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Full Length Research Paper

Anatomical and histochemical analysis of vegetative organs of Vernonia ferruginea Less. (Asteraceae)

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Vernonia ferruginea Less. is a perennial shrub species, present in several regions of Brazil, especially in the savanna. It is popularly used as a phytotherapeutic. This fact justifies the need to anatomically characterize the plant for its accurate identification and to conduct histochemical studies with the aim of identifying the chemical nature of its cellular constituents. The species-specific data will contribute significantly to pharmaceutical quality control and also provide information about the sites of specific chemical compounds. Samples of V. ferruginea vegetative organs were collected and submitted to the usual plant anatomy and histochemical techniques. The leaves are anhiphystomática with anomocytic stomata; have tector and glandular trichomes that store essential oils. The stem has collateral-type vascular bundles arranged in a eustele structure; it also has glandular and tector trichomes. The root has brachysclereids, endoderm with various chemical compounds and vascular bundles having axial elements and rays. Few differences were found in the structure of vegetative organs in relation to other species of the genus, confirming the importance of the details shown.

Key words: Plant anatomy, assapeixe-branco, essential oils.

INTRODUCTION

The family Asteraceae has about 25,000 species distributed in 1600 genera arranged in 17 tribes and three subfamilies (Bremer, 1994). In Brazil, the family is represented by about 275 genera and 2,045 species (Barroso et al., 1991; Nakajima et al., 2012). They may be herbs, subshrubs, shrubs, trees, liana/twiner/vine, terrestrial, ripicolous or epiphyte, found throughout Brazil, where 74 genera and 1,305 species are endemic (Nakajima et al., 2012). Medicinal plants are widely used in different countries for the treatment of diseases, and in Brazil some Asteraceae are used in folk medicine, such as Achyrocline satureioides (Fachinetto et al., 2007) Baccharis trimera; Mascagnia cordifolia, Equisetum kansanum (Ferreira et al., 2001) Stercorarius chilensis, Vernonia polyanthes, Chamomilla recutita, Conradina grandiflora, Muhlenbergia glomerata, Bidens pilosa (Pereira et al., 2005) and Vernonia ferruginea (Lorenzi and Matos, 2002).

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V. ferruginea Less., popularly known as assaípe-branco, assaípe-do-pará or cálcão-de-velho, is a perennial shrub or tree; it is erect, highly branched, with a woody stem, rugous at the base and has rusty hairiness at the apex. It measures 2 to 3.5 m high and is native to Brazil. It is one of the most common weeds in the pastures, occurring throughout Brazilian territory except in the Southern Region. V. ferruginea is more common in cerrado and low fertile soils, considered as standard for weak land (Lorenzi, 2000). It has been used over time for treatment, prevention and cure of diseases. Its leaves and roots are considered diuretic, balsamic and antirheumatic and are used in cases of bronchitis and persistent cough. Its roots are diuretic and are used for the treatment of hemoptysis and internal abscesses. It is also used externally for skin affections, muscle aches and rheumatism (Lorenzi and Matos, 2002).

The use of medicinal plants has been described throughout history for centuries; they are as old as the human species (Pereira et al., 2005). The knowledge about these plants is the only therapeutic resource in many communities and ethnic groups (Maciel et al., 2002). In the poorest regions of Brazil and even in large Brazilian cities, medicinal plants are sold in street and popular markets, and found even in residential backyards. Popular observations about the use and effectiveness of these plants contribute significantly to the dissemination of the therapeutic virtues of the plants. They are often prescribed due to their medical effects, even though their chemical constituents are unknown. Thus, users of medicinal plants throughout the world maintain the practice of herbal medicine consumption in vogue. This makes therapeutic information accumulated for centuries valid, indirectly arousing the interest of researchers in studies involving multidisciplinary areas such as botany, pharmacology and phytochemistry that together enrich the knowledge about the inexhaustible natural medicinal source: the world flora (Lorenzi et al., 2002).

Considering the importance of medicinal plants, the anatomical and histochemical study of the vegetative tissues of medicinal species is essential, and the correct identification and morphoanatomical characterization of these plants is crucial for the quality control of raw material used to produce herbal, ensuring their reliability (Martins and Appezzato-da-Gloria, 2006).

Therefore, this study aimed to identify the disposal of the tissues of V. ferruginea to give an accurate identification of this species, the chemical nature of its cellular constituents and its location through the usual techniques of plant anatomy and histochemistry. Plants collected in the Cerrado in the Municipality of Rio Verde were used for this study.

**MATERIALS AND METHODS**

The analysis was conducted at the Laboratory of Plant Anatomy, Federal Institute of Goiás - Campus Rio Verde, Goiás. We used vegetative fresh young organs, fixed in FAA70, except for the histochemical tests, collected from V. ferruginea individuals growing in a Cerrado area of the Patu Farm in the municipality of Rio Verde - GO. The identification of V. ferruginea Less. was performed by specialist and the voucher specimen N°. 502 was deposited at the herbarium of Rio Verde in the Goiano Federal Institute. Cross-sections with about 10 μm thickness were obtained using a microtome LPC for plant anatomy with a disposable steel blade. Microscopic analyses and photographic records were made in a Leica (DM500) optical microscope with camera (Leica ICC50). For structural analysis, sections were stained with safranin and astra blue (Bukatsch, 1972), and slides were mounted in Canada balsam.

For histochemical characterization, the sections were stained with Sudan IV for lipid compounds; lugol reagent for starch; phloroglucinol acid for lignin; ferric chloride (Johansen, 1940) and potassium dichromate (Gabe, 1968) for phenolic compounds; xylidine Ponceau for total proteins (O’Brien and McCully, 1981); vanillin HCl for tannin (Mace and Howell, 1974); Wagner reagent for alkaloids (Furr and Mahlberg, 1981); NADI reagent for essences and resin acids (David and Carde, 1964); sulfuric acid for sesquiterpene lactones (Geissman and Griffin, 1971), and PAS for carbohydrates (McManus, 1948). The clearing technique was done on the structural analysis in the frontal view leaf, using sodium hydroxide (Arnott, 1959), and staining subsequently with basic fuchsin.

**RESULTS AND DISCUSSION**

**Anatomical characterization**

The leaves of V. ferruginea have anomocytic stomata on both surfaces; they are more abundant on the abaxial surface, characterizing the leaf as amphi-hypostomatic. This is also found in Vernonia scorpioides (Toigo et al., 2004) and Vernonia polyanthes (Alves and Neves, 2003). In a frontal view, the epidermal cells of the abaxial surface show slightly sinuous walls; however on the adaxial surface they are sinuous, as seen also in some Asteraceae (Figure 1A to B).

In cross section, the epidermis of the leaves is uniseriated, covered by a thin cuticle. The epidermis shows cubic cells, slightly elongated over the veins. At the midrib, the epidermis consists of elongated cells of different sizes, usually smaller than the mesophyll cells. The cells of the epidermis have little thickening between the anticlinal walls.

On both surfaces of the leaves, numerous multicellular uniseriate tector trichomes are observed, especially on the abaxial surface, all with 3 to 8 cells. Multicellular glandular trichomes are present in less numbers, formed by two basal cells and a multicellular head with about four cells (Figure 1C to F). The presence of trichomes is a factor common to the genus Vernonia, and may vary with type and quantity in each species (Metcalfe and Chalk, 1972).

The mesophyll has two distinct layers of palisade parenchyma closest to the adaxial surface of epidermis; and the size of the cells of the first layer is twice that of the second layer. The spongy parenchyma has approximately five to eight cell layers of irregular shape and varied size, characterizing the mesophyll as dorsiventral.
Figure 1. Anatomical characterization of *Vernonia ferruginea* leaf. A-B, front view of adaxial and abaxial surfaces of epidermis, respectively. C-D, cross-sections of the midrib and mesophyll. E-F, trichomes. Co=collenchyma; Ep ad=adaxial epidermis; Ep ab=abaxial epidermis; St=stomata; VB=vascular bundle; SP=spongy parenchyma; PP=palisade parenchyma; GT=glandular trichome; Tr=tector trichome.

(Figure 1D). It is similar with most species of this genus such as *V. scorpioides* (Toigo et al., 2004), *V. polyanthes* (Alves and Neves, 2003) and *Vernonia brasiliana* (Filizola et al., 2003), differing only by the double layer of palisade parenchyma, also observed in *Vernonia condensata* (Lolis and Milaneze Gutierre, 2003). The mesophyll has slight intercellular spaces, and the cells have a compact arrangement. Around the bundles into the mesophyll, the parenchymal sheaths extend to both epidermis surfaces (Figure 1D).

At the midrib, immediately under the epidermal cells, two to four layers of angular collenchyma cells are observed, a common feature to other members of the family Asteraceae (Metcalfe and Chalk, 1972): *Ayapana triplinervia* (Nery et al., 2014) and *Mikania laevigata* (Budel et al., 2009). The fundamental parenchyma shows several layers of cells with different sizes. There are few intercellular spaces in the midrib region. The vascular bundles are collateral with evident procambium layers (Figure 1C).

The stem cross-section of *V. ferruginea* has a uniseriate epidermis showing flattened cubic cells with
straight walls or slightly convex external periclinal walls. They are covered by a thin cuticle, with a large number of tector trichomes and few glandular trichomes. Immediately below the epidermis, 2 to 3 layers of angular collenchyma (Figure 2C) and 6 to 9 layers of cortical parenchyma are observed. The stem has various collateral vascular bundles irregularly arranged into the few pith, characterizing a eustele structure (Figure 2A). Numerous secretory ducts of variable diameter with secretory epithelium of seven to eight cells are observed across the cortex among the fundamental parenchyma cells (Figure 2D). Aspects of rosalado and crystal idioblasts of calcium oxalate in form of druse are observed (Figure 2D).

_V. ferruginea_ root has many brachysclereids (stone cells) in the cortical parenchyma, over the vascular bundles; it consists of a sheath over the vascular cylinder. These are sclereids with moderately thick walls and numerous pits, resembling the parenchyma cells in shape (Apezzato-da-Glória and Carmello-Guerreiro, 2006). Between the cortex and vascular cylinder, there is the endoderm, storing various metabolites. This is mainly due to the compact appearance of its cells and the presence of Casparian strips that are little permeable to water and ions (Apezzato-da-Glória and Carmello-Guerreiro, 2006). The vascular cylinder presents a fascicular cambium giving rise to the axial elements and rays, and the inter fascicular cambium originating only rays. The cells of the radial parenchyma present lignified walls, alternating side by side with solitary and multiple radial and racemiform vessels and fibers of average thickness, which provides further rigidity to the root structure (Figure 3).

**Histochemical tests**

Table 1 shows the metabolites group and the reagent used in the leaf, stem, root and glandular trichomes to identify its presence/absence. The histochemical tests revealed the presence of essential oils in the glandular trichomes and a mixture of essences and resin acids inside the endoderm, vessel elements in the root and parenchyma fill of the leaves. Proteins are present in chlorenchyma, collenchyma, cortical parenchyma and phloem in the leaves and stems. The mesophyll and cortical parenchyma of the stem have a large amount of starch grains. Phenolic compounds are found in the mesophyll and parenchyma of leaves and stems. According to Castro et al. (2004), the phenolic compounds are related to antiulcerogenic properties and
Figure 3. Cross section of Vernonia ferruginea root. **A-B.** Cross section of the periderm and cortex. **C-D.** Cross sections of sclerenchyma and conductor vessels. **E-F.** Cross sections of the vascular cambium and parenchyma rays. VC = vascular cambium; Sc = sclereids; VE = vessel elements; Ph = phloem; Fi = fiber; PF = fundamental parenchymal; PR = radial parenchyma; Xy = xylem.

Table 1. Histochemical characteristics of organs and glandular trichomes of Vernonia ferruginea Less.

<table>
<thead>
<tr>
<th>Group metabolites</th>
<th>Reagent</th>
<th>Leaf</th>
<th>Stem</th>
<th>Root</th>
<th>Glandular trichomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid compounds</td>
<td>Sudan IV</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Starch</td>
<td>Lugol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lignin</td>
<td>Phoroglucine acid</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Phenolic Compounds</td>
<td>Ferric chloride and potassium Dichromate</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Total protein</td>
<td>Xilidine Ponceau</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>Vanillin hydrochloric</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Wagner</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Essences</td>
<td>NADI</td>
<td>+</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Sesquiterpene lactones</td>
<td>Sulfuric acid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total carbohydrates</td>
<td>PAS</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>-</td>
</tr>
</tbody>
</table>

(+) Indicates a positive result and (-) negative. The number of positive signs indicates the intensity of the metabolites into the organ.
scarring and antiseptic actions. Fibers, vessel elements and root rays showed lignified walls, with the phloroglucinol test. The PAS test was positive in the overall stem parenchyma and radial parenchyma of the root. Lipids were also detected in the root endoderm and the stem ducts, stained by Sudan.

In *V. polyanthes* Less., the phytochemical analysis revealed the presence of alkaloids, glycosides and flavonoids (Lorenzi and Matos, 2002). In a phytochemical screening conducted with *V. ferruginea*, the presence of flavonoids and terpenoids was detected, but the presence of tannins, saponins and alkaloids was not detected in the extracts studied (Barbastefano, 2007). The presence of chemical constituents such as essential oils, essences, resin acids, lipid compounds and phenolic compounds shows a high variation of primary and secondary metabolites of the species, which might confirm its potential in pharmaceutical production. The identification of the location of the chemical compounds in certain structures of the vegetative organs of the species significantly aids pharmaceutical industries in the extraction of metabolites. This leads to a better utilization of raw materials, to facilitate the process, and enables the use of only those tissues that store the product concerned.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES


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Full Length Research Paper

Chemical composition and mutagenic assessment of petrochemical effluents on onion (Allium cepa) root tip mitosis

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The continuous production and release of chemicals into the environment have led to the need to assess their chemical composition and genotoxic effects on cell reproduction. Two petrochemicals, air liquid and polyester resin effluents were assessed. The common onion of the purple variety was used as the test organism. The results of the chemical analysis of the wastewaters showed high concentration of some potentially mutagenic heavy metals. The effects of the wastewaters and their dilutions on root tip mitosis were mitodepressive. The mitotic index (MI%) were 14.0, 5.3 and 4.1 in control, air liquid and polyester resin effluents, respectively. The MI (%) values estimated across concentrations ranged from 15.1 in control to 5.5 in undiluted effluent. Percent abnormal dividing cells increased with increase in wastewater concentration. Abnormal dividing cells observed ranged from C-mitotic effects to precocious chromosomes and anaphase bridges. These findings indicate the cytotoxic and genotoxic effects of these wastewaters on Allium cepa chromosomes. Positive results on Allium chromosomes could serve as indicator of the deleterious effects of these wastewaters on other organisms at the point of discharge – either on land or water bodies. The need for sound sewerage system that would protect flora and fauna in the ecosystem is advocated.

Key words: Chromosome, ecosystem, heavy metal, mitosis, mutation.

INTRODUCTION

High rate of industrialization means high rate of development. Compounds such as agricultural products, food additives, pharmaceuticals, chemotherapeutic agents and several others produced in different industries are for the well-being of any nation, however, the processes they undergo in these industries to come out as useful products lead to discharge of effluents into the environment. Most of these toxic composites are released as wastewaters which are discharged into large lakes, rivers, canals or drains and even agricultural land without any further treatment to remove the toxic component (Grover and Kaur, 1999). The indiscriminate
discharge of effluents into the environment by local industries without treatment has been accompanied with pollution (Odeigah et al., 1997). These wastewaters are extremely complex mixtures. The complexity makes it almost impossible to carry out a hazard assessment based on chemical analyses only (El-Shahaby et al., 2005). Moreover, the possibility of bioaccumulation and biotransformation is a risk to environmental and human health. An important task has been to develop test systems which can be used to provide data as scientific basis for regulating the discharge of potentially hazardous substances into the environment. Higher plants have been proposed and also used as test organism for the detection of genotoxic substance in the environment (Rank et al., 2002; Maluszynska and Juclimiuk, 2005). Plant roots are excellent in toxicity assays because the roots are always the first to make contact with the chemicals in both soil and water (Odeigah et al., 1997). *Allium cepa* is one of those plants that have been used in different studies to detect chromosome aberrations induced by chemicals since it was first introduced by Levan (1938) and later was proposed as a standard method for the testing of chemicals, in environmental monitoring and in toxicity screening of wastewater and river water (Fiskesjö, 1985a; 1985b; 1993).

The *Allium* genetic material has been widely exploited for such purpose because of its excellent chromosome conditions. *Allium* test is easy to handle, has low cost and shows good correlation with mammalian test systems (Fiskesjö, 1985a). *A. cepa* root system is particularly sensitive to the harmful effects of such environmental contaminants (Vargas et al., 1993). Gross effects can be quantified by measurement of inhibition of growth of the newly developing root system while the examination of the chromosomes of the individual cells of the root tip could be a pointer to their mutagenic effect.

Environmental degradation along with subsequent agronomic con-strains is slowing down the growth in world’s food output. The investigations made by various researchers had pointed out that industrial pollution induced reduction in the number of populations and restricted the distribution of species from the fauna of the areas studied (Metchera et al., 1997). Heavy metals (Cd, Zn, Cu, Pb) are among the most toxic and environmentally dangerous pollutants (Luter et al., 2011; Adu et al., 2012). They are potentially mutagenic class of environmental pollutants and some of them are implicated in the induction of tumours in experimental organisms and exposed humans (Minissi and Lombi, 1997). Jiang et al. (2000) had reported that copper could cause rapid decrease of mitotic index. Cd had been reported to cause genome damages (Risso-de Faverney et al., 2001). Pd is capable of causing a wide range of biochemical and enzyme inhibitions, chromosome aberrations, DNA synthesis abnormality and mutation, it forms complexes with many biomolecules thereby influencing genetic structures (Johnson, 1998).

Dimitrova and Ivanova (2003) reported that the increased heavy metal amount in soil decreased not only the growth of vegetative organs but also the rate of cell division, inducing chromosome aberrations in *Linum usitatissimum*. Abu and Mba (2011) had also reported the induction of diverse chromosomal and cytokinetic abnormalities on *Allium* root mitosis by pharmaceutical effluents. Equally in previous studies chromatin clumping and denaturing effects on DNA by untreated wastewater samples had been reported (Abu and Ogbonna, 2009). Due to the high level of risk associated with the indiscriminate discharge of wastewater without adequate pretreatment and little or no concern by the industrialist and some environmental protection agencies in developing countries, these series of work has been designed to assess the cytoxic and genotoxic effects of wastewaters. The aim of this study was to assess the chemical, cytoxic and genotoxic effects of air liquid and polyester resin industrial effluents on *A. cepa* root tip mitosis as a pointer to their possible mutagenic effects on the ecosystem.

**MATERIALS AND METHODS**

**Wastewater collection**

The test effluents were collected from two industries in the Niger Delta, Southern Nigeria. The two chemical industries produce air liquid and polyester resin, respectively. The wastewaters were collected after primary treatment and stored in opaque plastic gallons in a refrigerator before use. Before the use of the wastewater, the temperature was restored to room temperature in the laboratory.

**Experimental material**

Approximately equal sized onion bulbs of the purple variety were purchased from the local market in Nsukka, Enugu State. The outer scale leaves were removed and the compressed stem bases of the bulbs were scrapped to remove dry roots and expose the root primordial before planting.

**Chemical analysis**

The chemical analyses of the effluents were determined at the Department of Civil Engineering, University of Nigeria, Nsukka. The wastewaters were analysed for zinc, lead, copper, cadmium, sulphate and nitrate. The pH of the samples were taken and recorded.

**A. cepa test**

All the already cleaned bulbs for the microscopic assessment were sprouted over tap water before they were transferred to appropriate test solution (Fiskesjö, 1993; Ukaegbu and Odeigah, 2009). The poorly sprouted bulbs in tap water were discarded. The efficient dilutions used for the experiment were 25, 50 and 75% effluent concentrations. The undiluted wastewater was 100% while tap
water served as the control (0%). Equal volumes of transparent plastic cups were properly positioned on the laboratory benches. Each sample with its five test solutions - 0, 25, 50, 75 and 100% were replicated three times for two treatment durations - 12 and 24 h, respectively. The bulbs with their roots immersed in the appropriate test solution were monitored for 12 and 24 h before harvesting the roots. The roots were fixed in Carnoy solution at the ratio of 3:1 absolute ethanol to glacial acetic acid and thereafter, transferred to 70% ethyl alcohol and stored at 4°C (Jones and Rickards, 1991). The roots were prepared for microscopic study through the conventional methods and acetoorcein was used as stain (Sharma and Sharma, 1965; Jones and Rickards, 1991). The stained and macerated milky root tips on glass slides were covered with cover slips and observed in a microscope starting from lower magnifications. Cell counts were made for both dividing and non-dividing cells. Number of dividing cells out of 1000 cells were counted and recorded. Equally, the number of abnormal cells was also counted. The mitotic index was estimated by calculating the number of dividing cells out of 1000 (one thousand) cells expressed in percentage. The abnormal dividing cells were also expressed as a percentage of abnormal dividing cells over number of dividing cells. Different types of aberrant cells were noted and recorded. The mitotic cells were photographed with motic camera fixed on ordinary light microscope.

### RESULTS AND DISCUSSION

The result of the chemical analysis presented on Table 1 showed that the pH values were 6.4 in air liquid effluent and 5.8 in polyester resin effluents while recommended safe levels for the environment ranged from 6 to 9 (FEPA, 1991). The pH values of the wastewaters were approximately within the range of safe environmental values recommended by the federal environmental protection agency except that polyester resin wastewater was slightly more acidic. The sulphate value (12813.4 mg/l) in polyester resin wastewater was very much higher than recommended environmental value of 500 mg/l. The nitrate, lead and iron contents in the effluent are within environmental safe levels ranging from 5.93 to 11.84, 0 to 0.22 and 12.17 to 17.73 mg/l in nitrate, lead and iron, respectively (Table 1). The values of cadmium and zinc in polyester resin industrial effluent were 170.67 and 58.84 mg/l. Copper was high in the two effluents, 11.29 and 36.13 mg/l for air liquid and polyester resin wastewaters, respectively. The presence of heavy metals in wastewater samples are implicated in diverse cytotoxic and genotoxic effects on organisms. The effects of mutagens on eukaryotic nuclei can be assessed cytologically by observing inhibition of cell growth and division, interruption of metaphase or the induction of numerical and structural chromosomal aberrations and changes among sister and other chromatids (Vieira and Vicentini, 1997).

Beyond the chemical analysis, the effects of the effluents were tested on living system by observing cell reproduction in A. cepa root tip cells. The analyses of variance table for mitotic indices estimates showed that the effluent, concentration, time and all the interactions were highly significant at P ≤ 0.001 (Table 2). This seems to suggest that the effluent and their dilutions, and the duration/length of time these effluents were allowed to remain in contact with biolife could significantly affect the mitotic indices. The significance of effluent dilutions is a pointer to their effects in natural systems where they may be diluted to varying concentrations by natural sources as rain or down the stream in water bodies. However, rain

### Table 1. Chemical analysis of the effluents.

<table>
<thead>
<tr>
<th>Effluent</th>
<th>pH</th>
<th>SO$_4$</th>
<th>NO$_3$</th>
<th>Pb</th>
<th>Cd</th>
<th>Fe</th>
<th>Zn</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air liquid effl</td>
<td>6.4</td>
<td>485.321</td>
<td>5.92</td>
<td>Nil</td>
<td>Nil</td>
<td>12.16</td>
<td>Nil</td>
<td>11.29</td>
</tr>
<tr>
<td>Polyester res. effl</td>
<td>5.8</td>
<td>12813.39</td>
<td>11.84</td>
<td>0.218</td>
<td>170.67</td>
<td>17.73</td>
<td>58.84</td>
<td>36.13</td>
</tr>
</tbody>
</table>


### Table 2. Analysis of variance (ANOVA) of the mitotic index values.

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>VR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effluent</td>
<td>2</td>
<td>9.40E+02</td>
<td>4.70E+02</td>
<td>72737.84</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Concentration</td>
<td>3</td>
<td>1.98E+02</td>
<td>6.61E+01</td>
<td>10241.49</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Duration</td>
<td>1</td>
<td>1.69E+01</td>
<td>1.69E+01</td>
<td>2620.16</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Effluent*Concentration</td>
<td>6</td>
<td>1.04E+02</td>
<td>1.73E+01</td>
<td>2671.56</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Effluent*Duration</td>
<td>2</td>
<td>9.09E+00</td>
<td>4.55E+00</td>
<td>704.03</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Concentration*Duration</td>
<td>3</td>
<td>4.77E+00</td>
<td>1.59E+00</td>
<td>246.05</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Effluent<em>Concentration</em>Duration</td>
<td>6</td>
<td>3.72E+00</td>
<td>6.20E-01</td>
<td>95.99</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Residual</td>
<td>24</td>
<td>1.55E-01</td>
<td>6.46E-03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>1.28E+03</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
fall may take longer periods depending on seasonal variations, thereby allowing contact between plants and effluents to last for a longer period. The significance of the interactions also indicate that effluent, its concentration and the length of the time of contact with biological systems can significantly affect cell reproduction in diverse ways.

The M1 in the effluents, their dilutions and across the treatment durations is shown on Figure 1. Significant differences (P = 0.001) were observed on the main effects of effluents on the MI (%). The control had the highest MI in both the wastewater and the treatment durations. The Polyester resin effluent at 100% (undiluted) was more deleterious to cell reproduction at both 12 and 24 h treatment time Figure 1. The main effects of the industrial wastewater and the control on cell reproduction as estimated by M1 (%) were 14.0, 5.3 and 4.1 in control, air liquid and polyester resin effluents, respectively (Figure 2). This indicates that the main effects of the effluents on MI (%) as it compares with the control value are 37.86 and 29.29% for air liquid and polyester resin, respectively. This places these wastewaters as being capable of causing sublethal effects on contact organisms (Antonsie – wiez, 1990; Panda and Sahu, 1985). Figure 3 shows the effects of concentration and treatment time on mitotic index. The MI (%) reduced significantly as the concentrations increased. The treatment time of 12 and 24 h duration
also caused significant reduction in cell reproduction as shown by MI (%) values. The 24 h treatment time had lower MI (%) across all the concentrations thereby suggesting that longer period of the wastewater with contact organisms are more deleterious.

The effects of the effluent across the different concentrations showed increase in percent abnormal cells as the concentration of the wastewater increased (Figure 4). The percentage abnormal dividing cells had direct relationship with the concentration of the effluents increasing with increase in concentrations. The abnormal dividing cells ranged from 0.2% in control to 97.2% in air liquid and 0.4 to 98.7% in polyester resin effluents (Figure 4). At 75% effluent concentrations, abnormal dividing cells were more than 80% of the cells dividing. On close observation all the dividing cells in the undiluted wastewater concentrations in both effluents were abnormal. This implies that all the cells estimated in M1% in Figure 1 were all abnormally dividing cells at 100% effluent concentration. Similarly, treatment time had significant effects in inducing abnormal cells, the longer the A. cepa root cells stayed in the wastewater the more the number of abnormal dividing cells (Figure 5). Polyester resin wastewater, which also had higher concentrations of lead, cadmium, zinc and copper, significantly induced higher number of abnormal dividing cells, even at lower concentrations. This agrees with several reports on the implication of heavy metals in inducing diverse cytotoxic and genotoxic effects in organisms (Bruning and Chronz, 1999; Kovalchuk et al., 2001).

The high reduction in cell reproduction and other observed abnormalities could lead to mutagenic effects of these effluents in the ecosystem. The reduced M1 and high rate of abnormalities could be correlated with the chemical compositions of the effluents. The chemical composition of the air liquid effluent was within the range of safe environmental levels with the exception of the copper which was as high as 11.3%. On the other hand, the polyester effluent had higher levels of sulphate, zinc and copper when compared to environmental standards. The M1 in undiluted polyester effluent was significantly lower than their values in both the control and air liquid effluent. The aberrant cells were also more in the polyester wastewater. The observed reduction in cell reproduction and increased aberrant cell could be linked to the toxic levels of chemicals and heavy metals in the effluent. It has been reported by several authors that heavy metals are deleterious and could cause diverse forms of aberrations (Minissi and Lombi, 1997; Johnson, 1998; Jiang et al., 2000). The very high levels of zinc and copper could be the reason for higher deleterious effects
Figure 4. Effects of the effluents and the different concentrations on abnormal cells.

Figure 5. Effect of effluent and treatment time on abnormal dividing cells.
observed in this polyester resin industrial effluent, however, the air liquid which had values within the range of environmental levels were also highly deleterious as can be seen in reduced cell reproduction and high percentage abnormal dividing cells. This is in line with an earlier report that wastewaters are complex mixtures and that chemical analysis cannot be effectively used to assess their toxicity level. Fiskesjö (1985a) and El-Shahaby et al. (2005) reported that wastewater are extremely complex mixtures containing numerous inorganic and organic compounds, the complexity makes it impossible to carry out a hazard assessment based on chemical analysis only. Therefore, the seemingly low levels of compounds observed in air liquid effluent based on chemical analysis may not be enough to quantify their risk in the ecosystem. The possibility of bioaccumulation and biotransformation equally increases their risk. Moreover, the action of wastewater in the natural system cannot be attributed to specific compounds in the mixture but to a set of chemical properties and interactions of groups of compounds in the wastewater.

Diverse forms of chromosomal aberrations were observed (Plate 1), these include: C-mitotic effects of diverse kinds signifying disturbance or breakdown effects on the spindle apparatus, precocious chromosomes at anaphase and anaphase bridges which could lead to chromatid breakage and formation of micronuclei or aneuploidy conditions in the parental cells. It has been
reported that cytokinetic failure in addition to diverse forms of chromosomal aberrations could lead to cancerous cells in organisms (Panda and Sahu, 1985; Bruning and Chronz, 1999; Kovalchuk et al., 2001). Another major abnormality observed was prophase accumulation. The effluents at high concentration significantly reduced cell division and equally there seemed to be a delay at prophase as most of the cells dividing at high concentration remained at prophase stage. This phenomenon has been attributed to a delay in the breakdown of the nuclear membrane due to a ‘carryover’ inhibitory effect from treatments (Wilson, 1965).

The significant reductions in cell reproduction as shown by low MI at high concentrations and diverse forms of chromosomal aberrations are pointers to the possible mutagenic effects of these wastewaters in the ecosystem. Positive results of Allium test should be considered as warning and an indication that the samples tested may constitute a risk to environmental health. Therefore, there is need for industries in the third world to maintain a sound sewerage system for proper pretreatment of effluents before discharge into the environment.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES


Full Length Research Paper

Management of *Fusarium* Wilt using mycolytic enzymes produced by *Trichoderma harzianum* (Th. Azad)

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The main aim of this study was to isolate the best chitinase and glucanase enzyme producing *Trichoderma* strain to manage the *Fusarium* wilt disease of *Cicer aritenum* under in vitro conditions. We also studied the effect of *Trichoderma* strains on the growth and development of *C. aritenum* plants. Seven strains of *Trichoderma* were screened against the *Fusarium* pathogen to isolate the best biocontrol agent causing maximum inhibition of *Fusarium* growth. *Trichoderma harzianum* (Th. Azad) was found to be the best strains among all the tested strains. *Trichoderma* treated plant exhibited the least disease incidence as compared to control plants. *Trichoderma* treated plant showed a significant stimulatory effect on all the tested eight parameters as compared to control.

Key words: *Trichoderma*, antagonistic activity, chitinase, glucanase, Biocontrol agent, phytopathogenic fungi.

INTRODUCTION

Plants are the major source of food, fibre, fodder, medicines and many other useful products (Naseby et al., 2000). Various insects, bacteria, virus, fungi and other pests attack plants at various stages of their development (Rifai, 1969; Elad, 1983). *Fusarium oxysporum* and *Sclerotinia sclerotiorum* are the major plant pathogens which cause rot, and wilt in plants. For the control of these phytopathogens different chemical fungicides are used (Papavizas, 1985). Extensive use of these chemical fungicides has lead to the development of fungicide resistant strains. Thus, there is a need for identifying alternative measures which can be efficiently used for the control of phytopathogens. *Fusarium* is an important disease which attacks chickpea, bean, wheat, barley and other grains worldwide, especially in humid and semi humid areas (Schroeder and Christensen, 1963; Howell et al., 2003; Haggag and Amin, 2001). The disease caused by this fungus is characterized by wilted plants, yellowed leaves and root rot, and minimal or absent crop yield (Nemec et al., 1976; Harman et al., 2000, 2006). In many regions of the world, chickpea (*Cicer arietinum* L.) is a popular vegetable and chief source of protein in the human diet. During chickpea cultivation problems have occurred that were connected to diseases which could reduce yield and crop quality. Chickpea is susceptible to *Fusarium* root rot strain (*Fusarium solani* (Mart.) Sacc. f.

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sp. eumartii (C. Carpenter) (Snyder and Hans) and Fusarium wilt strain (F. oxysporum Schlechtend.: Fr. f. sp. ciceris (Padwick) Matuo and K. Sato). Rhizosphere is the first line of protection for roots and rhizospheric microorganisms producing HCN, siderophore or leading to antibiotics, competition, parasitism and cell lysis can ideally be used as biocontrol agents (Shahid et al., 2012a). As chitinase and β-1, 3-glucanase are two main hydrolytic enzymes associated with fungal cell wall lysis, the purpose of this study was to isolate the best chitinase and glucanase producing Trichoderma strain and to screen their antagonistic activity against F. oxysporum (Elad, 1999; Pandey et al., 2014 b). So, we can employ Trichoderma species for the control of Fusarium wilt (Shahid et al., 2012b).

MATERIALS AND METHODS

All the microbes used in this study were isolated from the soil of different locations of UP. All the microbes were purified using serial dilution plate method and preserved on PDA media at 4°C.

Screening of Trichoderma strains

All the isolated strains were screened against Fusarium by dual culture method to identify the potential and effective strains. Out of different strains of Trichoderma screened against Fusarium Trichoderma harzianum (Th. Azad) showed the maximum inhibition against Fusarium under in vitro conditions. β-1, 3-Glucanase and chitinase were assayed in culture filtrates and reducing sugar was evaluated by using dinitrosalicylic acid (DNS) solution. One unit of β-1, 3-glucanase/ chitinase enzyme was defined as the amount which liberates 1 U/ml of reducing sugar per hour. Trichoderma harzianum (Th. Azad) has earlier been proved successful in their ability to biocontrol diseases in a broad range of plant species (Lorito et al., 1994). Czapek Dox broth (pH 7) was inoculated with T. harzianum and incubated for ten days for glucanase enzyme production. For chitinase production chitinolytic media was inoculated with T. harzianum under aseptic conditions and incubated at 120 rpm, 28°C. The enzyme activity was measured after seven days of incubation period. It was found that T. harzianum produced 2.01 U/mg of glucanase enzyme and 6.2 mg/ml of chitinase enzyme (Pandey et al., 2014 a and c). Trichoderma grown in PD broth at 28°C and 120 rpm for seven days were centrifuged at 6800 g for 12 min at 4°C. Pellet was collected and resuspended in distilled water to obtain a population density of 1 × 10^6 CFU/ml. 1% carboxy methyl cellulose (CMC) was mixed with the suspension to make slurry and were used to coat surface sterilized C. aritenum seeds. Seeds were allowed to dry overnight under sterile condition and CFU was counted by dilution plate method and found to be 3.6 × 10^6 CFU/seed (Mukesh Srivastav et al., 2014; Wells et al., 1972). Spore inoculum (10^6 conidia/ml) of the selected fungal strains was mixed with sterilized seeds. Sowing was done in pots filled with crystal sands. Three replicates of each treatment were designed: In treatment 1 only C. aritenum seeds (C) were used. In treatment second seeds were treated with only biocontrol agent (S) and in treatment third seeds were treated with bioagent as well as with pathogen (R). For each treatment four replicates were used. After 40 days the plants were uprooted and growth (root/shoot length, germination index, total weight, Dry wt of plant, total nitrogen and protein content) were recorded.

RESULTS AND DISCUSSION

In pot experiments, the germination of half of the seeds was inhibited by F. oxysporum. However, in the presence of bioagent all the seeds germinated successfully. Similarly, the germination index with F. oxysporum alone was only 25% (Table 1 and Figure 1a). The interaction with T. herzianum led to better increase in all the 9 attributes as compared to control. About 80% increase in the total weight was recorded when T. herzianum was inoculated in F. oxysporum infested seeds as compared to uninoculated pathogen. Protein and nitrogen content was also high in the R treatment as compared to S and C (Table 2 and Figures 1b and c). The extensive use of chitinase and glucanase producing microorganism as biological control agents against many fungal pathogens has been reported (Vipul et al., 2014, Vipul et al., 2015). Reports have indicated that application of different species of Trichoderma have been found (Vipul et al., 2015; Bell, 1982; Elad, 1999; Ramezani, 2009). Our work has demonstrated the ability of isolates (T. herzianum) to destroy the phytopathogens because of mycolytic enzymes production which were biologically active in soil conditions and showed excellent promise as biocontrol agent (Jayaraj and Ramakrishnan, 1991; Biswas and Das, 1999). Several workers (Jalakshmi et al., 2009; Muhammad and Amusa, 2003; Bunker and Mathur, 2001; Shabir et al., 2012) have reported the inhibition of soil borne fungi, F. oxysporum f. sp. ciceri by Trichoderma species, due to production of extracellular cell wall degrading enzyme such as chitinase, β-1, 3-glucanase, β-1, 6-glucanase, protease, cellulase and lectin, which help Trichoderma in colonizing the host.

Trichoderma species are the most studied biocontrol agents that are used against a variety of fungal plant pathogens. T. herzianum is among the most potential species of Trichoderma that are commonly used for phytopathogen control. Trichoderma employs several mechanisms to combat the effect of phytopathogens. The various mechanisms employed by Trichoderma are, secretion of CWDEs, secondary metabolite production, mycoparasitism, competition for food and space and induction of host defence response.

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEDGMENTS

The authors are grateful for the financial support granted by the ICAR under the Niche Area of Excellence on Exploration and Exploitation of Trichoderma as an antagoist against soil born pathogen, running in Department of Plant Pathology, C. S. Azad University of Agriculture and Technology, Kanpur.
Figure 1A. Effects of *Trichoderma* application on all the three treatments (C only *Cicer arietinum* seeds, S seeds treated with only biocontrol agent and R seeds treated with bioagent as well as with pathogen) of *Cicer arietinum* seeds. For each treatment four replicates were used. A: Seed treatment process of *Cicer arietinum* seeds and their growth in crystal sand.
Figure 1B. Effects of *Trichoderma* application on all the three treatments (C only *Cicer arietinum* seeds, S seeds treated with only biocontrol agent and R seeds treated with bioagent as well as with pathogen) of *Cicer arietinum* seeds. For each treatment four replicates were used. Uprooted *cicer arietinum* plants.
Figure 1C. Effects of *Trichoderma* application on all the three treatments (C only *Cicer aritenum* seeds, S seeds treated with only biocontrol agent and R seeds treated with bioagent as well as with pathogen) of *Cicer aritenum* seeds. For each treatment four replicates were used. Dried *Cicer aritenum* plants.

Table 1. Effect of mycolytic enzymes produced by *Trichoderma* on the different growth parameters of all the three treatments (C only *Cicer aritenum* seeds, S seeds treated with only biocontrol agent and R seeds treated with bioagent as well as with pathogen) of *Cicer aritenum* seeds.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Germination</th>
<th>Root length (cm)</th>
<th>Shoot length (cm)</th>
<th>Seedling length (cm)</th>
<th>Dry Weight (g)</th>
<th>Vigour Index I (g)</th>
<th>Vigour Index II (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>20</td>
<td>11.3</td>
<td>14.8</td>
<td>21.0</td>
<td>0.36</td>
<td>420.0</td>
<td>7.2</td>
</tr>
<tr>
<td>R2</td>
<td>24</td>
<td>4.0</td>
<td>17.0</td>
<td>24.8</td>
<td>0.50</td>
<td>595.2</td>
<td>12.0</td>
</tr>
<tr>
<td>R3</td>
<td>17</td>
<td>7.6</td>
<td>18.4</td>
<td>29.2</td>
<td>0.46</td>
<td>496.4</td>
<td>7.82</td>
</tr>
<tr>
<td>R4</td>
<td>17</td>
<td>4.8</td>
<td>16.9</td>
<td>25.1</td>
<td>0.39</td>
<td>426.7</td>
<td>6.63</td>
</tr>
<tr>
<td>Average</td>
<td>19.5</td>
<td>16.925</td>
<td>16.77</td>
<td>25.02</td>
<td>0.42</td>
<td>484.5</td>
<td>8.41</td>
</tr>
<tr>
<td>S1</td>
<td>13</td>
<td>6.2</td>
<td>14.2</td>
<td>19.0</td>
<td>0.38</td>
<td>341.9</td>
<td>5.33</td>
</tr>
<tr>
<td>S2</td>
<td>18</td>
<td>7.8</td>
<td>13.2</td>
<td>18.3</td>
<td>0.28</td>
<td>288.0</td>
<td>4.32</td>
</tr>
</tbody>
</table>
Table 1. Contd

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>S3</td>
<td>13</td>
<td>10.8</td>
<td>13.1</td>
<td>16.7</td>
<td>0.30</td>
<td>221.0</td>
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<tr>
<td>S4</td>
<td>20</td>
<td>8.2</td>
<td>16.9</td>
<td>22.5</td>
<td>0.38</td>
<td>288.0</td>
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<tr>
<td>Average</td>
<td>16</td>
<td>8.25</td>
<td>14.35</td>
<td>19.12</td>
<td>0.335</td>
<td>284.7</td>
</tr>
<tr>
<td>C1</td>
<td>12</td>
<td>4.8</td>
<td>15.00</td>
<td>26.3</td>
<td>0.41</td>
<td>228.0</td>
</tr>
<tr>
<td>C2</td>
<td>10</td>
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<td>12.0</td>
<td>16.0</td>
<td>0.24</td>
<td>183.0</td>
</tr>
<tr>
<td>C3</td>
<td>16</td>
<td>3.6</td>
<td>9.4</td>
<td>17.0</td>
<td>0.36</td>
<td>267.0</td>
</tr>
<tr>
<td>C4</td>
<td>14</td>
<td>5.6</td>
<td>9.6</td>
<td>14.4</td>
<td>0.31</td>
<td>315.0</td>
</tr>
<tr>
<td>Average</td>
<td>13</td>
<td>4.77</td>
<td>11.5</td>
<td>18.42</td>
<td>0.33</td>
<td>248.3</td>
</tr>
</tbody>
</table>

For each treatment four replicates were used.

Table 2. Biochemical effect of mycolytic enzymes produced by Trichoderma on all the three treatments (C only Cicer aritenum seeds, S seeds treated with only biocontrol agent and R seeds treated with bioagent as well as with pathogen) of C. aritenum seeds.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nitrogen (%)</th>
<th>Protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>0.370</td>
<td>2.31</td>
</tr>
<tr>
<td>R2</td>
<td>0.330</td>
<td>2.05</td>
</tr>
<tr>
<td>R3</td>
<td>0.300</td>
<td>2.18</td>
</tr>
<tr>
<td>R4</td>
<td>0.320</td>
<td>2.02</td>
</tr>
<tr>
<td>Average</td>
<td>0.318</td>
<td>1.98</td>
</tr>
<tr>
<td>C1</td>
<td>0.274</td>
<td>1.71</td>
</tr>
<tr>
<td>C2</td>
<td>0.266</td>
<td>1.66</td>
</tr>
<tr>
<td>C3</td>
<td>0.280</td>
<td>1.75</td>
</tr>
<tr>
<td>C4</td>
<td>0.260</td>
<td>1.62</td>
</tr>
<tr>
<td>Average</td>
<td>0.287</td>
<td>1.68</td>
</tr>
</tbody>
</table>

For each treatment four replicates were used.

REFERENCES


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Short Communication

β-Lactam and chloramphenicol-resistant enterobacteria in hospital surfaces

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The following study aimed to research the Enterobacteriaceae present on the material surfaces of a hospital environment in a Community Health Unit in Ceara-Brazil. Data was collected in 10 different rooms and facilities by rubbing sterile swabs in an enclosed area of 10 cm² for a minute. Bacterial growth was observed in all surveyed areas. However, Enterobacteriaceae were only found in surfaces from the kitchen and the reception. From the isolated strains (n = 10), the vast majority were identified as Enterobacter (n = 7). Four of those Enterobacter strains were found to be resistant, with the following resistance profiles: monoresistance to ampicillin (n = 2) and chloramphenicol (n = 1) and cross-resistance to beta-lactam (n = 1). The results serve as an alert to public health authorities, for enteric bacteria resistant to drugs were found in two environments in the facility.

Key words: Enterobacteriaceae, antimicrobial resistance, hospital environment.

INTRODUCTION

Hospital-associated bacteria have been commonly related to outbreaks (Brust et al., 2013) and environmental surfaces may contribute to transmission of nosocomial pathogens (Livshiz-Riven et al., 2015). Among contaminant bacteria found in those surfaces, enterobacteria are worth mentioning, since these microorganisms are associated with infections (Ito et al., 2015). For Loftus et al. (2015), Gram-negative bacteria (GNB) are an important health care concern due to increasing prevalence of infection and community spread. In this context, the isolation of Gram-negative enteric bacilli on surfaces from hospital environments (Thurlow et al., 2013; Freeman et al., 2014) has been previously reported, suggesting that these surfaces may contribute to the transmission of pathogens implicated in nosocomial infections. The alert is also extended to the spread of antibiotic-resistant strains, as these microorganisms may become part of the allochthonous microbiota.

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Abbreviations: GNB, Gram-negative bacteria; CRE, carbapenem-resistant enterobacteriaceae.

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of a hospital setting (Di Conza et al., 2014) if the disinfection procedures are not properly conducted. For Pelege and Hooper (2010), GNB are highly efficient at up-regulating or acquiring genes that code for mechanisms of antibiotic resistance.

In hospital environmental, the emergence of drug-resistant enterobacteria is currently a worldwide problem. Carbapenem-resistant Enterobacteriaceae (CRE) has been detected as contaminant of the environmental surfaces of hospital room (Weber et al., 2015). In a tertiary care hospital in Mexico City, Torres-Gonzalez et al. (2015) report an outbreak caused by a New Delhi Metallo-β-lactamase 1 (NDM-1) harboring plasmid spread by *Escherichia coli* (ST617) and *Enterobacter cloacae* (ST182). Thus, considering that understanding microbial populations in hospital environments is crucial for improving human health (Poza et al., 2012), the present study aimed to verify the occurrence of enterobacteria in hospital surfaces from a Community Health Unit in Ceará-Brazil, by isolating and identifying enteric bacteria, as well as proceeding with the antimicrobial susceptibility profile of the isolates.

**MATERIALS AND METHODS**

Samples for microbiological analysis were collected from a Health Unit located in Ceará-Brazil. They were collected from surfaces in ten different rooms: dining room table, kitchen counter, reception counter, reception chair, external knob of the bathroom door, internal knob of the bathroom door, seat in the nursing room, workbench in the nursing room, table in the doctor’s office and workbench in the doctor’s office. Collection procedure corresponded to rubbing sterile swabs for about a minute in an enclosed area (10 cm²) from each surface. After the sampling, swabs were placed in tubes containing Brain Heart Infusion Broth (BHI - Difco) medium, incubated at 35 ± 1.0°C for 24 h. Then, aliquots were removed, plated on McConkey agar and incubated at 35°C ± 1.0/24 h. Colonies with phenotypic characteristics consistent with those of enterobacteria were isolated on tryptone soy agar (TSA), followed by incubation at 35°C ± 1.0/24 h. Gram stain analysis and oxidation test were subsequently performed. The Gram-negative oxidase-negative bacilli cultures were selected for the identification process via EPM – MiLi – Simmons Citrate kit enterobacteriaceae identification (Indol, L-triptofano, sacarose, H₂S, gas glicose, I-lisina, motilitade), with incubation at 35°C ±1.0/ 24 h. Strains confirmed as enterobacteria were kept in TSA until the antimicrobial susceptibility testing.

The determination of antimicrobial susceptibility profiles were performed by the agar diffusion disk technique using Agar Mueller-Hinton (MH) medium, as detailed in the Clinical and Laboratory Standards Institute (CLSI, 2012). The following antimicrobials were tested: Amikacin (30 µg), Ampicillin (10 µg), Cefotaxime (30 µg), Cefepime (30 µg), Ceftriaxone (30 µg), Cefuroxime (30 µg), Chloramphenicol (30 µg), Streptomycin (10 µg), Gentamicin (10 µg), Imipenem (10 µg), Meropenem (10 µg), and Tetracycline (30 µg). All enterobacteria strains were diluted in 0.85% saline in order to match a turbidity of a McFarland 0.5 scale. Aliquots were removed from the diluted cultures and plated onto MH medium, followed by the application of antibiotic disks. Plates were incubated at 35°C ± 1.0 and the reading and interpretation of inhibition zones were in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2012).

**RESULTS AND DISCUSSION**

Microbial growth was observed on all analyzed surfaces. However, enterobacteria were only confirmed on surfaces 2 (kitchen counter) and 3 (reception counter). Out of the Enterobacteriaceae identification, seven out of 10 isolated strains were identified as *Enterobacter* (areas 2 and 3), two were of indeterminate profile and one was confirmed as *Citrobacter*.

The bacterial genus which represented 70% of total isolates - *Enterobacter* - is a common nosocomial microorganism (Tuon et al., 2015). Its isolation from samples taken on hospital surfaces is not an unusual fact: Matoušková and Holy (2014) conducted a monitoring of a transplant unit, hematology/oncology clinic in Czech Republic and detected, from samples taken from surfaces, that the second most frequently isolated Gram-negative strains were from the *Enterobacter* (28%) genus.

The occurrence of bacteria in hospitals has been commonly related to some possible sources of dissemination: bottle soap (Buffet-Bataillon et al., 2009), hands of healthcare professionals (Tan et al., 2013), gloves and gowns (Rock et al., 2014), mobile phones (Ustun and Cihangiroglu, 2012) paper money and coins (Angelakis et al., 2014). Besides those vectors, the occurrence of hospital pests must also be highlighted. Menasria et al. (2014) researched the bacterial load of a cockroach species (*Blattella germanica*) found in hospital environment, and isolated *Enterobacter* and *Citrobacter* from both external surface and digestive tract of the insect.

In the present study, enteric bacteria were isolated from the bench designed for processing the hospital's food. This is a fact of major concern, and it serves to alert the risk of food contamination through inadequate hygiene practices, since these microorganisms can be indicators of fecal contamination (Van Hoek et al., 2015). In a similar study, Staskel et al. (2007) evaluated the microbiota of foodservice surfaces in several Texas child-care centers, and isolated Enterobacteria (*Klebsiella pneumonia* and *Salmonella Paratyphi A*) that are considered nonopportunistic and can infect healthy individuals. The authors stated that it is vital that the staff wash their hands often and disinfect every surface, for even those that appear to be clean may harbor microorganisms.

The reception counter was another place where enterobacteria were detected. This may be related to the continuous flow of patients and professionals, which could cause not only the contamination per se, but its spread to other sectors in the facility.

All isolates confirmed as enterobacteria (n = 10) were submitted to an antibiogram. Four strains resistant to the following profiles were detected: monoresistance to ampicillin (n = 2), monoresistance to chloramphenicol (n = 1), and cross-resistance to the β-lactam Amp, Cro, Ipm e Mer (n = 1). No strain presented multi-resistance (Table
Among the group of β-lactam antibiotics, ampicillin was the antibiotic for which the resistance index was the highest (n = 3). Besides, resistance to second (cefuroxime) and third (ceftriaxone) generation of cephalosporin was observed (Table 1). In accordance to our findings, Vasques et al. (2011) isolated Enterobacter spp. in hospitals and detected resistance to ampicillin and ceftriaxone. To the authors, there is a high incidence of infections caused by betalactam-producing Gram-negative microorganisms in Brazil. These organisms are of clinical and epidemiological importance, since their mobile genetic elements helps in the cross-infection process.

Hawkey (2015) alerted to the fact that in Asian countries a consequence of high rates of ESBL production among Enterobacteriaceae is that there is a substantial use of carbapenem antibiotics, resulting in the emergence of plasmid-mediated resistance to this class of drugs. In the present study, one strain resistant to imipenem and meropenem (Table 1) was found, which characterizes the health unit studied as a potential source of a carbapenem-producing bacteria spread. The low isolation rate of Carbapenem-resistant Enterobacteriaceae (CRE) is in accordance to the findings of Werber et al. (2015), who researched the survival of CRE in inoculated surfaces of hospital rooms. The authors verified that three species of CRE (Klebsiella, Enterobacter, and Escherichia) survived poorly (>85% die-off in 24 h) when ~2 log10 CFU were inoculated onto 5 different environmental surfaces.

Increasing on the incidence of antibiotic-resistant Gram-negative infections has become the most pressing issue in bacterial resistance. Indiscriminate antimicrobial use in humans and animals, combined with an increased global connectivity fostered the transmission of Gram-negative infections harboring extended-spectrum β-lactam in the 1990s. Carbapenem-producing Enterobacteriaceae have been the latest affliction since late 1990s and early 2000s (Vasoo et al., 2015).

One strain of a mono-chloramphenicol-resistant Enterobacter was found (Table 1). Ćivljak et al. (2014) stated that chloramphenicol is a broad spectrum antibiotic that was abandoned in developed countries due to its association with fatal aplastic anemia. However, it is still widely used in 3rd world countries. In the light of the emerging problem of multi-drug resistant pathogens, its role should be reassessed. Susceptibility patterns for Gram-positives were good, although less favorable for Gram-negatives.

The findings in this study serve as a warning to political and social authorities in Public Health as an evidence that hospital surfaces can be antibiotic-resistant enteric bacteria reservoirs. Thus, it is important to be extremely cautious about the quality of the cleaning and disinfecting in hospital environments, providing orientation to both patients and visitors, as well as continuing education measures to all the health professionals in different units.

### Conflict of interest

The authors did not declare any conflict of interest.

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Short Communication

A direct and sensitive method for screening fructooligosaccharides-digesting microorganisms useful in food and health science

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Short-chain fructooligosaccharides (FOS) stimulate selectively the growth and activity of microorganisms in the colon providing positive health effects and well-being in humans and animals. The lack of accurate isolation methods, however, hampers the possibility of getting new potential fructooligosaccharides (FOS)-fermenting yeast or bacterial strains. A valuable screening procedure to visually detect bacterial and yeast strains able to ferment FOS in liquid or solid rich media supplemented with an innocuous pH indicator is described. Using this assay, 15 FOS-consuming strains isolated from different sources were successfully evaluated to prove the utility of the method. This screening procedure is a new and valuable tool in rapid large-scale detection of potential FOS fermenting-strains useful in food and health science.

Key words: FOS-fermenting microorganisms, prebiotics, probiotics, symbiotics.

INTRODUCTION

The selection of bacterial or yeast strains able to efficiently ferment short chains-carbohydrates with prebiotic properties for example, fructooligosaccharides (FOS), is a subject of permanent interest. However, the lack of direct screening methods hampers the rapid identification of such important microorganisms. The ability to ferment short chain oligosaccharides is a key property for any bacterial or yeast strain to provide desirable clinical effects (Kaplan and Hutkins, 2000). Main targets for FOS consumption in the colon are Bifidobacteria and Lactobacilli as these intestinal bacteria have shown several positive effects upon human or animals’ well-being (Kaplan and Hutkins, 2003).

According to that explained above, if certain carbohydrates, such as fructooligosaccharides, are fermented by only specific beneficial microbial strains, then a liquid or solid growth medium containing these “prebiotic” substrates as unique carbon sources could efficiently and directly select for those useful FOS-fermenters microbial strains.

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Abbreviations: FOS, Short-chain fructooligosaccharides; TLC, thin layer chromatography; HPLC, high pressure liquid chromatography; YNB, yeast nitrogen base.

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In previous research the ability to ferment FOS of some Bifido and Lactic acid bacterial strains was assayed (Kaplan and Hutkins, 2000). However, two main drawbacks still to date make it difficult to establish an accurate screening procedure based on FOS consumption. Firstly, FOS commercial preparations may have different polymerization degrees and/or contain contaminants like glucose, fructose, sucrose, or other fermentable sugars being rather difficult to establish if microbial growth in FOS-containing medium is really due to FOS metabolism.

This fact makes necessary the use of additional confirmatory analytical techniques such as thin layer chromatography (TLC) or High Pressure Liquid Chromatography (HPLC). Secondly, the selection of FOS-fermenter strains by using continuous culture (Sghir et al., 1998) or rich media such as De Man, Rogosa and Sharpe (MRS), to evaluate FOS consumption by bacteria or yeast undoubtedly could mask the final results. To overcome this problem, instead of a rich media like MRS or any rich media containing components in yeast extract or peptone due to product release. causing alkalinization (pH above 7) due to ammonia evolution, a simple visual inspection is sufficient to detect the slightly pH switches from acidic to basic conditions can be detected by the addition of the innocuous pH indicator Bromothymol blue (transition interval pH 6.0 to 7.6; yellow-blue, Sigma Co., USA Catalog number: M6030) to the minimal media but including 20 g of agar per liter. For the pH shift assays using any of the solid or liquid media mentioned above, 0.0025% of Bromothymol blue (transition interval pH 6.0 to 7.6: yellow-blue, Sigma Co., USA Catalog number: 34656), filter-sterilized solutions of glucose, fructose and sucrose (BDH) added at 2% final concentration or FOS solution obtained in this work 2 and 3% final concentration were incorporated to the adequate culture media according to the different experiments requirements. Final pH in the different media was adjusted to 6.5.

Microbial growth under anaerobic conditions

COY Chambers (COY Laboratory Products Inc.) were used to create and maintain anaerobic conditions as needed during growth of some of the microorganisms mentioned above in the different experiments.

FOS mixture composition

The initial FOS mixture was composed by: 4.6% nystose (GF3), 56% 1-kestose (GF2), 21% sucrose (GF), 17% glucose (G), and 1.4% fructose (F). This syrup was obtained according to the different experimental conditions. The mixture was further submitted to HPLC separation as described below to get an enriched FOS solution composed mainly by 1-kestose and nystose.

Sugars separation by high performance liquid chromatography (HPLC)

Sugar composition in the enriched FOS solution were separated in an Aminex HPX-42C column (0.78 by 30 cm; Bio-Rad Laboratories, Hercules, Calif.) and detected with a RI-410 (Waters) detector. The column temperature during the analysis was kept constant at 85°C, and water was used as the mobile phase at 0.6 ml/min.

RESULTS

Unlike yeasts, some lactobacilli and bifido bacterial strains were not able to grow in minimal media supplemented with FOS. Previously, syrup composed of 4.6% nystose (GF3), 56% 1-kestose (GF2), 21% sucrose (GF), 17% glucose (G), and 1.4% fructose (F) was produced during sucrose transformation by recombinant P. pastoris cells entrapped in Ca-alginate beads. After sugars separation, an enriched FOS solution containing

MATERIALS AND METHODS

Microorganisms

Bifidobacterium bifidum 15696, B. dentium 27678 were obtained from ATCC collection and E. coli TOP10F, Pichia pastoris GS115, P. pastoris X33 were purchased from Invitrogen SA. Other bacterial and yeast strains such as: Saccharomyces boulardii L/25/4/96, Saccharomyces cerevisiae L/25-7-82, S. cerevisiae L/25-7-76, Kluyveromyces fragilis L/12-8-1 K. fragilis L/12-8-6, Lactobacillus acidophilus B/103-5, Lactobacillus rhamnosus B/103-1-5, Lactobacillus reuteri B/102-1, Lactobacillus fermentum B/103-11-3, Lactobacillus casei B/103-11-6, Lactobacillus paracasei B/103-11-7, Lactobacillus bulgaricus B/103-12-6, Streptococcus thermophilus B/103-12-7, were isolated from different sources and belongs to the microorganism collection of the Cuban Research Institute for Sugarcane Derivates (ICIDCA) Havana, Cuba.

Culture media

The commonly used minimal Yeast Nitrogen base (YNB) and rich media YP (Yeast extract and Peptone) and LB (Luria Bertani) for yeast and bacterial growth, respectively, were prepared according to the Pichia expression vectors for constitutive expression and purification of recombinant proteins Catalog nos., V200-20 and V205-20, Invitrogen SA (USA). Minimal M9 media was purchased from Sigma Co., (USA) catalog number: M6030 and prepared according to the manufactures instructions. Solid media was prepared similar that liquid media but including 20 g of agar per liter. For the pH shift assays using any of the solid or liquid media mentioned above, 0.0025% of Bromothymol blue (transition interval pH 6.0 to 7.6: yellow-blue, Sigma Co., USA Catalog number: 34656), filter-sterilized solutions of glucose, fructose and sucrose (BDH) added at 2% final concentration or FOS solution obtained in this work 2 and 3% final concentration were incorporated to the adequate culture media according to the different experiments requirements. Final pH in the different media was adjusted to 6.5.
1-ketose (96%), nystose (3%) and sucrose (1%) was obtained, as judged by HPLC analysis (Figure 1A). To evaluate microbial FOS fermentation, the use of minimal media appears to be ideal because of the lack of carbohydrates or proteins as alternative energy sources for cell growth. On this basis, the enriched filter-sterilized FOS solution was incorporated to a final concentration of 2 and 3% into solid or liquid minimal Yeast Nitrogen Base (YNB, Invitrogen Co., USA) or minimal M9 (Sigma Co., USA) medium supplemented with 0.05% L-cysteine. Two yeasts (S. boulardii and K. fragilis) and 2 bacterial strains (B. bifidum and B. dentium) were streaked on YNB-FOS or M9-FOS plates and incubated 48 h at 30 or 37°C, respectively, under anaerobic conditions. The assayed yeasts were able to grow in solid or liquid YNB-FOS demonstrating that FOS was equally as good substrate as glucose (BDH), fructose (BDH) and sucrose (BDH) in supporting growth (Figure 1B). Additionally, HPLC analysis confirmed the complete depletion of the FOS fraction after microbial growth (Figure 1C). Unlike yeasts, the two Bifidobacteria and two additional FOS-consumers Lactobacillus strains used as controls, failed to grow in solid or liquid M9-FOS (results not shown). Likely, their limited growth on minimal media may not guarantee the initial production of enzymes for FOS transport inside the cell and further FOS hydrolysis.

Lactobacilli and Bifidobacteria, organisms that are generally considered to be desirable members of the colonic microbiota, release mainly lactic and acetic acids during FOS catabolism under anaerobic conditions (Collins and Gibson, 1999; Kaplan and Hutkins, 2000; 2003) so, provoking acidification of the culture medium reaching pH values below 5. As shown above, these bacteria are unable to grow in minimal media due to their high nutritional requirements. These characteristics prompted us to test whether the addition of an adequate pH indicator as Bromothymol blue (transition interval pH 6.0 to 7.6: yellow-blue) to the LB media supplemented with 2% FOS could produce visible changes in the medium depending on the bacterium ability to FOS consumption. As shown in Table 1 or Figures 2A and B, the growth of 10 of the screened bacterial strains, in solid and liquid LB media turned the medium color from initial green (pH 6.5) to yellow indicating acidification, pH below 6, due to FOS consumption (Table 1). On the other hand, when FOS was not added, the medium color turned to blue revealing alkalization due to ammonia release from utilization of the nitrogen-containing carbon sources in yeast extract and peptone so, pH values raised up from initial 6.5 to 8 (Table 1). The same results were obtained when five yeast strains were assayed (Figures 3A and B). Additionally, the use of YNB-FOS minimal medium supplemented with Bromothimol blue revealed also, by simple visual inspection, the capacity to consume these short chain carbohydrates by these yeasts (results not shown). As expected, the enteric bacterium E. coli and the methylotrophic yeast P. pastoris, which are unable to use FOS, turned the medium color to blue raising the pH values over 8 (Table 1) due to also utilization of the nitrogen-containing carbon sources in yeast extract and peptone.

FOS depletion was further verified by HPLC analysis demonstrating that the assayed bacteria and yeasts consumed totally the GF2 (1-ketose) and GF3 (nystose) fractions (results not shown). In addition, no accumulation of other monosaccharide was seen which suggest that they were also degraded by the microorganisms. These results agree with previous reports dealing with efficient consumption of GF2, GF3 together with mono and disaccharide fractions (Saulnier et al., 2007).

**DISCUSSION**

The intestinal flora is part of a complex ecosystem and many of its beneficial constituents remain unidentified despite there is strong evidence about their influences over the immune system of both, human and animals (Kohler et al., 2003; Patterson, 2005). FOS stimulates selectively the growth and activity of this beneficial ecosystem in the colon providing positive health effects and well-being in humans and animals. However, there are not reports regarding to rapid and accurate qualitative detection methods to get new potential fructooligosaccharides-fermenting yeast or bacterial strains based on consumption of these short chain carbohydrates widely used as prebiotics (Roberfroid, 1998; Tokunaga, 2004). During evaluation of microbial growth in FOS containing media, the use of FOS commercial preparations with different polymerization degrees, together with the presence of background levels of other carbohydrates and the use of rich media have been two of the major drawbacks that undoubtedly mask final results. So, it is not possible that, the implementation of an accurate screening procedure based on FOS consumption under these circumstances. Several bacterial strains, previously identified as FOS non-fermenters, grew in MRS-FOS broth (Kaplan and Hutkins, 2000), likely due to the catabolic oxidation of the yeast extract and peptone media constituents instead of FOS as carbon source.

In this research a direct qualitative screening method that overcomes these two problems is proposed. Firstly, the enriched 96% FOS preparation composed mainly by GF2 (1-ketose) and GF3 (nystose) is useful as a carbon source since guarantee that microbial growth is mainly due to FOS consumption as unique carbon source. Additionally, this FOS preparation has further commercial application as a food or feed additive to stimulate several potential probiotics strains in the gut or in new symbiotic designs.

Secondly, the addition of an innocuous pH indicator like Bromothymol blue (transition interval pH 6.0 to 7.6: yellow-blue) to any of the used media supplemented with2% FOS produced rapid and visible changes in the
Figure 1. FOS utilization by two yeasts currently used as probiotic. A) HPLC profile of FOS solution (96% purity) used in this and subsequent experiments. Letters represent nystose (GF3), 1-kestose (GF2), and remaining sucrose (GF). B) Growth in liquid minimal YNB medium supplemented with 2% sucrose, 2% fructose, 2% glucose, or 2% FOS. Culture growth was followed by measuring dry cell weight (DCW) at different time points during 35 h. C) HPLC profile of culture media samples containing 2% FOS before and after microbial growth. Letters represent: GF3: nystose, GF2: 1-kestose, GF: sucrose.
Table 1. pH values reached after growth in FOS-LB or YP rich media of bacterial and yeast strains screened with this method able or not to metabolize FOS as unique carbon source.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Source</th>
<th>pH FOS(+)</th>
<th>FOS(-)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Saccharomyces boulardii</em> L/254/96</td>
<td>Commercial preparation</td>
<td>3.44 ±0.02</td>
<td>8.02 ±0.03</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em> L/25-7-82</td>
<td>Honey</td>
<td>3.35 ±0.06</td>
<td>8.22 ±0.02</td>
</tr>
<tr>
<td><em>Saccharomyces cerevisiae</em> L/25-7-76</td>
<td>Honey</td>
<td>3.30 ±0.04</td>
<td>8.01 ±0.02</td>
</tr>
<tr>
<td><em>Kluyveromyces fragilis</em> L/12-8-1</td>
<td>Sugarcane industrial process</td>
<td>3.42 ±0.06</td>
<td>8.06 ±0.05</td>
</tr>
<tr>
<td><em>Kluyveromyces fragilis</em> L/12-8-6</td>
<td>Sugarcane industrial process</td>
<td>3.40 ±0.03</td>
<td>8.03 ±0.04</td>
</tr>
<tr>
<td><em>Lactobacillus acidophilus</em> B/103-5</td>
<td>yogurt</td>
<td>4.14 ±0.06</td>
<td>8.31 ±0.02</td>
</tr>
<tr>
<td><em>Lactobacillus rhamnosus</em> B/103-1-5</td>
<td>pigs faecal blend</td>
<td>4.45 ±0.04</td>
<td>8.33 ±0.01</td>
</tr>
<tr>
<td><em>Lactobacillus reuteri</em> B/108-1</td>
<td>human faecal blend</td>
<td>4.09 ±0.02</td>
<td>8.03 ±0.04</td>
</tr>
<tr>
<td><em>Lactobacillus fermentum</em> B/103-11-3</td>
<td>human faecal blend</td>
<td>4.01 ±0.05</td>
<td>7.98 ±0.03</td>
</tr>
<tr>
<td><em>Lactobacillus casei</em> B/103-11-6</td>
<td>human faecal blend</td>
<td>4.51 ±0.02</td>
<td>8.13 ±0.02</td>
</tr>
<tr>
<td><em>Lactobacillus paracasei</em> B/103-11-7</td>
<td>Cheese process</td>
<td>4.48 ±0.04</td>
<td>8.32 ±0.06</td>
</tr>
<tr>
<td><em>Lactobacillus bulgaricus</em> B/103-12-6</td>
<td>Cheese process</td>
<td>5.19 ±0.01</td>
<td>7.94 ±0.05</td>
</tr>
<tr>
<td><em>Streptococcus thermophilus</em> B/103-12-7</td>
<td>Cheese process</td>
<td>5.31 ±0.03</td>
<td>8.03 ±0.04</td>
</tr>
<tr>
<td><em>Bifidobacterium bifidum</em> 15696</td>
<td>ATCC</td>
<td>4.35 ±0.04</td>
<td>8.06 ±0.06</td>
</tr>
<tr>
<td><em>Bifidobacterium dentium</em> 27678</td>
<td>ATCC</td>
<td>4.32 ±0.02</td>
<td>8.04 ±0.03</td>
</tr>
<tr>
<td><em>Escherichia coli</em> TOP10F</td>
<td>Invitrogen SA</td>
<td>8.52 ±0.02</td>
<td>8.31 ±0.04</td>
</tr>
<tr>
<td><em>Pichia pastoris</em> GS115</td>
<td>Invitrogen SA</td>
<td>8.44 ±0.01</td>
<td>8.43 ±0.03</td>
</tr>
<tr>
<td><em>Pichia pastoris</em> X33</td>
<td>Invitrogen SA</td>
<td>8.46 ±0.04</td>
<td>8.42 ±0.05</td>
</tr>
</tbody>
</table>

Figure 2. Growth of five of the assayed bacteria: *L. paracasei* (Lp), *rhamnosus* (Lr), *acidophilus* (La), *B. bifidum* (Bb) and *dentium* (Bd) under anaerobic conditions at 37°C in a covered 12 wells plates with solid (A) and liquid (B) LB medium supplemented or not with 2% FOS and 0.025% (final concentration) of the pH indicator Bromothymol blue and initial pH 6.5. The FOS non-consumer enteric bacterium *Escherichia coli* were used as negative control.
medium color depending on the bacterium or yeast ability to consume or not FOS as unique carbon source. Fifteen of the assayed strains were able to switch to yellow the media color due to FOS consumption. According to the results described above, the proposed screening method is a valuable tool in large-scale detection of pure cultures of potential FOS fermenters—yeast or bacterial strains.

Conflict of interests

The author(s) did not declare any conflict of interest.

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