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A sustainable use of Ricotta Cheese Whey for microbial biodiesel production

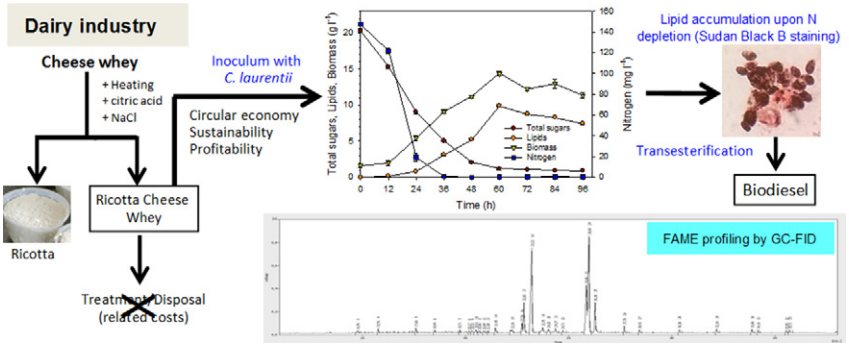
Eleonora Carota, Silvia Crognale, Alessandro D'Annibale *, Anna Maria Gallo, Silvia Rita Stazi, Maurizio Petruccioli

Department for Innovation in Biological, Agro-food and Forest Systems (DIBAF), University of Tuscia, Via S. Camillo De Lellis, 01100 Viterbo, Italy

HIGHLIGHTS

- Ricotta cheese whey (RCW) used as a lipid-production medium
- Oleaginous yeasts (OY) screened for lipid-producing ability on RCW
- *C. curvatus* and *C. laurentii* produced substantial amounts of lipids on RCW
- Dominant fatty acids were oleic, linoleic and palmitic acids in *C. laurentii*
- *C. laurentii* lipid production was faster in STR than shaken flask

GRAPHICAL ABSTRACT



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ABSTRACT

The increasing demand of plant oils for biodiesel production has highlighted the need for alternative strategies based either on non-food crops or agro-industrial wastes that do not compete with food and feed production. In this context, the combined use of wastewater and oleaginous microorganisms could be a valuable production option. Ricotta cheese whey (RCW), one of the major byproducts of the dairy industry, is produced in very high and steadily increasing amounts and, due to its high organic load, its disposal is cost-prohibitive.

In the present study, in order to assess the adequacy of RCW as a growth medium for lipid production, 18 strains of oleaginous yeasts were investigated in shaken flask for their growth and lipid-producing capabilities on this substrate. Among them, *Cryptococcus curvatus* NRRL Y-1511 and *Cryptococcus laurentii* UCD 68-201 adequately grew therein producing substantial amounts of lipids (6.8 and 5.1 g L^{-1}, respectively). A high similarity between the percent fatty acid methyl esters (FAME) composition of lipids from the former and the latter strain was found with a predominance of oleic acid (52.8 vs. 48.7%) and of total saturated fatty acids (37.9 vs. 40.8%). The subsequent scale transfer of the *C. laurentii* UCD 68-201 lipid production process on RCW to a 3-L STR led to significantly improved biomass and total lipid productions (14.4 and 9.9 g L^{-1}, respectively) with the biodiesel yield amounting to 32.6%. Although the *C. laurentii* FAME profile was modified upon process transfer, it resembled that of the Jatropha oil, a well established feedstock for biodiesel production. In conclusion, *C. laurentii* UCD 68-201, for which there is very limited amount of available information, turned out to be a very promising candidate for biodiesel production and wide margins of process improvement might be envisaged.

* Corresponding author.
E-mail address: dannib@unitus.it (A. D'Annibale).

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1. Introduction

Ricotta cheese whey (RCW) is a by-product of dairy industry derived from ricotta cheese production. During this process, the whey is heated at 80–90 °C and generally added with organic acids and salts to induce the denaturation and consequent precipitation of whey proteins. The curd thus obtained is allowed to cool and filtered to separate the solid part (ricotta) from the liquid waste which is referred to as RCW (Lavarda, 1972).

Although the large majority of RCW is produced in the Mediterranean basin (about 1 Mton per year only in Italy) (Sansonetti et al., 2009), the manufacturing of ricotta cheese is also widespread in USA, where this product is often referred to as ricottone. Therefore, the technical hurdles related to either upgrading or disposal of this dairy byproduct are not only a regional issue. The typical composition of RCW is 4.8–5.0% lactose, 1.0–1.3% salts, 0.15–0.22% proteins, 0.20–0.25% organic acids and 0.20% fats with a COD ranging between 50.000 and 80.000 mg L^{-1} (Sansonetti et al., 2009). However, despite the presence of residual nutrients, the aforementioned presence of additives impairs its nutritional value and, consequently, its profitable use as a feed for livestock, causing additional costs for the dairy industry due to the need of its disposal. Moreover, RCW might cause a considerable environmental impact in case of an inappropriate disposal procedure.

Despite the appreciable sugar content in RCW which makes it a putative candidate as a growth medium in microbial production processes, only few studies have been conducted for this purpose. These studies include bio-ethanol production by *Kluyveromyces marxianus* (Sansonetti et al., 2009; Zoppellari and Bardi, 2013) and lactic acid production by mixed and pure bacterial cultures (Secchi et al., 2012). Most of research has focused instead on cheese whey, which, however, differs substantially from RCW, due to its lower concentration of salts and organic acids and higher content of whey proteins (Ykema et al., 1988; Takakuwa and Saito, 2010).

Microbial biodiesel production is among the most promising upgrading options of low cost feedstocks characterized by a high content in carbohydrates associated with low nitrogen content. In this respect, RCW seems to meet these nutritional requirements. Biodiesel is a mixture of fatty acids alkyl monoesters derived from renewable resources such as higher plant oils, or, to a lesser extent, animal fats and waste cooking oil (Srivastava and Prasad, 2000) produced from transesterification reactions of triacylglycerides (TAG). Compared to conventional diesel, it has several advantages, being non-toxic, renewable and biodegradable. However, the rising costs of plant oils, from which the large majority of biodiesel is derived (around 95% of total world production), and the increasing demand for biofuels have given rise to concerns about land-use practices, increase of food price and oil production strategies (Atabani et al., 2012). Therefore, the use of microbial oils might represent a promising alternative to mitigate the problems associated with the "food vs. fuel" issue. Several microorganisms, belonging to yeasts, molds and microalgae, have the reported ability to accumulate intracellular lipids up to 70% (w/w) of their dry weight; the microbial fatty acid composition is often similar to that of vegetable oils used for biodiesel (Christophe et al., 2012). Among them, oleaginous yeasts (OY) seem to be more suitable for an industrial production, being easy to cultivate, fast-growing and receptive to scale-up (Li et al., 2008).

Lipid accumulation in OY occurs through two different mechanisms depending on the nature of growth medium. "Ex-novo" synthesis is observed on hydrophobic substrates, while "de novo" synthesis occurs on sugar-based media in concomitance with a shortage of a key element, usually nitrogen.

In this study, the adequacy of RCW as a growth medium for the production of microbial oil was assessed. In fact, apparently the chemical composition of RCW characterized by high C/N ratios might be compatible with microbial "de novo synthesis" of lipids (Pirozzi et al., 2013). The exploitation of RCW in this direction would be in line with sustainability since it would enable a partial replacement of edible oils as feedstock for biodiesel, giving back land use to food crops. Furthermore, in view of a possible development of a circular economy (Cicatiello et al., 2016), RCW upgrading would be beneficial for both dairy industries, due to a reduction of production costs, and for the environment since the spent medium derived from fermentation, would have a negligible organic load.

To this aim, a screening was initially performed with several OY belonging to well known lipid-producing species, some of which previously isolated from dairy products (Corbo et al., 2001), to assess their respective abilities to grow and produce lipids on that dairy byproduct and to determine productivities. The fatty acid methyl ester compositions derived from transesterification of lipids produced by some selected strains were analyzed and compared with well established feedstocks for biodiesel production. The most promising strain was then tested in a stirred tank reactor in view of a preliminary assessment of the process scale transfer feasibility.

2. Materials and methods

2.1. Microbial strains and maintenance

The strains under study were obtained from various culture collections or were isolated from environmental matrices and were chosen on the basis of their reported lipid accumulation ability on synthetic media. *Candida rugosa* NRRL Y-95, *Cryptococcus curvatus* NRRL Y-1511, *Lipomyces starkeii* NRRL 11557, *Rhodosporidium torouloides* NRRL Y-1091, *Rhodosporidium torouloides* NRRL Y-17902, *Trichosporon fermentans* NRRL Y-1492, *Yarrowia lipolytica* NRRL YB-423, *Y. lipolytica* NRRL Y-1095 and *Y. lipolytica* NRRL Y-7208 were provided by the ARS Culture Collection (NRRL, Peoria, IL). *Cryptococcus albidus* UCD 68-150, *C. albidus* UCD 68-174, *Cryptococcus laurentii* UCD 68-201, *Rhodotorula glutinis* UCD 68-255 and *Rhodotorula minuta* UCD 68-280 were obtained from the UCD Collection (Davis, California), while *Rhodotorula glutinis* DBVPG 3853 from DBVPG Collection (Perugia, Italy). *Pichia guilliermondii* 1067 and *Pichia anomala* AN/4 were a kind gift of Prof. Cardinali (University of Perugia; Italy). *Pichia membranifaciens* 6C1 was isolated from olive brine (Crognale et al., 2012) and identified on the basis of its ITS sequence (GenBank Accession number JN900498).

During the study, the strains were maintained on potato dextrose agar (PDA) slants at 4 °C and sub-cultured every month.

2.2. Growth medium

RCW was collected from a cheese processing plant (Formaggi Boccea S.r.l., Rome, Italy) and stored at −20 °C until used. RCW had the following characteristics (g L^{-1}): dry weight, 48.2 ± 4.10; Chemical Oxygen Demand (COD), 43.5 ± 3.8; Total Organic Carbon (TOC), 16.3 ± 1.4; lactose, 40.2 ± 0.8; galactose, 1.6 ± 0.2, total nitrogen, 0.053 ± 0.04; protein, 0.008 ± 0.001; C/N, 307; ash, 4.5 ± 0.4. Initial pH was 5.8. After thawing, the RCW was centrifuged (8000 × *g*, 15 min), two-fold diluted with deionized water, added with $(NH_4)_2SO_4$ so as to reach a C/N ratio of 55 and finally its pH was adjusted to 5.5 with 0.1 M NaOH.

2.3. Culture conditions

2.3.1. Shaken flask experiments

The microorganisms mentioned above were firstly screened in shaken flasks to select the best strain in terms of biomass and lipid production. Regardless of the strain, each inoculum was obtained by suspending 72-h-old PDA slants with sterile physiological solution. Inocula were added to 250-mL Erlenmeyer flasks containing 50 mL of RCW-based medium so as to yield an initial value of optical density of 600 (OD_{600}) equal to 0.2. After inoculation, flasks were incubated at 30 °C in an orbital shaker (185 rpm) for 5 days. Samples were collected on a daily basis. All experiments were performed in triplicate.