Journal de Mycologie Médicale (2012) 22, 21—29

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ORIGINAL ARTICLE/ARTICLE ORIGINAL

Microbial fermentation of cabbage by a bacterial strain of *Pectobacterium atrosepticum* for the production of bioactive material against *Candida* species

Production de composés actifs contre diverses espèces de Candida *obtenus à partir de choux fermentés par* Pectobacterium atrosepticum

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Received 22 August 2011; received in revised form 3 November 2011; accepted 12 November 2011
Available online 29 December 2011

KEYWORDS
Microbial fermentation;
Cabbage (*Brassica oleracea* var. *capitata*);
Pectobacterium atrosepticum;
Bioactive materials;
Antifungal activity;
Candida species

Summary
Objective. — The objective of this study was to produce the bioactive fermented product by the microbial fermentation of cabbage (*Brassica oleracea*) using a bacterial strain *Pectobacterium atrosepticum* which was assessed for its antimycotic efficacy against pathogenic isolates of *Candida* species.
Materials and methods. — An approach of microbial fermentation of cabbage using a bacterial strain *P. atrosepticum* was applied to obtain the bioactive fermented product. Antimycotic efficacy of bioactive fermented product of cabbage was evaluated by disc diffusion assay, minimum inhibitory (MIC) and minimum fungicidal (MFC) concentrations, cell viability assay and scanning electron microscopy (SEM) analysis.
Results. — The bioactive fermented product (500 μg/disc) revealed promising antimycotic effect against the tested *Candida* species as a diameter of inhibition zones (10 ± 0.2 to 13 ± 0.4 mm) along with its MIC and MFC values, ranging from 250 to 1000 and 250 to 2000 μg/ml, respectively. Exposure of 140 min of bioactive fermented product exerted potential antimycotic effect on the viable counts of the tested fungal isolates with about 85 to 100% inhibitory effect. Further, the study of SEM revealed potential detrimental effect of bioactive product on the morphology of *C. albicans* KACC 30003 at MIC concentration. Elaborative study of

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1156-5233/$ — see front matter
doi:10.1016/j.mycmed.2011.11.004

GC-MS analysis conducted on bioactive fermented product of cabbage revealed transformation products present in fermented product.
Conclusion. — These results confirmed the therapeutic potential of microbially bioconverted/fermented products of cabbage for using in medicinal and pharmaceutical preparations.

MOTS CLÉS
Fermentation microbienne ;
Choux (*Brassica oleracea* var. *capitata*) ;
Pectobacterium atrosepticum ;
Métabolites ;
Activité antifongique ;
Candida

Résumé
Objectif. — Produire des métabolites actifs contre diverses espèces de *Candida* à partir de la fermentation de choux (*Brassica oleracea*).
Matériel et méthodes. — La fermentation de choux est faite à l'aide d'une souche de *Pectobacterium atrosepticum* dans des conditions de culture définies. L'activité antifongique des métabolites obtenus est analysée par la méthode des disques chargés à 500 mg, la CMI, la CMF, la survie des cellules fongiques en culture et la MEB.
Résultats. — La méthode des disques montre une activité antifongique avec des zones d'inhibition comprises entre 10 et 13 mm. La valeur des CMI et des CMF sont respectivement comprises entre 250 et 1000 mg/ml et 250 et 2000 mg/ml. Une exposition à ces métabolites pendant 120 minutes exerce une action inhibitrice sur la vitalité des cellules fongiques comprise entre 85 et 100 %. La MEB révèle des altérations morphologiques des cellules de la souche de *Candida albicans* KACC 30003 pour des concentrations en métabolites égales à la CMI. La chromatographie en phase gazeuse montre la présence de ces métabolites dans les produits de fermentation.
Conclusion. — Ces résultats confirment l'activité antifongique potentielle de ces métabolites pour un usage thérapeutique.

Introduction

Systemic fungal infections have emerged as important causes of morbidity and mortality in immunocompromised patients. *Candida* species are characterized by the ecological features depending on the species that represent the most common fungal pathogens among yeasts that affect humans. Although filamentous fungi can be of importance [16], the growing problem of mucosal and systemic candidiasis reflects the enormous increase in the number of patients at risk and the increased opportunity that exists for *Candida* species to invade deep tissues [29]. The increased prevalence of local and systemic disease caused by *Candida* species has resulted in numerous new clinical syndromes. *Candida* species produce a wide spectrum of diseases, ranging from superficial mucocutaneous disease to invasive illnesses, such as hepatosplenic candidiasis, *Candida* peritonitis, and systemic candidiasis [31]. The management of serious and life-threatening invasive candidiasis remains severely hampered by the lack of reliable antifungal drugs that allow both fungemia and tissue invasion by *Candida* species. Patients who are critically ill or immunosuppressed and in medical and surgical intensive care units, have been the prime targets for opportunistic nosocomial fungal infections, primarily due to *Candida* species. In persons with systemic infections, *Candida* species are now the fourth most commonly isolated pathogens from blood cultures [26]. Clinical and autopsy studies have confirmed the marked increase in the incidence of disseminated candidiasis, reflecting a parallel increase in the frequency of candidemia [9,24].

Despite advances in antifungal therapy, the treatment of infections caused by *Candida* species with amphotericin B, the azoles or flucytosine has not been uniformly successful. Although use of echinocandins in antifungal therapy has met a desired level of satisfaction to serve as potent anticandidal agents [12], the management of *Candida* infections still faces a number of problems including limited number of effective antifungal drugs, toxicity of the available antifungal drugs, increased resistance of *Candida* to commonly used antifungal drugs, relapse of *Candida* infections and the high cost of antifungal drug therapies [15,17,18,34]. In order to overcome these problems, several attempts have been made and a new concept of microbial fermentation of vegetable products has been emerged with new and effective microbially fermented products to serve as better antifungal agents. A literature data on microbially bioconverted and/or fermented products have confirmed their efficacy to limit the propagation of harmful microbes. Previously, the *in vitro* and *in vivo* antifungal efficacy of other types of microbially bioconverted products has been confirmed [4]. However, only few reports are available on the antimycotic effect of microbially fermented products of vegetables against pathogenic *Candida* species [3]. In our continuous efforts to develop potent antifungal agents, recent findings on development of bioconverted product of cabbage using different microbial strain Pcc21 confirmed that microbially fermented products potently inhibited the growth of *Candida* pathogens including a clinical isolate [2]. Recently, microbial fermentation technology has become the major focus of research for the production of antifungal materials using vegetables or waste products as substrates [2,3,32]. Hence, microbially fermented products seem to act as a possible alternatives or complementary therapeutic compounds to treat serious fungal infections caused by *Candida* species.

The objective of this research was to develop more potent bioactive fermented product from the microbial fermentation of cabbage using a bacterial strain *Pectobacterium atrosepticum* (Pecto-a), and to determine it antimycotic efficacy against clinical isolates of *Candida* species. Also, we analyzed the biotransformation products of bioactive fermented product of cabbage by GC-MS analysis.