From the Mayan Highlands to the Bioreactors: *In Vitro* Tissue Culture of the Mexican Medicinal Plant *Solanum chrysotrichum*

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ABSTRACT

***Solanum chrysotrichum* of the Solanaceae family was selected for investigation as according to ethnomedical knowledge it represents the plant most widely used by the Highland Maya from Chiapas, Mexico for the treatment of skin mycosis. Research with a multidisciplinary focus has been applied to study the pharmacological, phytochemical, clinical and biotechnological aspects of this plant species, and is reviewed here, in this paper. *In vitro* pharmacological studies demonstrated the efficacy of this plant for inhibiting the growth of dermatophytes (*Trycophyton mentagrophytes*, *T. rubrum* and *Microsporum gypseum*) in culture. Clinical tests were conducted and confirmed the efficacy of plant extracts for treating patients suffering from *tinea pedis*. Phytochemical studies achieved the isolation and purification of the antimycotic principles, which were found to be present in a family consisting of six novel spirostanol saponins, designated as SC-1 to SC-6. In order to obtain higher yields of the saponins, we applied a number of biotechnological procedures including micropropagation and the establishment of cell and hairy root cultures. Cell suspensions were scaled-up in 10 L airlift bioreactors. Novel fittings on 2 and 10 L airlift reactors were designed and evaluated to up the scale of *S. chrysotrichum* hairy roots, permitting the production of higher yields of the most active saponins; SC-2 and SC-4. This Mexican plant represents an important popular remedy, whose cultivation in bioreactors was made possible for the first time. Here we review the procedures involved in the bio-production of antimycotic chemicals from the cells and hairy roots of the plant on a larger scale.**

IMPORTANCE OF THE PLANT

A group of plants commonly known as "sosas" are used among the Highland Maya of Chiapas, Mexico, for the treatment of skin ailments and dermatological infections. Traditional healers describe the plant *S. chrysotrichum* (Schldl) from the Solanaceae family, as the most effective herbal remedy for the treatment of tinae (*Tinae pedis*), scabies and other mycosis (Zurita and Zolla, 1986; Lozoya and Aguilar, 1987). Mayan ethnic groups apply different names to this plant: "*kúx peul*" among the Tzotzil, "*kúxbal chíx*" among the Tzeltal, and "*pajutiek*" among the Chol.

Herbal medication produced from this plant is normally prepared by boiling fresh leaves in water and administering the solution topically as plasters or poultices, but it is also sometimes prescribed as an oral infusion (Zurita and Zolla, 1986). The plant is a perennial herb which is able to grow up to 2 m in height and has spiny stems. The leaves are rough to touch, 20-30 cm long and 10 cm wide and covered with large hairs. The flowers are white with a star-like appearance (Figure 1).

Figure 1. Adult specimen of *Solanum chrysotrichum* (Schldl.).

IN VITRO PHARMACOLOGICAL TESTING

Initial pharmacological research concerning plants collected in Chiapas, consisted of testing for their ability to inhibit the growth of bacteria and dermatophytes in culture. Organic solvent extracts were prepared from leaves and tested against Gram positive and Gram negative bacteria, as well as against the yeast *Candida albicans* and the dermatophyte *Microsporum gypseu.* Promising activity against these last two microorganisms was evident. When the extracts were specifically tested against the main causative agents of athlete's foot: *T. mentagrophyts*, *T. rubrum* and *M. gypseum,* they exhibited significant antifungal activity (Lozoya et al., 1991).

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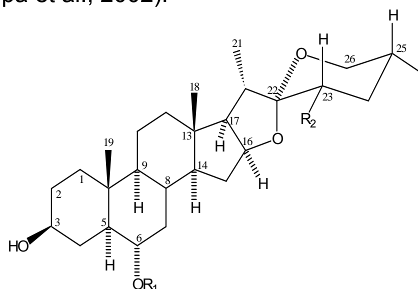
ISOLATION AND PURIFICATION OF ANTIFUNGAL COMPOUNDS

A major bioactive constituent, obtained from the methanolic extract prepared from the leaves of *S. chrysotrichum* was purified by means of bioassay guided fractionations using *T. mentagrophytes* as the biological monitor. The molecular structure of this compound designated as SC-1 was established on the basis of spectral analyses, mainly proton and ^{13}C-NMR, including two-dimensional techniques, i.e. ^{1}H-^{1}H COSY, HMQC and HMBC, and was identified as consisting of a novel spirostanol saponin with glycoside moieties. The chemical structure of SC-1 was established as 3-O-{β-D-quinovopyranosyl (1→6)-β-D-glucopyranosyl (1→6)-β-D-glucopyranosyl} chlorogenin (Alvarez et al., 2001). In another series of studies and using bioactivity-directed isolation procedures, five new spirostan saponins and two sterol glycosides were isolated. The structure of the new saponins designated as SC-2 – SC-6 (Figure 2) was established, based upon spectroscopic measurements, especially ID and 2D NMR data referring to their peracetate derivatives (Zamilpa et al., 2002). All the isolated compounds were tested against dermatophytes in culture (*T. mentagrophytes* and *T. rubrum*) and all manifested antifungal activity. The most active compound was shown to be SC-2 (MIC values of 12.5 µg L^{-1} each) followed by SC-4 (MIC values of 25 and 50 µg mL^{-1} against *T. mentagrophytes* and *T. rubrum* respectively (Zamilpa et al., 2002).



	R_1	R_2
SC-1	Qui(1→6)-Glc(1→6)-Glc	H
SC-2	Xyl(1→3)-Qui	H
SC-3	Xyl	H
SC-4	Qui	H
SC-5	Rha(1→3)-Qui	H
SC-6	Rha(1→3)-Qui	OH

Figure 2. Chemical structure of saponins SC-1 – SC-6 from *Solanum chrysotrichum*

CLINICAL STUDIES

In order to evaluate the effectiveness of plant extracts in humans, a pilot clinical study was carried out, using extracts from leaves of *S. chrysotrichum* among patients with *tinae pedis* who attended the Regional Hospital of the Mexican Social Security Institute in Cuernavaca, Morelos, Mexico. A group of 18 ambulatory patients was selected and treated with a cream containing 5% of the methanolic extract of the leaves, which was applied topically during 4 weeks of treatment, and these were then compared with a similar number of infected patients that were treated with miconazole. The results showed that after one week, 42% of the patients from the group receiving the plant extract recovered, while no cure was observed among those receiving miconazole during this early period. Remission of symptoms was observed among both groups when each treatment was completed (García-Cruz, 1988). In a controlled and randomized clinical investigation conducted recently, the effectiveness and tolerability of a standardized phytodrug prepared from *S. chrysotrichum* was tested among 101 patients diagnosed with *tinae pedis*. A standardized solution was prepared from saponin SC-2, obtained from the plant and applied to the experimental group, while 2% ketoconazole was administered to the control group. Both treatments were applied topically during a period of four weeks. After the treatment, the results showed a clinical effectiveness of 96% in the experimental group, and 92% for the ketoconazole group, with good tolerability (100%) in the case of both groups (Herrera-Arellano et al., 2003).

BIOTECHNOLOGICAL INVESTIGATIONS

Micropropagation and Callus Formation

Due to the fact that *S. chrysotrichum* grows in a restricted area of Chiapas Mexico, and is currently threatened by over-harvesting, we established the micropropagation of this species, as well as the development of calluses, using axillary buds. Explants were grown in Murashige and Skoog's (MS) medium, supplemented with various growth regulators. Induction of rooted plants was initiated, only when indol-3 acetic acid (IAA) was present as an auxin in combination with either of two cytokinins: kinetin (KN) or benzyladenine (BA); however, the combination of IAA (0.1 mg L^{-1}) + BA (0.2 mg L^{-1}) was found to be best suited to the purpose of morphogenesis. Adaptation among *in vitro*-derived rooted plants was high (94%), and twelve months after adapting, the plants flowered (Villarreal and Muñoz, 1991). Micro-propagated plants have been used as a source of raw material in order to carry out chemical and pharmacological studies.

Cell Suspension Cultures

Once the first bioactive saponin SC-1 was isolated and elucidated and with the aim of producing high and controlled levels of the antifungal compound, we decided to establish *in vitro* cell culture systems for *S. chrysotrichum*. Initially, we developed cell suspension cultures from friable calluses that were cultivated in MS medium, in combination with 0.1 mg L^{-1} naphtalene acetic acid (NAA) + 0.2 mg L^{-1} KN (Villarreal and Muñoz, 1991). The suspensions were established in 100 mL MS media (250 mL Erlenmeyer flasks), supplemented with 2 mg L^{-1} KN, and with four different auxins; MS1 [2 mg L^{-1} 2,4 dichlorophenoxyacetic acid (2,4-D)], MS2 (0.5 mg L^{-1} NAA), MS3 (1.3 mg L^{-1} IAA) and MS4 [1 mg L^{-1} 2,4,5-trichlorophenoxyacetic acid (2,4,5-T)]. The flasks were incubated in a batch mode at 130 rpm during 25 days of culture, and maintained at 28 $\pm$ 2°C with a daily photoperiod of 16 h, and with a light intensity of approximately 25 µmol m^{-2} s^{-1} (Villarreal et al., 1997a). The kinetic parameters concerning growth and metabolite production were measured every 5 days (three replicates):

dry weight (DW), fresh weight (FW), pH, cell viability, medium carbohydrates and SC-1 concentration. Identification and quantification of SC-1 were carried out by HPLC analysis, using an R1-71 Merck refractive index detector, a Lichrosfer Si60 258 μm 4 mm, and a 5 mm Merck column; with a mobile phase of methanol:chloroform:water (29:70:1), and a flow rate of 1.2 mL min^{-1}. Retention times of SC-1 peaks (2.20 min) from cell cultures and wild plant material were compared using co-chromatography. Authentication of SC-1 in the cultures was corroborated by applying infrared to the *in vitro* spectra and comparing this with that obtained from wild plants. Cell growth was registered in the four types of media employed; however, MS1 was selected, because a finer and more homogeneous suspension was obtained. The effects of inoculum size and sucrose concentration on the biomass accumulation and synthesis of the active metabolite were studied. The maximum cell biomass was 12.9 g DW L^{-1}, which represents a 5.6-fold increase over the inoculum. The specific growth rate (μ) was 0.15 d^{-1}. The maximum concentration of SC-1 was 14.6 mg DW g^{-1} (representing fifty times that of field grown plants) which was reached after 20 days using a 2% inoculum, complete MS1 medium and sucrose, consisting of between 30 and 45 g L^{-1}. The culture reached stationary phase after 10 days, even though a high level of sugar (ca. 22 g L^{-1}) still remained in the medium. Doubling times, based on fresh and dry weights were 4.5 and 5.0 d respectively (Villarreal et al., 1997a).

Large-Scale Cell Suspension Cultures

In order to obtain higher biomasses and to increase productivity level, scale-up of batch suspension cultures in bioreactors was investigated. Two cell lines ccvx (cotyledon derived) and ccvz (hypocotyl derived) of *S. chrysotrichum* were cultivated in 10 L airlift bioreactors for a period of 3 to 4 weeks, using two inocula of 2 and 3 g DW L^{-1}. A draw-fill batch culture mode was also put to the test by harvesting 50% of the cell culture and replacing this with fresh medium. The cell cultures grew in the bioreactors, forming a homogeneous white to yellow suspension. Batch growth and accumulation of SC-1 over a 21 day period in culture, in the case of both cell lines, when using 2 g L^{-1} as inoculum showed a maximum biomass concentration of 14.1 and 5.9 g DW L^{-1} for ccvx and ccvz. Cell suspensions manifested doubling times of 3.15 and 6.9 days respectively. Accumulation of SC-1 in bioreactors was non-growth associated and reached maximum values of 21 and 19 mg g^{-1} for ccvx and ccvz (Villarreal et al., 1997b). Using 3 g L^{-1} of inoculum and the same culture conditions as described above; maximum biomass concentrations reached 14.6 and 7.7 g DW L^{-1} for ccvx and ccvz, manifesting doubling times of 5.3 and 5.8 d, respectively. Maximum SC-1 concentration for ccvx and ccvz were 23 and 20 mg DW g^{-1} after 17 and 24 d in culture (Villarreal et al., 1997b). When a draw-fill batch culture mode was introduced, a steady state of concentration of specific SC-1 was obtained, consisting of about 25 mg DW g^{-1}, during the second stage of the culture. The productivity reached in the bioreactors was between 2.33 and 2.0 times higher than in shake-flask cultures (Villarreal et al., 1997 b). These results show that the use of draw-fill batch culture modes with *S. chrysotrichum* cell suspensions is able to significantly increase productivity, whilst eliminating dead periods such as the time required to sterilize the bioreactor as well as initial lag phases in the cultures.

Hairy Root Cultures

Once the new saponins (SC-2 — SC-6) had been isolated and elucidated, a systematic study was conducted among field cultivated plants, in order to determine the content of the active principles, throughout the year. The yield of the antifungal saponins, harvested from wild and cultivated specimens is low and their accumulation in the leaves fluctuates, depending on stationary and ontogenic variables (Zamilpa et al., 2002). This situation prompted us to initiate research aimed at inducing plant genotypes with the capacity to express higher and controlled levels of the antifungal compounds. It is well known that hairy root cultures transformed using the soil born pathogen *Agrobacterium rhizogenes* are considered a potentially valuable resource for synthesizing and in some cases they also secrete an important number of secondary metabolites. These systems exhibit stable and fast growth rates, comparable to those found in cell suspensions and also exhibit genetic and biochemical stability, as well as producing a greater quantity of certain secondary compounds (Flores et al., 1995; Yoshimato et al., 2003).

Transformed root cultures of *S. chrysotrichum* were established by infecting nodal segments with *A. rhizogenes* C58C1/pRi15834 and A4/pRiA4pESC4 (Nieto, 2003). Root lines were grown in solid B5 medium (Figure 3) and following five passages they were cultured in liquid B5 nutrient medium without growth regulators, showing the typical hairy roots phenotype over four years of continuous sub-culturing.

Genetic transformation of the cell line C58-431 was confirmed by PCR analysis showing the integration of the *rol*A into the plant genome (Nieto, 2003). The hairy root cell line was cultivated in 250 mL flasks (100 mL of B5 medium), supplemented with sucrose (30 g L^{-1}) without hormones and incubated at 26°C, under uniform conditions (8-10 μmol m^{-2} s^{-1}), at 115 rpm in a gyratory shaker. Forty day old batch cultures were established, and growth (fresh and dry weight) and production of saponins were evaluated every five days. Carbohydrate consumption (sucrose, fructose and glucose) was analyzed by HPLC using an IR detector. The column was of the NH_2 type measuring 3.9 x 300 mm, with 125 A pore size and a 10 μm particle diameter from Waters. Predetermined conditions were: mobile phase 20:80 acetonitrile: water with an operational flow of 1.5 mL min^{-1}. Identification and quantification of saponins SC2- SC6 was carried out by HPLC analysis. Saponins representing the standards were obtained from *S. chrysotrichum* wild plants, as previously described (Zamilpa et al., 2002). Extracts were analyzed on a Waters Delta prep 4000 modular HPLC system, consisting of a U6K injector, 600E pump system controller and 9 Millenium 3.2 software), and a RI detector. The analysis was carried out on two Chromolith TM RP-18 (100 x 4.6 mm, 2 μm) columns

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connected in series; the mobile phase was 35:65 acetonitrile:water at a flow rate of 1.5 mL min $^{-1}$ for SC-2 and, 1.7 mL min $^{-1}$ for SC-3 and SC-4. For SC-5 and SC-6, the mobile phase was 37:63 acetonitrile:water at a flow rate of 1.5 mL min^{-1} (Caspeta et al., 2005a). After 40 days, the density of roots was 4.3 g DW L^{-1} which was 6 times greater than the inoculum. Conductivity of the culture medium dropped in proportion to root tissue growth (Figure 4a). Root growth followed first order kinetics with a mean specific growth rate (μ) of 0.08 d^{-1}. Sucrose was hydrolyzed on day 20 when the exponential growth phase ended (Figure 4b).

Production of the active extract (a mixture of saponins extracted from chloroform) in the roots was growth-associated. During the culture time, only three of the saponins (SC-2, SC-3 and SC-4) were recovered from root biomasses, exhibiting discontinuous patterns in their peaking, but with differences in their accumulation profiles, so that maximum yields for saponin SC-2 (0.37 mg DW g^{-1}) and SC-4 (0.851 mg DW g^{-1}) were registered on day 15, when saponin SC-3 was not present; while maximum yield for saponin SC-3 (0.189 mg DW g^{-1}) was recovered on day 10, when the levels of SC-2 and SC-3 had diminished (Figure 4c) (Caspeta et al., 2005a). Saponins were not released into the culture medium and SC-5 and SC-6 were not observed, either in the biomass or in the culture medium. None of the saponin yields extracted from hairy roots cultured in flasks was higher than those observed for plant leaves.

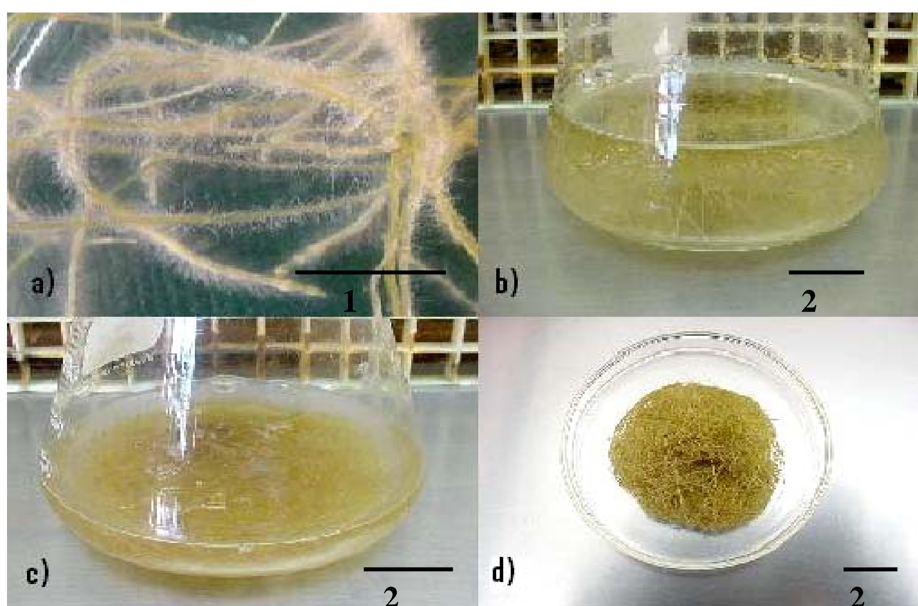

Figure 3. *Solanum chrysotrichum* hairy root cultures: a) roots in solid medium b) roots growing in liquid B5 medium c) after 15 days biomass, reaching 2 g FW L^{-1}, d) packet of root tissue.

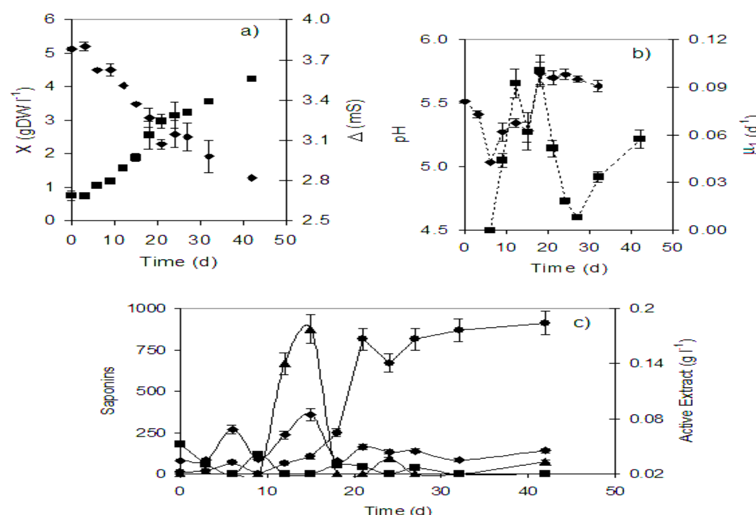

Figure 4. *Solanum chrysotrichum* hairy root cultures in 250 mL flasks with 100 mL of B5 medium: a) biomass X (■) and conductivity (◆); (b) carbohydrate consumption sucrose (▲), glucose(◆) and fructose (•); (c) active extract (•), SC-2(◆),

SC-3 (■) and SC-4 (▲). (Reprinted with permission from Caspeta et al (2005) *Solanum chrysotrichum* hairy root cultures: characterization,scale-up, and production of five antifungal saponins for human use. Planta Med **71**: 1081-1084. Copyright (2008), Georg Thieme Verlag KG.

Large-Scale Hairy Root Cultures

To scale-up the growth and production of the hairy root cell line C58-431, 2 and 10 L airlift basic design reactors (BDRs) were used. The reactor systems consisted of concentric draft-tube, internal-loop airlift reactors (Figure 5) with a d_D/d_c of 1.96 and h_D/h_c of 1.94. Gas sparging was carried out on the draft-tube bottom. The downcomer had a cross-sectional area three times greater (A_d) than the riser (A_r). Three adaptations for the BDR were constructed and evaluated (Figure 5). A stainless steel mesh draft, with the same downcomer diameter/column diameter (d_D/d_C) and downcomer height/column height (h_D/h_C) relationship, as those on the BDR was introduced, and basic hydrodynamics were maintained. Root tissue inoculation and distribution in the downcomer was able to be promoted at low velocities, and growth was promoted without any disruption of riser dynamics. A mesh opening of 2.4 mm was chosen, in order to allow free root enlargement from the downcomer to the riser and in order to promote radial growth (Caspeta et al., 2005b) (Figure 5b). Other fittings consisted of: a mesh draught tube with extensions (Figure 5b) and mesh draught tube with extensions and helixes (Figure 5c) (Caspeta et al., 2005b). Reactors were filled with a sterilized B5 medium plus 3% sucrose, without growth regulators.

Small pieces of hairy root tissues were suspended in 0.5 L of culture medium for inoculation, using gravity. Tissue distribution in the downcomer was performed at 0.001 vvm. In the case of the mesh-draft with helixes, this structure was rotated, using 3.2 mm tube during the inoculation process. Reactors were operated at 0.05 vvm for 3 days and then at 0.1 vvm until harvest on day 42, when draft tubes were removed, and root beds were cut. In order to measure homogeneity and distribution, downcomer and riser beds were divided into three sections (Caspeta et al., 2005b). Root tissue growth was indirectly monitored using conductivity measurements as previously described (Tescione et al., 1997).

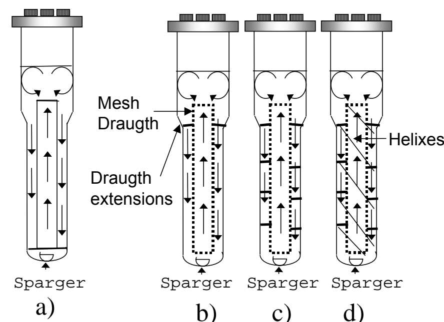

Figure 5. Reactor and modifications: a) basic design (concentric draft-tube internal-loop airlift reactor) b) mesh draft tube, c) mesh draft tube with extensions, d) mesh draft tube with extensions and helixes. (Reprinted with permission from Caspeta et al., (2005) Novel airlift reactor fitting for hairy root cultures: developmental and performance studies. Biotechnology Progress **21**:735-740. Copyright (2008), American Chemical Society.

Influence of tissue geotropism was observed in the BDR. After some hours, roots got trapped on the top and bottom draft extensions, and grew there in dense tangles, manifesting necrotic tissue (Figure 6). After 42 days in culture, a maximum concentration of 2.04 g DW L^{-1} was obtained. During the time in culture, growth did not appear to follow first-order kinetics (Caspeta et al., 2005b).

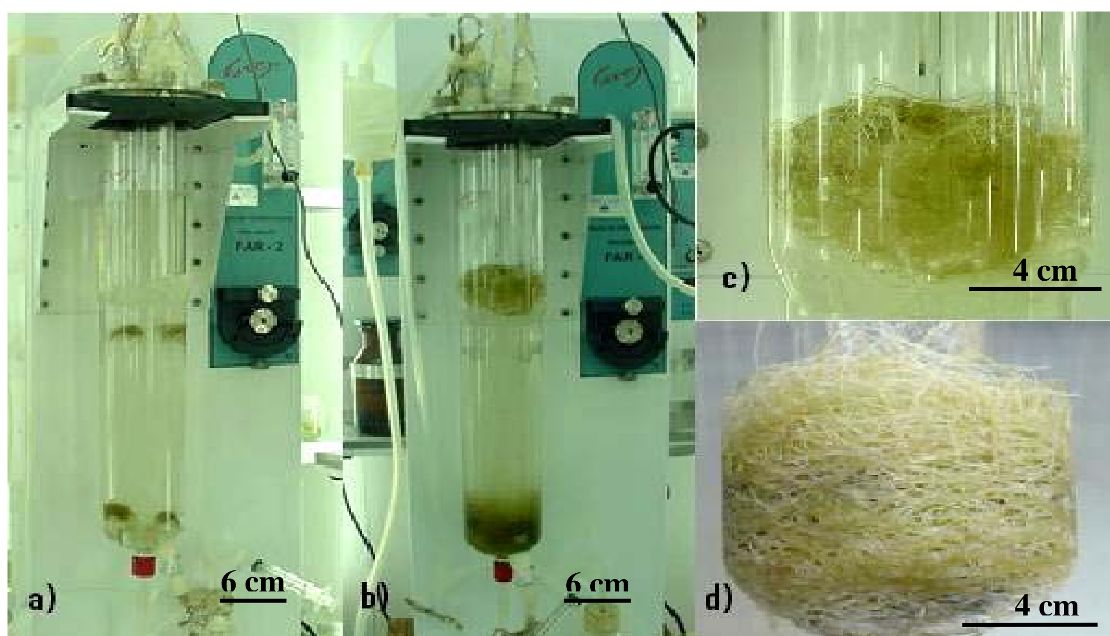

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Figure 6. Hairy root culture of *Solanum chrysotrichum* in 2 L basic design airlift bioreactors (BDR). One gram of FW L^{-1} was inoculated at the top and bottom of the bioreactor, a) poor distribution of root tissue impaired homogeneous growth, resulting in only 2.1 g DW L^{-1} of root biomass after 42 days in culture, b), c), and d).

Modifications on the 2 L reactor were undertaken (Figure 5). For distributing the roots, the original glass draft tube was exchanged for a mesh that allowed radial growth. The hydrodynamic patterns were practically the same as in the BDR. At 0.01, vvm roots were better distributed, although when the gas flow operation was undertaken, roots moved to the arms for draft support as happened in the BDR, so more arms were placed in the draft, and when the gas flow operation was carried out, roots remained distributed throughout the reactor. For a better suspension of roots at the bottom of the reactor, helixes were placed on the draft arms (Figure 5d) forming a perpetual screw, and their spin helped in the movement of roots from the top to the bottom of the reactor. With the last fitting roots were cultivated, introducing inoculum by gravity to the downcomer and turning the draft simultaneously to the right and left to distribute the root tissue evenly. In the first two days, roots were deposited in small packets on draft extensions, and after a few days, roots started to grow along empty spaces between consecutive arms, and a little radial growth was also observed. Mean specific growth rate was 0.115 d^{-1}, corresponding to a doubling time of 6 days; which represented between 2 and 4 days less than that observed in the case of shake flasks and the BDR, respectively. Roots were able to grow radially through the mesh apertures, and on day 15, roots crossed the mesh draft and began to fill the entire disposable area (Caspeta 2005b) (Figure 7).

Figure 7. Hairy root culture of *Solanum chrysotrichum* in a 2 L airlift modified bioreactor: a) after two days and b) after 45 days of culture.

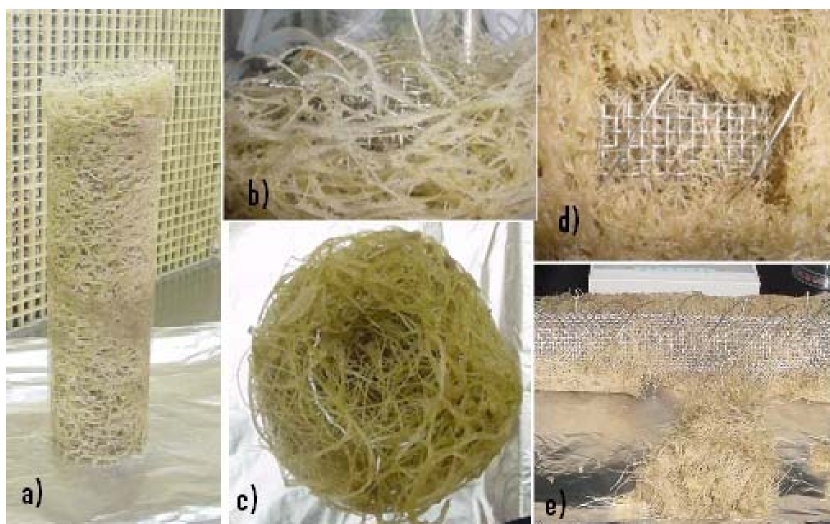

Figure 8. Hairy root tissue growing in 2 L airlift reactor modified with mesh draft tube extensions and helixes, were harvested after 45 days: a) and b) lateral views, c) view from the bottom, d) and e) cut off from growth at the middle of the root package.

After the culture period (45 days), the biomass concentration doubled in comparison with that obtained in the BDR. Roots were harvested (Figure 8) and local root density was measured at the top (10.2 g DW L^{-1}) middle (10.8 g DW L^{-1}) and bottom (11.88 g DW L^{-1}), and no significant differences were found after an arc-sine transformation of the percentages, and the application of a Z test ($p<0.13$) (Caspeta et al., 2005b). Considering only the disposable area for root growth, a gross density of 8.7 g DW L^{-1} was obtained.

Accumulation of SC-2 (the most active saponin) in root biomasses grown in 2 L reactors was of 7.17 mg DW g^{-1} (0.7% DW), which was 19 and 6 times that of flask cultures or that obtained from plant leaves, SC-4 yield was lower than the one from flask cultures, and SC-3, SC-5 and SC-6 were not produced by biomasses. In the culture medium, small concentrations of SC-5 and SC-6 were recovered (Caspeta et al., 2005a)

Table 1. Yields of biomass and saponins in flasks and 2 L reactors of *Solanum chrysotrichum* hairy roots

Volume	Maximum biomass (g DW L^{-1})	Specific growth rate (μ) d^{-1}	Saponin content (mg DW g^{-1})		
				Biomass	Medium
Flasks (100 mL)	4.3	0.08	SC2	0.32 ± 0.05	nd
			SC3	0.19 ± 0.03	nd
			SC4	0.85 ± 0.12	nd
Airlift reactor (2 L)	4.4	0.11	SC2	7.17 ±1.4	nd
			SC3	nd	nd
			SC4	0.137 ± 0.03	nd
			SC5	nd	0.028 ± 0.012
			SC6	nd	0.056 ± 0.014

nd = not detected

The scale-up of root cultures from 2 to 10 L using modified reactors was evaluated. The relationship between total draft area and total helix longitude was maintained as in the 2 L modified reactor. Therefore, the space between two consecutive helixes was twice as long as the one in the 2 L. Inoculum pouring and distribution were undertaken as in the 2 L. Root tips grounded in the same way as those in 2 L, but little empty spaces were observed and therefore root distribution was less homogeneous than in the 2 L reactor (Figure 9).

Growth took place in the downcomer as well as radially, inside the riser. After 20 days in culture, the growth kinetic was similar to that observed in flasks and in the 2 L modified reactor (Caspeta et al., 2005b). After 45 days in culture, a final biomass of 3.6 g DW L^{-1} was obtained.

CONCLUSIONS AND OUTLOOK

Solanum chrysotrichum, a plant species used to treat skin mycosis was selected in the light of traditional medicinal knowledge, provided by Mayan ethnic groups from Chiapas, Mexico. The pharmacological value of this plant was demonstrated and we were able to isolate an antifungal spirostanol saponin named SC-1, which represents a new molecule. In a more recent study, five new bioactive saponins (SC-2 ─ SC-6) were isolated and elucidated. Of these compounds, SC-2 and SC-4 were shown to be the most efficacious for eliminating dermatophytes in culture. Clinical studies conducted among patients suffering from *tinae pedis*, demonstrated the effectiveness and tolerability of a standardized cream prepared with saponin SC-2, when compared with a control group, treated with ketoconazole.

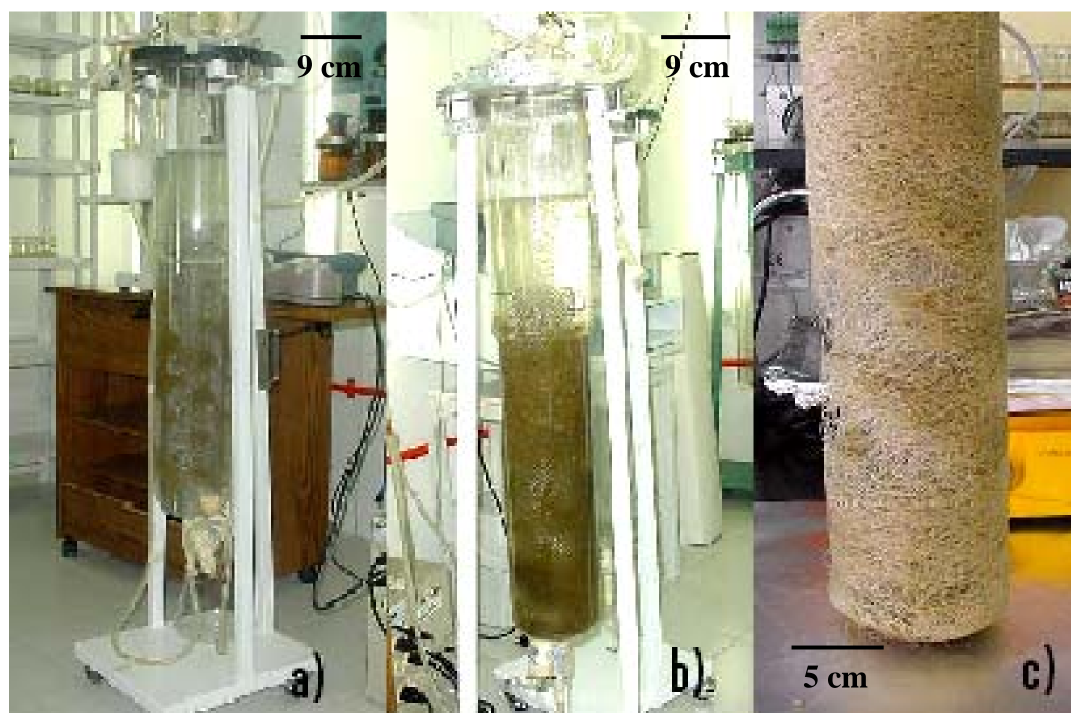

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Figure 9. Hairy root culture of *Solanum chrysotrichum* in 10 L airlift modified bioreactor: a) root distribution following inoculation, b) at 45 days, biomass reached 3.4 g DW L^{-1} and c) the pack of root tissue.

Even though *S. chrysotrichum* is widely used as a popular medicine and grows in a very limited region in Mexico, the plant has never been cultivated, and the content of the antifungal compounds varies seasonally and according to the age of the plant. The biotechnological approaches described here may help promote the permanent and controlled production of this antifungal medicine. The plant was micropropagated, and the cell suspension cultures of this species were scaled-up to 10 L, demonstrating that the airlift bioreactors used for this purpose were adequate. Hairy root cultures established in Erlenmeyer flasks were promising, as the most active saponin SC-2 was produced with a maximum yield of 0.04% DW. Novel fittings on 2 and 10 L airlift reactors were established to scale up *S. chrysotrichum* hairy roots. The modified reactor with a mesh replacing the glass draft tube and with additional extensions and helixes exhibited improved root distribution, achieving the highest root concentrations and manifesting more adequate dynamic behavior. Moreover, the geometric scale-up of the fitting provided a reproducible method for distributing inoculum, as well as an easier method for harvesting and recovering biomass. Among root biomasses grown in the reactor, the yield of SC-2 was 0.7% DW, a value representing 19 and 6 times that from flask cultures or that obtained from plant leaves. The results obtained with the hairy root cultures of *S. chrysotrichum* offer feasible alternatives for the production of bioactive saponins. These *in vitro* culture procedures have made an important contribution towards establishing the sustainable exploitation of this unique Mexican medicinal plant. Thus the importance of these investigations becomes clear as this species is transferred "from the Maya highlands to the bioreactors"

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