

## Use of dicloxacillin for the isolation and counting of *Bifidobacteria* from dairy products

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**Summary** — The use of the antibiotic dicloxacillin for isolating and counting the *Bifidobacteria* present in fermented milks is described. The addition of 2 µg/ml of dicloxacillin to TPY medium was found to inhibit the growth of *Lactobacilli* and *Streptococci* whereas most *Bifidobacteria* grew well. TPY medium was found to be more suitable than MRS agar to select *Bifidobacteria*.

**selective media / *Bifidobacteria* / fermented milk**

**Résumé** — **Emploi de la dicloxacilline pour l'isolement et le dénombrement des bifidobactéries dans les produits laitiers.** L'utilisation de l'antibiotique dicloxacilline pour isoler et compter les bifidobactéries présentes dans les produits laitiers fermentés est décrite. L'addition de 2 µg/ml de dicloxacilline au milieu TPY s'est révélée efficace pour inhiber totalement la croissance des lactobacilles et des streptocoques, mais par contre sans effet négatif sur la plupart des bifidobactéries. Le milieu TPY s'est révélé plus adéquat que le milieu MRS agar pour la sélection des bifidobactéries.

**milieu sélectif / bifidobactérie / lait fermenté**

*Bifidobacteria* are Gram-positive, non-sporing anaerobic rods. Generally of irregular shape, the rods are bifurcated or club-shaped with cell protuberances and branchings (Scardovi, 1986). The typical habitat of this bacterial group is human and animal intestinal tract, where they play a protective role against intestinal infections caused by *Salmonella*, *Shigella* and *Escherichia coli* enteropathogens (Sandine *et al*, 1972; Poupard *et al*,

1973). In order to establish a proper identification at genus level, fructose-6-phosphate phosphoketolase, the key enzyme of "bifid shunt" (Lauer and Kandler, 1976) must be determined in cellular extracts. Species identification requires DNA-DNA hybridization (Scardovi, 1986). For the purpose of proper and correct identification, various attempts have been made to obtain a selective medium for isolating *Bifidobacteria* from other intestinal micro-

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organisms, especially *Streptococci* and *Lactobacilli*. A number of differential media have been suggested based on the addition of several antimicrobial compounds to complex media (Teraguchi *et al*, 1978; Resnick and Levin, 1981; Munoa and Pares, 1988; Cole and Fuller, 1989; Wijsman *et al*, 1989). None of these media have proven to be sufficiently selective as all were found to inhibit part of the viable bifidobacterial population.

The problem of properly enumerating *Bifidobacteria* in dairy products containing *Lactobacilli* and *Streptococci* as well as *Bifidobacteria* themselves, has become an important issue over the last 10 years as *Bifidobacteria* have become widely employed in preparing fermented milks (Rašić and Kurmann, 1983). The NPLN agar medium proposed by Teraguchi *et al* (1978), which contains lithium chloride, nalidixic acid, neomycin sulphate and paranomycin sulphate as antimicrobial agents, has, to date, been the most suitable for this purpose. The preparation of this medium is, however, extremely time-consuming and entails the use of 24 components. In consideration of this drawback and given the *Bifidobacteria* sensitivity to various antimicrobial agents as reported by Sutter and Finegold (1976) and Matteuzzi *et al* (1983) the authors have screened for an antibiotic capable of being active on the *Lactobacilli* and on the *Streptococci* employed in dairy products, especially fermented milks, so as to quickly isolate, identify and count *Bifidobacteria*.

The antimicrobial activity of 30 antibiotics toward the above-mentioned bacteria has been examined using MRS (Merck no 10661) and TPY (Scardovi, 1986) media. The afore-mentioned compounds were examined at 0.5, 1.0, 2.0 and 3.0 µg/ml concentrations. Of the tested compounds,

only nafcillin and dicloxacillin were found to possess lower MIC (minimal inhibitory concentration) towards *Streptococci* and *Lactobacilli* than to *Bifidobacteria*. Subsequent testing showed that dicloxacillin was more effective than nafcillin for *Bifidobacteria* selection. Table I shows dicloxacillin MIC values towards 35 strains, mainly of human origin, of *Bifidobacteria* as well as towards the major species of *Lactobacilli* and *Streptococci* used in fermented milks. Clearly then, only 1 out of the 36 *Bifidobacteria* strains examined are inhibited by dicloxacillin at a concentration of 1 µg/ml. For all other strains, the MIC values were equal to or more than 3 µg/ml. All strains of lactic acid bacteria of the species *Lactobacillus acidophilus*, *L. bulgaricus*, *L. helveticus*, *Streptococcus thermophilus* and *S. cremoris* showed MIC values equal to or less than 1.5 µg/ml. Inhibition of the *L. casei* species takes place at concentrations of 2.0 and 2.5 µg/ml. Table II illustrates the efficacy of dicloxacillin at a concentration value of 2 µg/ml in the selection of *Bifidobacteria* present in some commercial fermented milk samples. It is clear from this table that the use of dicloxacillin allows the identification and the count of the *Bifidobacteria* present. Other tests conducted by us (data not reported in this paper) have shown that MRS or TPY media added with dicloxacillin at a concentration of 2 µg/ml behave similarly to the NPLN agar medium tested by Teraguchi *et al* (1978). The latter however, as has already been noted, is particularly difficult and complicated to prepare. In conclusion therefore, it can be stated that the addition of dicloxacillin at a concentration of 2 µg/ml to MRS or, better still, to TPY media can most certainly be recommended for *Bifidobacteria* isolation and count in fermented milks or dairy products in general.

**Table I.** Comparative activities of dicloxacillin against bifidobacteria and some species of lactic acid bacteria.*Activité comparée de la dicloxacilline sur les bifidobactéries et sur quelques espèces de bactéries lactiques.*

| <i>Species</i>                              | <i>Strains</i>   | <i>Source</i>    | <i>MIC (µg/ml)</i> |
|---|--|------------------|--------------------|
| <i>Bifidobacterium longum</i>               | ATCC 15707   | adult intestine  | > 5                |
|   | ATCC 15708   | infant intestine | > 5                |
|   | * B1672 – B1678 – B2055  | infant intestine | ≥ 3                |
|   | B2407 – B2432 – B2664<br>B2577   |                  |                    |
| <i>Bifidobacterium breve</i>                | ATCC 15700   | infant intestine | > 5                |
|   | B622, B626, B632   | infant intestine | ≥ 4                |
|   | B644, B648, B1303  |                  |                    |
| <i>Bifidobacterium bifidum</i>              | B1958, B1968   | infant intestine | ≥ 5                |
|   | B2004, B2531   |                  |                    |
|   | B2412  | infant intestine | 1                  |
| <i>Bifidobacterium adolescentis</i>         | ATCC 15705, ATCC 15706   | adult feces      | 3                  |
|   | *A202–16, A216–21  | infant feces     | 3                  |
|   | A201–3, *115–7   |                  |                    |
| <i>Bifidobacterium infantis</i>             | B1269  | infant feces     | 4                  |
| <i>Bifidobacterium animalis</i>             | ATCC 27536   | chicken feces    | > 5                |
| <i>Bifidobacterium boum</i>                 | ATCC 27917   | cattle rumen     | 3                  |
| <i>Bifidobacterium thermophilum</i>         | ATCC25866, *RU294, RU458   | cattle rumen     | ≥ 4                |
|   | * F333, F454, F466   | sewage           | ≥ 4                |
| <i>Lactobacillus acidophilus</i>            | La1, La3, La10, Lal3899  | dairy products   | ≤ 1.5              |
|   | **D212, **N5, **CS2,<br>**T125a, *Y14  | animal intestine | 1.0                |
|   | DSM20552, DSM20079,<br>NRRL B–15260  | adult intestine  | ≤ 1.5              |
|   |  | adult intestine  | 1.0                |
| <i>Lactobacillus bulgaricus</i>             | YL1, YL2, YL5, YL7   | dairy products   | 0.5                |
|   | LB2, LB50  |                  |                    |
| <i>Lactobacillus bulgaricus</i><br>"filant" | LFi 1, LFi 5   | dairy products   | 0.5                |
|   | LFi12, LFi 20  |                  |                    |
| <i>Streptococcus thermophilus</i>           | YS1, YS3, YS4, YS7, YS8<br>YS10, YS11, YS17, YS20,<br>YS23, YS27, YS28, YS31<br>YS32, YS33, D1 | dairy products   | ≤ 0.5              |

|   |   |                |            |
|---|---|----------------|------------|
| <i>Streptococcus thermophilus</i><br>"filant" | SFi 3, SFi 9, SFi 12,<br>SFi 16, SFi 18, SFi 19,<br>SFi 21, SV3, STV 1,<br>STV 12, STV 20, STB 2, STB 6 | dairy products | ≤ 1        |
| <i>Streptococcus cremoris</i>                 | STB 1, SCB 9  | dairy products | ≤ 1.5      |
| <i>Lactobacillus casei</i>                    | ATCC 7469, NCIB 8001,<br>**SC2, DSM 20024, DSM 20011  | –              | 2.5<br>2.0 |
| <i>Lactobacillus helveticus</i>               | S10.10, S29.9, S30.16<br>S30.18, S30.14, S34.17,<br>S39.4, S40.8  | dairy products | 1.0        |

\* From the collection of the Institute of Agricultural Microbiology, University of Bologna, Bologna, Italy. \*\* From Prof V Bottazzi Faculty of Agriculture, Piacenza – Italy. All other strains derived from International Culture Collections or from our laboratories.

**Table II.** Presence of bifidobacteria, lactic streptococci and lactobacilli on TPY agar containing 2 µg/ml dicloxacillin in some commercial fermented milks.

*Recherche de bifidobactéries, streptocoques lactiques et lactobacilles dans quelques laits fermentés du commerce sur milieu TPY gélosé contenant 2 µg/ml de dicloxacilline.*

| Product  | Streptococci | Lactobacilli | Bifidobacteria |
|--|--------------|--------------|----------------|
| Bio-Danone (Gervais<br>Danone Italiana, Turin) | absent       | absent       | +++            |
| Kyr (Parmalat,<br>Parma, Italy)                | absent       | absent       | +++            |
| Latte Natura<br>(Granarolo, Bologna,<br>Italy) | absent       | absent       | +++            |
| Ofilus (Yoplait,<br>France and Switzerland)    | absent       | absent       | +++            |
| BA (GIE BA, France)                            | absent       | absent       | +++            |
| OH (Chambourcy, France)                        | absent       | absent       | +++            |
| BI (Laiteries de Savoie,<br>France)            | absent       | absent       | +++            |
| Yogurt-Bifidus<br>(Migros, Switzerland)        | absent       | absent       | +++            |
| Fromage-Bifidus<br>(Migros, Switzerland)       | absent       | absent       | +++            |

+++ : Abundant growth (> 1 x 10<sup>5</sup> CFU/ml).

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