris rhizomes, plant cell cultures of iris: in this case, the iris callus culture may be initiated from an explant on conventional culture media (as listed above) using standard procedures, and the so-obtained plant cells are finally combined with a culture of the microorganism.

The rhizomes are, as outlined above, suitably pretreated.

The suitable temperature range is about 15 to 40 °C, with about 25 to 35 °C being preferred.

The suitable reaction time is about 1 to 20 days, with about 3 to 6 days being preferred.

25 The suitable range of the ratio rhizome: medium is about 1:20 to 2:1 with about 1:10 to 1:1 being preferred.

In case of supplementation by nutrient broth, a reasonable ratio of plant cell culture medium to total medium (i.e. plant cell culture medium + nutrient broth) is about 0.5 - 0.95:1.

For semi-solid media, a solidifying agent, preferably agar, is added at a final concentration of about 6-15 g/liter with about 8-12 g/liter being preferred.

30 The isolation of the final product is conveniently effected by the conventional methods as used nowadays for the isolation of the volatiles from plants or for the production of fragrances and flavors, i.e. steam distillation or the Likens-Nickerson technique involving simultaneous steam distillation and extraction and using any volatile organic solvent, preferably methylene chloride diethyl ether, hexane, etc.

35 Naturally, the enzymatic treatment may also be effected in bioreactors, e.g. as batch cultures or continuous cultures. In all of these cases, conventional methods of fermentation may be applied including the use of separated phase cultures.

40 Instead of using bacteria, one can also use the active enzyme fractions obtained therefrom. Such active enzyme fractions are obtained in the usual manner by preparing aqueous extracts of the bacteria, and, if desired, cleaning such extracts by, e.g. chromatographic methods, e.g. ion exchange chromatography, liquid chromatography, gel filtration (preferred), gel electrophoresis, affinity chromatography, etc. One works conveniently in buffered solutions, e.g. between pH 5.5-6.5. Very often, cleaning is not even necessary, and the crude extract can be used.

45 The separation of the γ -irone from the remaining irones is usually not required. If necessary, it can however be accomplished in the usual way, by, e.g. chromatographic techniques, see also Y. Rautenstrauch und G. Ohloff, *Helv. Chim. Acta*, 54(7) 189 (1971).

Example 1

50 Production of γ -irone by treatment of iris rhizomes with a culture of *Serratia liquefaciens* on a semi-solid medium

Rhizomes of *Iris pallida* are taken from a plant in the vegetative state. They are first cleared from excess earth and peeled in order to remove the outer epiderm. Afterwards they are decontaminated by immersion, first in an aqueous alcoholic solution (70% EtOH/30% H₂O Vol.) for 30 seconds, then in an aqueous solution of HgCl₂ (1 g/l) for 30 seconds and finally by rinsing them three times in distilled water. [Another even more simple means of decontamination is autoclaving at 120 °C for 20 minutes].

The decontaminated rhizomes (5 g) are cut into small pieces of about 0.5 g and placed on the surface of a semi-solid Murashige and Skoog medium (Agar: 10 g/l) (note 1) which medium had previously been inoculated by puncture with the bacterial suspension of *Serratia liquefaciens* (note 2). As the control experiment a non-inoculated (i.e. non-inoculated with bacteria) solid Murashige and Skoog medium is used under otherwise identical conditions. The incubation is carried out at 30 °C for 15 days. At the end of this period the rhizomes and the corresponding medium are extracted together or separately (i.e. by separating the rhizome parts, e.g. by filtration on a nylon cloth, pore size 50 µm), by simultaneous distillation and extraction (Likens-Nickerson technique, note 3). The combined extracts (ex rhizomes + medium), as analysed by GC and GC/MS (note 4), show the following approximate results: trans- α -irone 5.4%, cis- α -irone 33% and cis- γ -irone 61%, unidentifiable: 0.6% (assuming the total amount of the irones to be 100%). If compared with the control experiment (2.8 µg/g in the combined extracts/g of fresh rhizomes used as substrate), the amount of cis- γ -irone (67 µg/g fresh rhizomes), the major natural isomer, produced by the treatment of fresh rhizomes with *Serratia liquefaciens* has thus been increased by a factor of 24.

Note 1:

Murashige and Skoog medium (T. Murashige and F. Skoog, *Physiologia Plantarum* 15, 473 (1962)) supplemented with 1 mg/l of 6-furfurylaminopurine (kinetin) and 1 mg/l of 2,4-dichlorophenoxyacetic acid; 12 ml of medium and 0.5 g of iris rhizome per test tube.

Note 2:

Isolation and identification of the bacteria *Serratia liquefaciens*.

A rhizome of *Iris pallida* is cleared from excess earth, peeled and decontaminated (30 seconds in an aqueous alcoholic solution, 70% EtOH/30% H₂O). An internal fragment of about 0.5 g of this rhizome, placed in 10 ml of nutrient broth (peptone 5 g, meat extract 1 g, yeast extract 2 g, NaCl 5 g, H₂O 1 l), is incubated at 27 °C during 15 days. From this culture the bacterium *Serratia liquefaciens* is isolated and purified on a Petri dish containing nutrient Agar of similar composition (Agar: 10 g/l), and identified by biochemical test strips API 20E/API SYSTEM S.A., 38390 Montalieu-Vercieu, France. The strain is maintained by a weekly transfer on Petri dishes containing nutrient Agar and can be preserved by any standard method.

Note 3:

Likens-Nickerson technique following the procedure described by T.H. Schultz & al. *J. Agric. Food Chem.* 25, 446 (1977) using a volatile solvent, preferably methylene chloride.

Note 4:

Gas chromatography: DELSI DI 700, capillary column Carb. 20M, vector gas 4.5 ml/mn Helium, programming 70/180 °C 2 °C/mn. Mass Spectrometer: FINNIGAN 4500, electronic impact.

Example 2

Production of γ -irone by treatment of iris rhizomes with a culture of *Serratia liquefaciens* in a liquid medium

For these experiments three different types of rhizomes have been used:

- Batch I: fresh rhizomes in a vegetative state, cleared from excess earth, peeled and cut into small pieces as described in example 1.
- Batch II: dry unpeeled rhizomes harvested in Italy in August 1987, stored at 4 °C before utilization (3-6 month) then powdered just before use.
- Batch III: dry unpeeled rhizomes, matured for 3 years at room temperature and several months at 45 °C and then treated as above (batch II).

a) Experiments With the Murashige and Skoog medium

The powdered and cut rhizomes respectively are sterilized in 250 ml Erlenmeyer flasks (5 g rhizomes per flask) by autoclaving at 120 °C during 30 min. After cooling, 100 ml of Murashige and Skoog medium

and 10 ml of nutrient broth (see example 1 and Note 5) are added to each flask under sterile conditions. With the exception of the control experiments each of the flasks is inoculated with 0.5 ml of a 24 hours old culture (nutrient broth, 25°C, giratory shaker) of *Serratia liquefaciens* (see example 1 for the isolation) and incubated on a giratory shaker (100 revolutions/mn) at 25°C during two weeks (Note 6). At the end of this period both the rhizomes and the medium are extracted and analysed as described in example 1.

The results (batch I, II, III) expressed in mg of irones/kg of dry-weighed rhizomes are given in Table I. The average distribution of irones in these extracts is the following:

- trans- α -irone 6.5%,
- cis- α -irone 34%,
- cis- γ -irone 58%.
- β -irone 1.5%,
- unidentifiable irone(s) 0.2%

In contradistinction thereto, the classical Likens-Nickerson extraction (without microbiological treatment) of mature rhizomes (batch III) yields 700 mg of irones/kg of dry-weighed rhizomes exhibiting approximately the following distribution:

- trans- α -irone 4%,
- cis- α -irone 44%,
- cis- γ -irone 50%,
- β -irone 1.2%.
- unidentifiable 0.8%

Table I

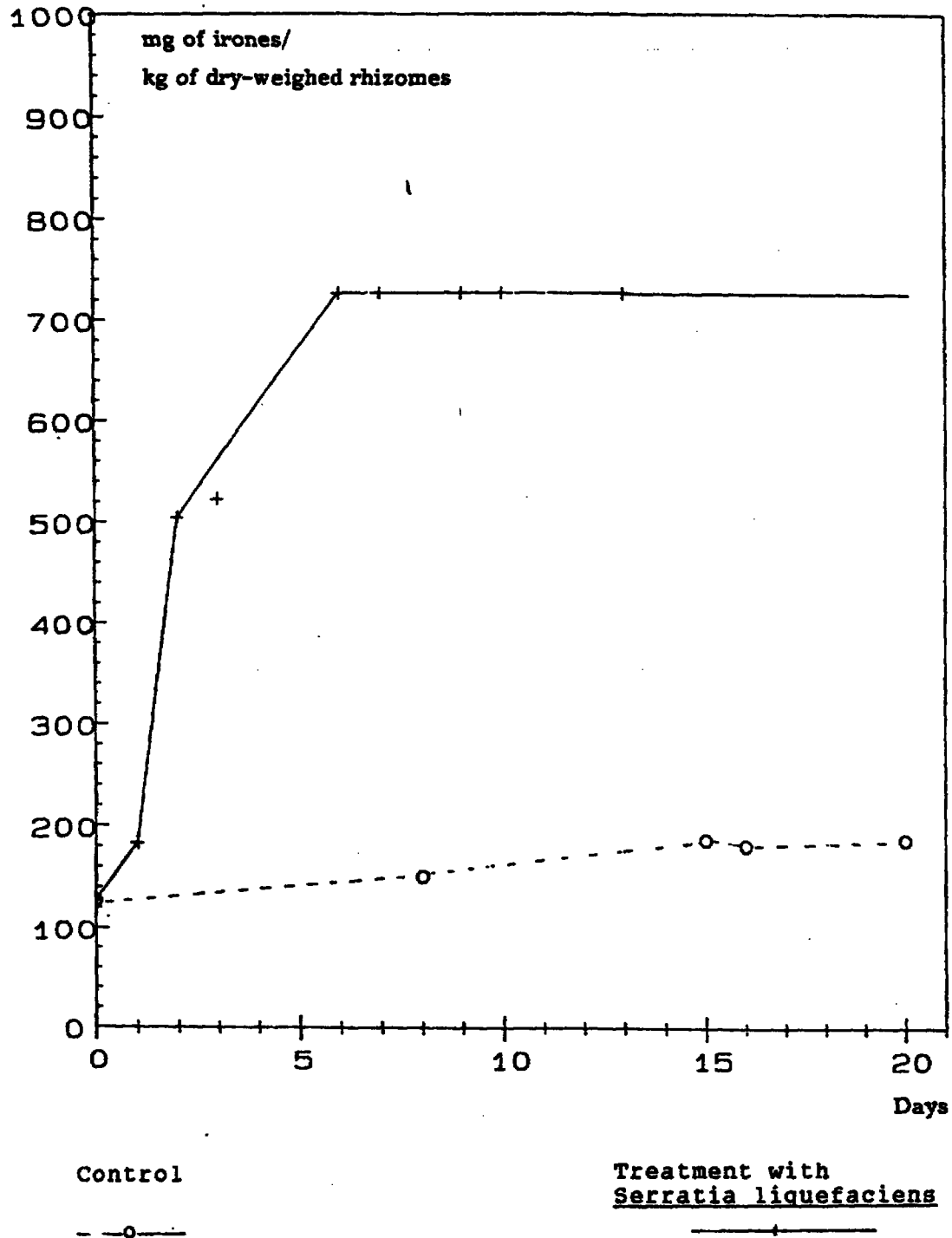
	Batch I	Batch II	Batch III
(T) Treatment with <i>Serratia liquefaciens</i>	720	675	822
(C) Control experiment	68	198	623
(R) = T/C	10.5	3.5	1.3

Note 5:

Under normal conditions of bacterial growth, the treatment of iris rhizomes with *Serratia liquefaciens* by using a nutrient broth only (i.e. a conventional nutrient broth for the bacterial growth) does not lead to any increase of the content of irones.

Note 6:

A kinetic study has shown that for batch I the maximum content (720 mg of irones/kg of dry-weighed rhizomes) was obtained after 5 days of incubation. (cf. Fig. 1).

Fig. 1Kinetic study of the production of γ -irone in liquid mediumb) Experiments with several other types of plant cell culture media

The replacement of the Murashige and Skoog medium by several further types of plant cell culture media (Gamborg also called medium B5, modified Heller, modified Skoog, cf. Plant Tissue Culture, Theory

and Practice, S.S. Bhojwani and M.K. Razdaw, Elsevier 1983), using otherwise unchanged reaction conditions (cf example 2a), leads also to a significant increase of the content of irones, see Table II.

Table II

Variation of the medium				
	Gamborg	Modified Heller*	Modified Skoog**	Murashige and Skoog
mg of irones/kg of dry-weighed rhizomes	760	360	630	685

* Modified Heller: Heller medium with Morel Vitamins (Wetmore R.H., Am. J. Bot., 38 (1951), 141-143; 2-4D (2,4-dichlorophenylacetic acid) (1 mg/ml).

** Modified Skoog: Murashige and Skoog medium with Morel Vitamins, 2-4D (1 mg/l), kinetin (1 mg/l).

The strain *Serratia liquefaciens* made use in the Examples was deposited as S.I.I.v under the number I-780 on July 12, 1988 at the Institut Pasteur, Collection Nationale de Cultures de Microorganismes (C.N.C.M.), 25, rue de Docteur ROUX 75724-Paris Cedex 15.

Example 3

Production of γ -irone by treatment of iris rhizomes with several other bacterial strains.

Fresh rhizomes (batch I, example 2) are treated as in example 2a for one week using one of the following bacteria:

Enterbacteriaceae: *Enterobacter cloacae* *
Escherichia coli **

Pseudomonaceae: *Pseudomonas fluorescens* *
Pseudomonas maltophilia (ATCC 17866)

After incubation both the rhizomes and the corresponding medium are extracted and analysed as described in example 1a. The results, expressed in mg of irones/kg of dry-weighed rhizomes are given in Table III.

Table III

Influence of strains				
	<i>Enterobacter cloacae</i>	<i>Escherichia coli</i>	<i>Pseudomonas fluorescens</i>	<i>Pseudomonas maltophilia</i>
mg of irones/kg of dry-weighed rhizomes	1073	535	187	174

* Isolated from iris rhizomes (procedure for the isolation as in example 1, note 2), the particular strain of *E.cloacae* was deposited as *E.cloacae* 1 under the number I-779 on July 12, 1988 at the C.N.C.M.

** Strain 54127, subcultured from the collection of the "Unité de Bactéries de l'Institut Pasteur" and supplied by Dr. C. Michel, Laboratoire d'Ichtyopathologie, INRA, Route de Thiverval, 78850 Thiverval-Grignon. The strain has been redeposited as *E.coli* 2 under the number I-794 on July 28, 1988 at the C.N.C.M.
I 779, I 780 and I 794 are Budapest deposits.

Example 4Production of γ -irone by treatment of other substrates derived from *Iris pallida* with *Serratia liquefaciens* in liquid medium.a) plant cell culture of *Iris pallida*

Callus cultures of *Iris pallida* have been initiated on Murashige and Skoog medium (cf example 1, note I) and on Gamborg medium (cf example 2b) according to a classical procedure (Plant Tissue, Theory and Practice. S.S. Bhojwani and M.K. Razdaw, Elsevier (1983)). The two strains I1 and I2 of *Iris* cell cultures are maintained on Petri dishes containing 45 ml of semi-solid Murashige and Skoog medium and are subcultured monthly on fresh medium. These strains are transferred to a liquid medium (Murashige and Skoog or Gamborg without Agar), maintained on a giratory shaker (26 °C, 100 revolutions/min, 15 days), filtered on a nylon cloth (pore size 50 μ m) and subcultured every two weeks on fresh medium. Once the cultures are two weeks old, 5 g of fresh-weighed cells are placed in 250 ml Erlenmeyer flasks containing 100 ml of Murashige and Skoog medium and 10 ml of nutrient broth (cf example 1). Each flask, except those used for the control experiments, is inoculated with 0.5 ml of a 24 h old culture of *Serratia liquefaciens*. They are then incubated on a giratory shaker (100 revolutions/mn) at 25 °C during two weeks. At the end of this period both the biomass and the medium are extracted and analysed as described in example 1. The results expressed in mg of irones/kg of dry-weighed *iris* cells are given in table IV.

Table IV

	Iris cell strain I1	Iris cell strain I2
Treated iris cells	35	160
Control experiment	0	0

b) Treatment of iris wastes

These wastes are the residues recovered after the industrial hydrodistillation of powdered *iris* rhizomes. These wastes are treated, as described in example 2a, with *Serratia liquefaciens*. In table V the results of the analysis of the treated wastes (mg of γ -irone/kg of wastes) are compared to those of the control experiment (without bacteria).

Table V

	Control experiment C	Treatment with <i>Serratia liquefaciens</i> T	T/C
mg of γ -irone/kg of waste	40	102	2.6

c) Treatment of iris extracts

A crude extract of *iris* rhizomes (from Batch II, see example 2) was prepared as follows: finely divided rhizomes (1000 g) are extracted twice with 1000 ml of methanol under agitation for 4 hours. After filtration, the combined methanolic solutions are concentrated.

A 5 g sample of this resulting crude extract is dissolved in methanol (10 ml) and portions of this solution (0.5 ml), sterilized by microfiltration (Sartorius NMLPF 0.22 μ m membrane; SARL, BP 27, 11 avenue du ler mai, 91122 Palaiseau cedex) are added to Erlenmeyer flasks, each of them containing 100 ml of sterile Murashige and Skoog medium and 10 ml of sterile nutrient broth. After inoculation with 0.5 ml of a 24 hours old culture (nutrient broth, 25 °C, giratory shaker) of *Serratia liquefaciens*, the flasks are incubated for 7 days. The total biomass is extracted and analysed as described in example 1. The results are listed in Table VI.

Table VI

	Control experiment	Treatment with <i>Serratia liquefaciens</i>
mg of irones/liter culture	0.17	0.45

Example 5

Production of γ -irone by treatment of fresh iris rhizomes with a culture of *Serratia liquefaciens* in bioreactors under batch or double phase separating conditions.

a) Production of irones in a batch stirred bioreactor

Powdered rhizomes (150 g) from Batch II (see example 2) and three liters of Murashige and Skoog medium are placed in a 5 liter bioreactor and autoclaved at 115°C during 25 minutes. After addition of 300 ml of sterilized nutrient broth (see example 1) the mixture is inoculated with 50 ml of a bacterial suspension of *Serratia liquefaciens* (cf example 1). The culture is maintained at 25°C for 8 days under mechanical stirring (100 r/mn) and an aeration of 0.2 V.V.M. (0.2 volumes air/volume of medium/min.). Samples of 100 ml are taken after 1, 2 and 5 days for analysis. A control experiment without bacterial inoculation (i.e. medium not inoculated with *Serr. liq.*) is run into an Erlenmeyer flask on a giratory shaker under otherwise identical conditions. Extraction of the samples furnished the results as given in Table VII (analysis carried out as described in example 1).

Table VII

Sampled after:	mg of irones/kg of dry-weighed rhizomes
24 h	411
48 h	404
120 h	370
120 h (control)	160

b) Production of γ -irone under double phase separating conditions

Operating conditions for keeping separated of the rhizomes from the bacteria by a dialysis membrane:

Small pieces of peeled iris rhizomes (Batch I, example 2) are placed in 10 cm dialysis tubes (Prolabo [BP 369; 75525 Paris Cedex]. Ref. 24132, 5 g/tube) and both ends of the tubes are closed with special plastic joints (Spectra [Spectrum Medical Industry Inc. 60916 Terminal Annex, Los Angeles, California 960]; ref. 132736). Each one of the so prepared dialysis tubes is placed in a 500 ml flask containing 250 ml of Murashige and Skoog medium and the whole is autoclaved at 115°C during 20 minutes. After cooling and addition of nutrient broth (30 ml) each flask, except those used for the control experiments, is inoculated with 1.5 ml of a bacterial suspension of *Serratia liquefaciens* (cf example 1). After one week of incubation at 25°C on a giratory shaker (100 r/mn), both the total biomass (rhizomes ex tube and bacterial ex flask) and the medium (ex tube and ex flask) are extracted and analysed as described in example 2a. The results are shown in Table VIII.

Table VIII

	Control experiment * Treatment with	
	(T/C)	Serratia liquefaciens
	(C)	(T)
mg of irones/ kg of dry- weighed rhizomes	63	1074
		17

* M & S medium + nutrient broth is not inoculated

Example 6

Production of γ -irone by treatment of iris rhizomes with an enzyme fraction, as represented by a bacterial cell free medium

The process is carried out in two steps:

- first step: preparation of a "conditioned medium", i.e. incubation of iris rhizomes with *Serratia liquefaciens* in a liquid plant cell culture medium + nutrient broth and separation of the residual rhizomes this medium now contains the enzymes.
- second step: incubation of iris rhizomes with the "conditioned medium".

a) First step:

Dry rhizomes (batch II, example 2) are incubated with *Serr. liq.* as in example 2a (4 x 250 ml Erlenmeyer flasks) for one week except that the Murashige and Skoog medium is replaced by a buffered plant cell culture medium which is prepared in the following way:

- a biological buffer (M.E.S.) i.e. 2-(N-morpholino)ethanesulfonic acid; Sigma ref.: M 8250) is added to non-sterilized Murashige and Skoog medium to obtain a concentration of 50 mmol/l and the pH is adjusted to 6 using a 3 N KOH solution. The mixture is autoclaved at 115°C during 20 min. or sterilized by microfiltration (Millipore membrane, 0.2 μ m).

After one week of incubation no bacterial viability was observed any more in the four flasks. At the end of this period the residual rhizomes were separated from the medium by filtration on a nylon cloth of 50 μ m porosity. In the following the thus obtained filtrate (medium + dead bacterial cells + enzymes) is called "conditioned medium".

b) Second step

The "conditioned medium" (100 ml) is centrifuged at a temperature of 0-4°C under 12000 g during 10 min or under 43140 g during 20 min. in order to separate first the dead bacterial cells from the medium.

- The supernatant is used to incubate 5 g of iris rhizomes (batch II of Example 2, sterilized by autoclaving at 120°C during 30 mn) in a 250 ml Erlenmeyer flask on a giratory shaker (100 revolutions/mn, at 25°C during one week.

- The centrifugation residue is mixed with 100 ml of the previously prepared buffered Murashige and Skoog medium whose pH had been adjusted to 5 and then used as above to incubate 5 g of iris rhizomes.

Control experiments using the "conditioned medium" with and without rhizomes were run under otherwise identical conditions.

Extraction of the samples and analysis as described in example 1 afforded the results as given in table IX.

Table IX

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	mg of irones/kg of dry-weighted rhizomes
Control experiments (a)	
- Conditioned medium	29
- Conditioned medium + rhizomes	868
Experimental cultures (b)	
- Supernatant + rhizomes	772
- Centrifugation residue + rhizomes	174
Centrifugation 43140 g/10 mn	
- Supernatant + rhizomes	703
- Centrifugation residue + rhizomes	229

Conclusions:

40

a) establishes once more the formation of γ -irone ex (non-mature) rhizomes in a liquid medium (see also Ex. 2).

45

b) established the fact that the major part of the active enzymes are soluble in water, are extra-cellular enzymes.

The strains I-780, I-779, I-794 will be available to the public as from February 5, 1990.

50

Example 7

Production of γ -irone by treatment of iris rhizomes with an enzyme fraction

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The procedure is as described in example 6, except that the "conditioned medium" prepared in the first step of Example 6 is replaced by a so-called "preculture" (without using rhizomes) obtained in the following way:

100 ml of a buffered plant cell culture medium prepared as described in example 6 and 10 ml of

nutrient broth (see example 1) are added to each flask of a group of 4 x 250 ml Erlenmeyer flasks. Each of the flasks is inoculated with *Serratia liquefaciens* and incubated as in example 2. After an incubation period of 24 h this culture, called preculture, is used for the second step, instead of the "conditioned medium", as described and used in example 6. The results are as given in table X.

Table X

	mg of irones/kg of dry - weighed rhizomes
Control experiment	236
Experimental culture	
Centrifugation 43140 g/20 mn	
- Supernatant + rhizomes	615

Claims

1. Process for the preparation of γ -irone by bioconversion, comprising treating iris rhizomes, parts of such rhizomes iris, extracts iris, extraction wastes or plant cell cultures of iris, with bacteria, preferably of the genera Enterobacteriaceae or Pseudomonaceae, or their active enzyme fractions in the presence of a plant cell culture medium.
2. Process according to claim 1, wherein the treatment of the rhizomes is performed with a culture of *Serratia liquefaciens*, *Enterobacter cloacae*, *Escherichia coli*, *Pseudomonas fluorescens* or *Pseudomonas maltophilia* or an active enzyme fraction thereof.
3. Process according to claim 1 or 2, wherein the bacterial culture is isolable from iris rhizomes.
4. Process according to claims 1, 2 or 3, wherein the rhizomes stem from *Iris pallida*, *Iris fluorentina*, *Iris germanica* or *iris of Verona*.
5. Process according to any one of claims 1 to 4, wherein fresh rhizomes are used.
6. Process according to any one of claims 1 to 4, wherein iris extracts serve as a starting material.
7. Process according to any one of claims 1 to 4, wherein iris extraction wastes serve as starting material.
8. Process according to any one of claims 1 to 4, wherein plant cell cultures of iris serve as starting material.
9. Process according to any one of claims 1 to 8, wherein said culture medium is the Murashige and Skoog, the Gamborg, the modified Heller or the modified Skoog medium, preferably used in the presence of a nutrient broth.
10. Process according to any one of claims 1 to 9, wherein the rhizomes are treated in a semi-solid medium.
11. Process according to any one of claims 1 to 9, wherein the rhizomes are treated in a liquid medium.



nutrient broth (see example 1) are added to each flask of a group of 4 x 250 ml Erlenmeyer flasks. Each of the flasks is inoculated with *Serratia liquefaciens* and incubated as in example 2. After an incubation period of 24 h this culture, called preculture, is used for the second step, instead of the "conditioned medium", as described and used in example 6. The results are as given in table X.

Table X

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Claims

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2. Process according to claim 1, wherein the treatment of the rhizomes is performed with a culture of *Serratia liquefaciens*, *Enterobacter cloacae*, *Escherichia coli*, *Pseudomonas fluorescens* or *Pseudomonas maltophilia* or an active enzyme fraction thereof.
3. Process according to claim 1 or 2, wherein the bacterial culture is isolable from iris rhizomes.
4. Process according to claims 1, 2 or 3, wherein the rhizomes stem from *Iris pallida*, *Iris fluorentina*, *Iris germanica* or iris of Verona.
5. Process according to any one of claims 1 to 4, wherein fresh rhizomes are used.
6. Process according to any one of claims 1 to 4, wherein iris extracts serve as a starting material.
7. Process according to any one of claims 1 to 4, wherein iris extraction wastes serve as starting material.
8. Process according to any one of claims 1 to 4, wherein plant cell cultures of iris serve as starting material.
9. Process according to any one of claims 1 to 8, wherein said culture medium is the Murashige and Skoog, the Gamborg, the modified Heller or the modified Skoog medium, preferably used in the presence of a nutrient broth.
10. Process according to any one of claims 1 to 9, wherein the rhizomes are treated in a semi-solid medium.
11. Process according to any one of claims 1 to 9, wherein the rhizomes are treated in a liquid medium.



12. Process according to any one of claims 1 to 9 and 11, wherein the enzymatic preparation of γ -irone is carried out in a bioreactor.
- 5 13. Process according to any one of claims 1 to 12, wherein cis- α -irone, trans- α -irone, and/or β -irone are produced together with the cis- γ -irone.
14. Process according to any one of claims 1 to 13, wherein the treatment of the rhizomes is performed at room temperature or slightly elevated temperatures (about 15-40 ° C).
- 10 15. Process according to any one of claims 1 to 14, wherein the treatment of the rhizomes is carried out for 1 to 15 days.
16. Process according to any one of claims 1 to 15, wherein the irones are isolated by way of extraction and/or distillation.

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Patentansprüche

- 20 1. Verfahren zur Herstellung von γ -Iron durch Biokonversion, dadurch gekennzeichnet, dass man Irisrhizome, Teile von solchen Rhizomen, Irisextrakte, Abfälle von Irisextrakten oder Irispflanzenzellkulturen mit Bakterien, vorzugsweise der Genera *Enterobacteriaceae* oder *Pseudomonaceae*, oder deren aktive Enzymfraktionen in Anwesenheit eines Pflanzenzellkulturmediums behandelt.
- 25 2. Verfahren nach Anspruch 1, dadurch gekennzeichnet, dass die Behandlung der Rhizome mittels einer Kultur von *Serratia liquifaciens*, *Enterobacter cloacae*, *Escherichia coli*, *Pseudomonas fluorescens* oder *Pseudomonas maltophilia* oder eine aktive Enzymfraktion davon erfolgt.
3. Verfahren nach Anspruch 1 oder 2, dadurch gekennzeichnet, dass die Bakterienkultur aus Irisrhizomen isolierbar ist.
- 30 4. Verfahren nach einem der Ansprüche 1, 2 oder 3, dadurch gekennzeichnet, dass die Rhizome von *Iris pallida*, *Iris fluorentina*, *Iris germanica* oder *Iris* von Verona stammen.
5. Verfahren nach einem der Ansprüche 1 bis 4, dadurch gekennzeichnet, dass frische Rhizome verwendet werden.
- 35 6. Verfahren nach einem der Ansprüche 1 bis 4, dadurch gekennzeichnet, dass Irisextrakte als Ausgangsmaterial dienen.
- 40 7. Verfahren nach einem der Ansprüche 1 bis 4, dadurch gekennzeichnet, dass Abfälle von Irisextrakten als Ausgangsmaterial dienen.
8. Verfahren nach einem der Ansprüche 1 bis 4, dadurch gekennzeichnet, dass Irispflanzenzellkulturen als Ausgangsmaterial dienen.
- 45 9. Verfahren nach einem der Ansprüche 1 bis 8, dadurch gekennzeichnet, dass als Kulturmedium das Murashige und Skoog-, das Gamborg-, das modifizierte Heller- oder das modifizierte Skoogmedium, vorzugsweise in Anwesenheit einer Nährbrühe, verwendet wird.
- 50 10. Verfahren nach einem der Ansprüche 1 bis 9, dadurch gekennzeichnet, dass die Rhizome in einem halbfestem Medium behandelt werden.
11. Verfahren nach einem der Ansprüche 1 bis 9, dadurch gekennzeichnet, dass die Rhizome in flüssigem Medium behandelt werden.
- 55 12. Verfahren nach einem der Ansprüche 1 bis 9 und 11, dadurch gekennzeichnet, dass die enzymatische Herstellung von γ -Iron in einem Bioreaktor durchgeführt wird.



13. Verfahren nach einem der Ansprüche 1 bis 12, dadurch gekennzeichnet, dass man zusammen mit dem cis- γ -Iron cis- α -Iron, trans- α -Iron und/oder β -Iron herstellt.
14. Verfahren nach einem der Ansprüche 1 bis 13, dadurch gekennzeichnet, dass die Behandlung der Rhizome bei Zimmertemperatur oder leicht erhöhten Temperatur (ungefähr 15-40 ° C) durchgeführt wird.
15. Verfahren nach einem der Ansprüche 1 bis 14, dadurch gekennzeichnet, dass die Behandlung der Rhizome 1 bis 15 Tage dauert.
16. Verfahren nach einem der Ansprüche 1 bis 15, dadurch gekennzeichnet, dass die Irone durch Extraktion und/oder Destillation isoliert werden.

Revendications

1. Procédé de préparation de γ -irone par bioconversion, comprenant le traitement de rhizomes d'iris, de parties de ces rhizomes, d'extraits d'iris, de déchets d'extraction d'iris ou de cultures cellulaires végétales d'iris, avec des bactéries, de préférence du genre enterobacteriaceae ou pseudomonas, ou leurs fractions enzymatiques actives, en présence d'un milieu de culture cellulaire végétale.
2. Procédé selon la revendication 1, dans lequel le traitement des rhizomes est réalisé avec une culture de Serratia liquefaciens, Enterobacter cloacae, Escherichia coli, Pseudomonas fluorescens ou Pseudomonas maltophilia, ou une fraction enzymatique active de celle-ci.
3. Procédé selon la revendication 1 ou 2, dans lequel la culture bactérienne peut être isolée de rhizomes d'iris.
4. Procédé selon la revendication 1, 2 ou 3, dans lequel les rhizomes proviennent d'Iris pallida, d'Iris fluorentina, d'Iris germanica ou d'Iris de Vérone.
5. Procédé selon l'une quelconque des revendications 1 à 4, dans lequel on utilise des rhizomes frais.
6. Procédé selon l'une quelconque des revendications 1 à 4, dans lequel des extraits d'iris servent de matière première.
7. Procédé selon l'une quelconque des revendications 1 à 4, dans lequel des déchets d'extraction d'iris servent de matière première.
8. Procédé selon l'une quelconque des revendications 1 à 4, dans lequel des cultures cellulaires végétales d'iris servent de matière première.
9. Procédé selon l'une quelconque des revendications 1 à 8, dans lequel ledit milieu de culture est le milieu de Murashige et Skoog, le milieu de Gamborg, le milieu de Heller modifié ou le milieu de Skoog modifié, utilisé de préférence en présence d'un bouillon nutritif.
10. Procédé selon l'une quelconque des revendications 1 à 9, dans lequel les rhizomes sont traités dans un milieu semi-solide.
11. Procédé selon l'une quelconque des revendications 1 à 9, dans lequel les rhizomes sont traités dans un milieu liquide.
12. Procédé selon l'une quelconque des revendications 1 à 9 et 11, dans lequel la préparation enzymatique de γ -irone est réalisée dans un bioréacteur.
13. Procédé selon l'une quelconque des revendications 1 à 12, dans lequel de la cis- α -irone, de la trans- α -irone et/ou de la β -irone sont produites avec la cis- γ -irone.



14. Procédé selon l'une quelconque des revendications 1 à 13, dans lequel le traitement des rhizomes est réalisé à la température ambiante ou à des températures légèrement supérieures (environ 15-40 °C).
- 5 15. Procédé selon l'une quelconque des revendications 1 à 14, dans lequel le traitement des rhizomes est réalisé pendant 1 à 15 jours.
16. Procédé selon l'une quelconque des revendications 1 à 15, dans lequel les irones sont isolées par extraction et/ou par distillation.

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