Oxygen sensitivity of bifidobacteria isolated from human faeces

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1. Introduction

From faeces samples taken from adult volunteers bifidobacteria were isolated and differentiated by amplified ribosomal DNA restriction analysis (ARDRA) and pulsed field gel electrophoresis (PFGE) (1). Bifidobacteria are obligate anaerobes, however, they differ in their sensitivity towards oxygen. *Bifidobacterium adolescentis* has been described to be more sensitive than *Bifidobacterium infantis, Bifidobacterium breve* and *Bifidobacterium longum* (2). Ahn et al. (3) showed that *B. longum* strains isolated from human faeces samples were less oxygen sensitive than *B. adolescentis* isolated from the same samples. Meile et al. (4) isolated from yogurt a rather oxygen tolerant bifidobacteria strain, which they named as *B. lactis* sp. nov. according to molecular biological analysis. The strain is supposed to be very similar to *Bifidobacterium animalis* DSM 20105/ATTC 27536 (4) and is nowadays designated as *B. animalis* ssp. *lactis* DSM 10140. Survival of probiotic bacteria, especially of bifidobacteria in yogurt depends on temperature, acidity, and sensitivity to oxygen, whereas oxygen permeability of the packaging material does not appear to play a significant role (5-8).

In order to select oxygen tolerant strains from the large number of bifidobacteria isolates, oxygen sensitivity was determined for representatively selected isolates from faeces, which had been differentiated to the strain level. Sensitivity was compared with well described bifidobacteria strains from strain collections.

2. Material and Methods

2.1 Origin of bifidobacteria strains

2.1.1 Strains from culture collections

The 37 bifidobacteria strains which had been obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany, are listed in Table 1 and are identified by DSM numbers. They represent 13 different species or subspecies of the genus *Bifidobacterium*. Strains were stored at –80 °C in the CRYOBANK™ system of Mast Diagnostica, Reinfeld, Germany.

2.1.2 Isolates from faeces

Faeces samples from 20 adult persons were taken in 4 intervals of ca. 2 weeks. From each sample usually several isolates with identical colony morphology were selected,

microscopically inspected and differentiated by ARDRA (11) and PFGE (9, 14), provided these samples could be cultured after initial isolation. Differentiated isolates were stored under the same conditions as described for strains from culture collections.

2.2 Isolation and cultivation of strains

After delivery, faeces samples were either immediately processed for isolation of bacteria or stored over night at 37 °C under anaerobic conditions. 1g of each faeces sample was suspended in 99 ml Ringer solution. This suspension was further diluted in several 1:10 steps. 0.1ml of each dilution (10^{-4} – 10^{-8}) was plated onto AMCTW agar plates (9, 10). Plates were anaerobically incubated for 5 d (if necessary up to 7 d) at 37 °C. Members of the genus *Bifidobacterium* show either 2 to 3 mm large, white colonies with reddish centre or entirely red colonies. After prolonged incubation, stressed organisms may form red colonies with a diameter of just 1 mm. From each faeces sample, colonies showing identical morphology were further differentiated with ARDRA (11) and PFGE (9, 14) after preliminary microscopic assignment to the group of bifidobacteria. (The results on molecular differentiation will be published elsewhere).

2.3 Determination of oxygen sensitivity

Determination of oxygen sensitivity was carried out for DSM strains as well as for a representative selection of isolates from faeces samples. When several identical strains had been isolated from the faeces of one volunteer, oxygen sensitivity was determined for 2 through 6 isolates of this strain. The bifidobacteria were grown anaerobically once or twice in TPY bouillon (12) at 37 °C until the bouillon turned turbid. 0.1 ml of these cultures were transferred to ca. 10 ml of 48 °C warm, molten TPY agar (TPY bouillon plus 1.5% agar) with or without L-cysteine hydrochloride in glass tubes (16x160 ml) and evenly distributed in the molten agar (by vortexing for at least 5 sec). The glass tubes were allowed to cool down until solidification of the agar was complete. Incubation was done aerobically for 3 through 5 d at 37 °C. For determination of oxygen sensitivity the distance between the agar surface and the region of visible bacterial growth (indicated either by turbid agar or by colonies visible in the agar; see Fig. 1) was measured (13).

3. Results and Discussion

Oxygen sensitivity of bifidobacteria was tested with a simple agar diffusion assay (13), as described in Materials and Methods. Fig. 1 shows as examples six glass tubes containing TPY agar incubated with bifidobacteria isolates under aerobic conditions. In the four tubes to the left the clear zone (representing the zone of inhibition) between agar surface and the region of growth, indicated by turbidity of the agar, is easily seen. In the two tubes to the right determination of the growth zone and thus of the zone of inhibition is rather difficult, since only single colonies are visible in the agar.

In Table 1 results on oxygen sensitivity of 37 different DSM strains belonging to 13 species within the genus *Bifidobacterium* are shown. These strains had probably been cultivated many times in the laboratory since the date of their isolation. It is conceivable that they have been in contact with oxygen from the air several times, e. g. during storage and handling (inoculation etc.). One cannot exclude that these repeated contacts have caused adaptation to oxygen and have thus resulted in reduction of oxygen sensitivity.

Fig. 1: Assay for determination of oxygen sensitivity. The agar surface is indicated to the right. The region of growth inhibition is seen between agar surface and dotted lines. Growth is indicated either by turbidity of the agar (four left tubes) or by colonies within the agar (two right tubes). Increased turbidity at the border between growth and inhibition zones is most likely due to better supply with nutrients diffusing from the region of growth inhibition.

The zone of growth inhibition was between 4 to 5 mm for *Bifidobacterium thermophilum, B. animalis* and *Bifidobacterium bifidum* and 13 mm for *Bifidobacterium magnum*. With the exception of *B. adolescentis* no large differences in oxygen sensitivity were observed for different strains the same species. Among the strains with small zones of inhibition (4-6 mm) was strain DSM 10140 (*B. animalis* subsp. *lactis*) which showed a PFGE pattern identical to *B. animalis* DSM 20105/ATTC 27536 (data not shown). It should be noted that strains with PFGE patterns identical to *B. animalis* DSM 20105 had been isolated almost exclusively from probiotic dairy products containing bifidobacteria (14). The low oxygen sensitivity of this strain is probably the reason why it has been used for production of probiotic dairy products, since low oxygen sensitivity results in higher survival rates in products which are stored under conditions where oxygen is not absolutely excluded. Relatively large zones of inhibition (>10 ml) were found for *B. magnum* and *B. adolescentis*. However, of the three *B. adolescentis* strains tested one showed a zone of inhibition of just 5 mm.

Tab. 1: Oxygen sensitivity of strains from culture collection, measured as distance [mm] of the region of visible growth from agar surface

Species and strains	inhibition [mm]	Species and strains	inhibition [mm]
B. adolescentis		*B. angulatum:*	
DSM 20083T	5	DSM 20098T *	10
DSM 20086	10	DSM 20225	7
DSM 20087	11		
		B. bifidum	
B. animalis		DSM 20082	5
DSM 20104T (animalis) [1]	6	DSM 20215	7
DSM 20105 ***	5	DSM 20239	6
		DSM 20456T	4
B. animalis (lactis) [1]			
DSM 10140 ***	5	*B. catenulatum*	
		DSM 20103T *	8
B. breve		DSM 20224	9
DSM 20091	8		
DSM 20213T	7	*B. infantis*	
		DSM 20088	9
B. dentium		DSM 20090	6
DSM 20084	8	DSM 20218	9
DSM 20221 **	9	DSM 20223	8
DSM 20436T **	8		
		B. magnum	
B. longum		DSM 20220	13
DSM 20097	5	DSM 20222T	12
DSM 20219	9		
DSM 20211 (suis) [1]	7	*B. pseudolongum*	
		DSM 20092T (globosum) [1]	9
B. pseudocatenulatum		DSM 20094 (pseudol.) [1]	6
DSM 20438	9	DSM 20095 (pseudol.) [1]	7
DSM 20439	9	DSM 20099T	7
B. thermophilum			
DSM 20209	4		
DSM 20210T	6		
DSM 20212	5		

*, **, *** Strains indicated by identical numbers of asterisks are identical according to PFGE (see (14) for reference).

[1] Species names in brackets indicated subspecies.

In Table 2 the data on oxygen sensitivity of bifidobacteria isolated from human faeces are listed. Faeces samples were obtained from 20 different volunteers at different times (see Materials and Methods). For these isolates, exposure to oxygen during isolation, cultivation and storage was reduced to a minimum. In the first column of the table species are listed based on differentiation by ARDRA and PFGE. Strain designations shown in the next column indicate identical strains whenever they are attributed to one species. In the third column the range of inhibition zones is presented. Samples with numbers 062 to 071 had been spiked with *B. bifidum* 94151. From volunteers 2 (042B4 and 042B5) and 5 (045C1 to 045C5) *B. animalis* strains were isolated which showed PFGE patterns

identical to those of strains DSM 20105/ATTC 27536 or DSM 10140, respectively. The isolates, which represent just one strain, showed low oxygen sensitivity with inhibition zones of just 4 to 5 mm. In contrast, *B. adolescentis* isolates exhibited zones of inhibition larger than 10 mm. *B. adolescentis* apparently is characterized by rather high oxygen sensitivity. A summarizing overview over oxygen sensitivity of the different *Bifidobacterium* species is shown in Table 3. Values from Tables 1 and 2 were combined and compared. Three groups of oxygen sensitivity were defined based on the size of the inhibition zone. Low oxygen sensitivity is characterized by inhibition zones between 4 and 7 mm, medium sensitivity by inhibition zones between 6 and 10 mm, and high oxygen sensitivity by inhibition zones larger than 10 mm. The overlap between low and medium sensitivity is caused by the fact that oxygen sensitivity of isolates belonging to the same strain (as defined by identical PFGE patterns) show some variation. E.g. the *B. longum* isolates obtained from volunteer 9 are identical and show a range of sensitivity between 4 and 7 mm (low range sensitivity), whereas the *B. longum* isolates obtained from volunteer 14 are also identical but show a range of sensitivity between 6 and 8 mm (medium range sensitivity). All *B. animalis* isolates exhibited low oxygen sensitivity. This is true for strains from strains collections as well as for those isolated from faeces. However, it has to be noted that of the five strains/isolates tested, four appeared to be identical according to PFGE. *B. bifidum* strain 94151 had been isolated from tablets. It also shows low oxygen sensitivity which corresponds to the sensitivity of strains of this species obtained from culture collections. In contrast the two isolates from faeces exhibited medium oxygen sensitivity. The same appeared to be true for *B. longum* and *B. adolescentis*. Due to the low numbers of strains from culture collections available for analysis, however, the conclusion that strains from culture collections are generally less sensitive to oxygen must be made with care. Most strains isolated belonged to the species *B. longum* and the strains tested showed medium to high oxygen sensitivity. According our results *B. adolescentis* strains appear to belong mostly to the group of high oxygen sensitivity.

Tab. 2: Oxygen sensitivity of bifidobacteria isolated from faeces of 20 healthy human volunteers

Species and strains	Isolates tested	Inhibition zone [mm]
Volunteer 1:		
B. adolescentis	041A1, 091A3	12
B. longum	001A1, 001B1, 021A2	8-10
Volunteer 2:		
B. adolescentis	022B1, 072A2	10-15
B. adolescentis	042A4, 072A3, 072A5	13
B. bifidum	002A1, 002A5, 002B1, 022C3	6- 8
B. longum	002A2, 002B3, 022A1, 042A3, 072A1	10-12
B. pseudocatenulatum	022C1	11
B. pseudocatenulatum	042B1	10
B. animalis	042B4, 042B5	4
Volunteer 3:		
B. longum	003A2, 003B5, 023A1, 043A1	10
B. pseudocatenulatum	003A4, 003B1, 073B2, 073B4	9-13
B. bifidum (94151) [1]	066A1, 066B1, 066B2	5- 6

Tab. 2 continued

Species and strains	Isolates tested	Inhibition zone [mm]
Volunteer 4:		
B. longum	004A1, 024A1, 024B1	4- 7
B. pseudocatenulatum	004B1, 044A1, 044B1, 062A1, 074B2	8-12
B. bifidum (94151) [1]	062B1	5
Volunteer 5:		
B. adolescentis	025B1, 025B3	15
B. adolescentis	075B2	13
B. longum	025A1a, 025A5, 045B1	8-10
B. animalis	045C1, 045C2	4- 5
B. bifidum (94151) [1]	067A1, 067A3	6
Volunteer 6:		
B. bifidum	046B2, 064C4	7- 8
B. longum	046B1, 076B1, 076B2	7- 9
B. pseudocatenulatum	006A1, 026A1, 046A1, 064B1, 076A1	8-10
B. bifidum (94151) [1]	064A1, 064C1	5- 6
Volunteer 7:		
B. longum	007A2, 027B2, 047B1, 065A1	7- 9
B. pseudocatenulatum	007A1, 027A1, 047A1, 047A2, 077B1, 077B4	7-10
Volunteer 8:		
B. longum	008A2, 008B5, 078A1	10-12
B. longum	048A1, 048B1, 078A2	7- 8
Volunteer 9:		
B. adolescentis	049B1	10
B. longum	009A1, 029A1, 049A1, 063B1	5- 7
B. bifidum (94151) [1]	063A1, 063A3	5
Volunteer 10:		
B. adolescentis	080B4	11
B. longum	010A1, 030A1, 050B2, 050B5	9-11
Volunteer 11:		
B. adolescentis	011C1, 031B4, 051B1, 081A2	10-12
B. longum	051C3, 081B1, 081B3, 081C1	7- 9
B. longum	011A4, 081A3	8- 9
B. longum	068B3, 068B4	9
B. pseudocatenulatum	051C3, 081B1, 081B3, 081C1	7- 9
B. bifidum (94151) [1]	068A1	5
Volunteer 12:		
B. longum	012A1, 012B1, 032A1, 82A1	9-12
Volunteer 13:		
B. adolescentis	069B2	12
B. adolescentis	069B3	8

Tab. 2 continued

Species and strains	Isolates tested	Inhibition zone [mm]
Volunteer 13:		
B. longum	013A1, 013B3, 053A1, 053B4, 069B1, 083B1	10-13
B. longum	013A3, 013B2	7
B. longum	033A1, 083A1	10-12
B. bifidum (94151) [1]	069A1	6
Volunteer 14:		
B. adolesecentis	034A3, 084C3, 084C4	8-13
B. longum	014A1, 014A4	10-15
B. longum	014A2, 014B1, 084A1, 084A5	10-14
B. longum	034A1, 054A1	10-11
B. longum	054A2, 084B3	10
Volunteer 15:		
B. longum	055A1, 070B2, 85A1	6- 8
B. bifidum (94151) [1]	070A1, 070B1	5
Volunteer 16:		
B. adolescentis	036A2, 036B4, 056A1, 086A3	12-13
B. adolescentis	036A4, 036B5, 086A5	10-11
B. longum	036B1	6
B. longum	086B2	6
Volunteer 17:		
B. adolescentis	057A1, 087A4	10-11
B. adolescentis	057A2, 087A3	10-11
B. longum	017A4, 037A1, 087B1	8-10
B. longum	057B1, 057B2	10
Volunteer 18:		
B. longum	018A1, 038A1	9-11
B. longum	018A2, 058A1, 058A6,	6-10
B. longum	018C1	14
B. longum	018B4, 038A2, 058A2	9-10
B. pseudocatenulatum	018B1, 038B2, 038B3	11-14
Volunteer 19:		
B. adolescentis	039B1, 089A1, 089B1	13-15
B. longum	019A1, 039A1, 059B1	10-12
B. longum	019A4, 059A1	9-11
Volunteer 20:		
B. adolescentis	090A4, 090A5	11
B. longum	020A1, 040A9, 090A2	10-12
B. longum	060B1, 060B2	9-10

[1] Faeces samples had been spiked with this strain.

Tab. 3: Comparison of oxygen sensitivity between strains from culture collection and isolates from human faeces[1]

Species	4 – 7 mm		6 – 10 mm		> 10 mm	
B. adolescentis[2]	(1)	0	(1)	3	(1)	15
B. bifidum	(4)	0	(0)	2	(0)	0
B. longum	(1)	1	(0)	22	(1)	13
B. pseudocatenulatum	(0)	0	(2)	5	(0)	6
B. animalis	(3)	1	(0)	0	(0)	0

[1] Numbers of strains shown represent different PFGE patterns; isolates with identical PFGE patterns have been combined to one strain. Since isolates belonging to one strain show some variation in oxygen sensitivity, the three groups are overlapping to some extend.

[2] Numbers of strains from culture collection are shown in brackets.

Tab. 4: **Comparison of oxygen sensitivity of isolates from human faeces in TPY-medium with or without addition of 0,05 % cysteine hydrochloride**

Species	Isolate	zone of inhibition [mm] with cysteine hydrochloride	zone of inhibition [mm] without cysteine hydrochloride
B. adolescentis	031B4	6	10
B. adolescentis	069B2	10	12
B. adolescentis	072A5	6	13
B. adolescentis	086A5	6	11
B. adolescentis	084C4	8	13
B. animalis	042B5	2	4
B. animalis	045C2	4	5
B. bifidum	002A5	4	6
B. longum	013A3	5	7
B. longum	013B3	7	11
B. longum	054A2	7	10
B. longum	040A9	6	10
B. longum	078A2	4	8
B. longum	058A6	5	10
B. longum	058A1	3	6
B. longum	068B4	4	9
B. longum	014A4	6	10
B. pseudocatenulatum	044A1	7	8
B. pseudocatenulatum	074B2	7	9
B. pseudocatenulatum	077B4	5	7
B. bifidum	94151*****	5	6
B. boum	DSM 20432T******	6	8
B. denticolens	DSM 10105******	7	10
B. gallicum	DSM 20093**	5	8
B. indicum	DSM 20214T******	4	6
B. minimum	DSM 20102T******	2	3

***** Isolate from tablets
****** DSM strains

TPY agar (11) is a medium frequently applied for isolation and cultivation of bifidobacteria. Since these microorganisms require anaerobic conditions for growth, cultivation media contain in addition to nutrients and growth promoters cysteine hydrochloride as oxygen scavenger. Therefore, it was interesting to see how effective this substance would be in our test system on oxygen sensitivity. The results are listed in Table 4 for some selected bifidobacteria from human faeces as well as for some strains from culture collections and for one isolate from tablets. As expected, presence of cysteine hydrochloride reduced oxygen sensitivity of basically all isolates/strains analysed. In some cases the sizes of zones of inhibition were reduced by cysteine hydrochloride to less than half of the sizes in the absence of this substance. Presence of cysteine hydrochloride, however, often caused a continuous transition between inhibition zone and growth zone. This made determinations of sizes of the inhibition zones rather difficult.

In conclusion, our results indicate that *Bifidobacterium* species differ in their sensitivity to oxygen. While *B. animalis* appears to be rather insensitive, *B. adolescentis* appears to be highly sensitive. This may be the reason why *B. animalis* has been frequently added to dairy products labelled “probiotic”. If oxygen tolerance is indeed considered to be an important technological prerequisite potential probiotic bifidobacteria have to meet, our results indicate that strains of other species, e.g. *B. longum*, may be promising candidates for testing probiotic properties. The species *B. longum* appears to be characterized by medium to high oxygen sensitivity. However, one of the isolated strains shows low oxygen sensitivity. This could be a candidate. Furthermore, since most bifidobacteria from culture collections belong to the low sensitivity group, adaptation to oxygen appears to be rather common among bifidobacteria, at least among those tested in this study. Thus, strains with known probiotic properties may be adapted to elevated levels of oxygen. However, it remains to be demonstrated that adaptation to oxygen does not negatively affect the probiotic properties of those strains.

4. Literatur

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5. Summary

Engel, G., Rösch, N. , Heller, K.J.: **Oxygen sensitivity of bifidobacteria isolated from human faeces.** Kieler Milchwirtschaftliche Forschungsberichte **58** (2) 81-92 (2006)

26 Microbiology (faeces, bifidobacteria, oxygen sensitivity)

Bifidobacteria were isolated from faeces samples obtained from 20 adult volunteers and were differentiated by amplified ribosomal DNA restriction analysis and pulsed field gel electrophoresis. Representative isolates were tested for oxygen sensitivity and compared to 37 bifidobacteria strains from cultures collections representing 13 species. According to the sizes of inhibition zones, i.e. distance between agar surface and visible growth in the agar column, three groups with respect to oxygen sensitivity were formed. The group with low sensitivity was characterized by inhibition zones ranging from 4 to 7 mm, the group with medium sensitivity by inhibition zones of 6 to 10 mm, and the group with high sensitivity by inhibition zones larger than 10 mm. *B. adolescentis* was found to belong to the latter group, whereas strains of *B. animalis* were rather insensitive to oxygen. *B. animalis ssp. lactis* DSM 20105, also exhibiting low oxygen sensitivity, had been shown to be identical with most isolates from probiotic yoghurts. Since other isolates from human faeces are characterised by medium to high sensitivity, this apparently indicates that the rather low oxygen sensitivity of *B. animalis ssp. lactis* DSM 20105 was the reason for its frequent application in dairy products.

Comparison of oxygen sensitivities between strains freshly isolated from faeces samples and those obtained from culture collections indicated that frequent cultivation in collections associated with handling under oxygen-containing conditions may have resulted in reduction in oxygen sensitivity. However, according to the low number of strains tested, this conclusion must be considered preliminary.

Addition of 0,05 % cysteine hydrochloride to the growth medium resulted in reduced oxygen sensitivity. However, determination of the sizes of inhibition zones was difficult in the presence of this oxygen scavenger, since it caused a fluid transition from inhibition to growth zone.

Zusammenfassung

Engel, G., Rösch, N. , Heller, K.J.: **Sauerstoffempfindlichkeit von Bifidobakterien aus Human-Faeces.** Kieler Milchwirtschaftliche Forschungsberichte **58** (2) 81-92 (2006)

26 Mikrobiologie (Faeces, Bifidobakterien, Sauerstoffempfindlichkeit)

Von 20 erwachsenen Personen wurden 4 mal im Abstand von ca. 2 Wochen aus Faecesproben Bifidobakterien isoliert und molekularbiologisch mit ARDRA und PFGE analysiert. Von einer repräsentativen Auswahl dieser Stämme wurde die Sauerstoffempfindlichkeit bestimmt und mit der von 37 Bifidobakterien-Stämmen (13 Arten) aus einer Stammsammlung verglichen. Nach Auswertung der Hemmhofgrößen (Dicke bzw. Höhe der wachstumsfreien Schicht) kristallisierten sich drei verschiedene Gruppen bezüglich der Sensibilität gegenüber Sauerstoff heraus. Mäßig sensitive Stämme wiesen Hemmhöfe zwischen 4 und 7 mm auf, die nächste Gruppe besaß Hemmhöfe zwischen 6 -10 mm und die empfindlichste Gruppe hatte Hemmhöfe, die über 10 mm hoch waren. Zu Letzteren gehörte vor allem *B. adolescentis*. Dagegen war *B. animalis* mit 5-6 mm, einschließlich des Stammes *B. animalis ssp. lactis* DSM 20105 relativ unempfindlich gegen Sauerstoff. Die in der PFGE zu diesem Stamm identischen Isolate wurden von uns fast ausschließlich in probiotischem Joghurt gefunden. Die am häufigsten aus Faeces isolierten Stämme von *B. longum* wiesen ebenso mittlere bis starke Sensibilität gegen Sauerstoff aus, wie die von *B. pseudocatenulatum*. Es ist daher nicht verwunderlich, dass seit Jahren fast ausschließlich ein sauerstoffunempfindlicher Stamm von *B. animalis* als probiotisches Bifidobakterium in fermentierten Sauermilchprodukten eingesetzt wird.

Vergleiche der Sauerstoffempfindlichkeiten zwischen relativ frischen Feacesisolaten und länger gezüchteten Stämmen aus Stammsammlungen deuten darauf hin, dass die fortgeführte Züchtung, mit „handling" in sauerstoffhaltiger Atmosphäre, ein Verlust dieser Empfindlichkeit mit sich führen könnte. Um hierzu jedoch genauere Aussagen machen zu können, müssten noch mehr Stämme aus Stammsammlungen analysiert und mit den Isolaten aus Faeces verglichen werden.

Zusatz von 0,05 % Cysteinhydrochlorid zum Nährmedium führte zu einer Herabsetzung der Sauerstoffempfindlichkeit. Allerdings beeinflusste diese Substanz die exakte Bestimmung der Größe der Hemmzonen, da vereinzelt ein fließender Übergang zwischen Hemm- und Wachstumszone beobachtet wurde.

Résumé

Engel, G., Rösch, N., Heller, K.J.: **Sensibilité à l'oxigène des bifidobactéries provenant de selles humaines.** Kieler Milchwirtschaftliche Forschungsberichte **58** (2) 81-92 (2006)

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4 fois, des bifidobactéries étaient isolées 20 d'échantillons de selles dans un intervalle d'environ 2 semaines et soumises à un examen moléculaire-biologique avec ARDRA (analyse des fragments d restriction de l'ADN ribosomal amplifié et avec ECP (Electrophorèse en Champs Pulsé). La sensibilité à l'oxygène était définie à partir d'une sélection représentative des ces souches, et comparée à celle de 37souches de bifidobactéries (13 espèces) d'une collection de souches. Après l'évaluation des tailles des zones d'inhibition (épaisseur et/ou hauteur de la couche sans croissance), trois

groupes différents se sont cristallisés pour la sensibilité par rapport à l'oxygène. Les souches modérément sensibles avaient des zones d'inhibition entre 4 et 7 mm, le prochain groupe de souches avait des zones d'inhibition entre 6-10 mm, et le groupe le plus sensible des zones d'inhibition d'une hauteur de plus de 10 mm. Surtout tout *B. appartenus adolescentis*. appartenait au dernier groupe. Par contre *B. animalis* (5 6 mm,) y compris *le tronc B. animalis* ssp. *lactis* DSM 20105 étaient relativement insensibles à l'oxygène. Pendant l'Electrophorèse en Champs Pulsé (ECP) des isolats identiques ont été détectés uniquement dans du yaourt probiotique. Les souches de *B. longum* les plus fréquemment isolées des selles ont démontré une sensibilité moyenne à forte à l'oxygène, comme par ex. *celles de B. pseudocatenulatum*. Il n'est pas donc étonnant que depuis des années, presque exclusivement une souche de *B. animalis*, insensible à l'oxygène, est utilisée comme bifidobactérie probiotique dans des produits laitiers fermentés.

Des comparaisons de la sensibilité à l'oxygène entre des échantillons de selles relativement fraîches et des souches cultivées depuis plus longtemps provenant de collections de souches indiquent que la culture continue dans une atmosphère oxygénée pourrait mener à la perte de cette sensibilité. Pour pouvoir faire des déclarations plus précises, il faudrait davantage d'analyses de souches provenant de collections de souches et les comparer aux isolats de selle.

L'addition de 0,05% de cystéine hydrochloride au substrat a conduit à une réduction de la sensibilité à l'oxygène. Toutefois, cette substance a exercé une influence sur la détermination exacte de la taille des zones d'inhibition, comme une transition courante a été observée entre la zone de croissance et d'inhibition.