# DECOMPOSITION OF SOYBEAN OLIGOSACCHARIDES BY INTESTINAL BACTERIA*

## III Comparison of Eighteen Strains of *Escherichia coli* for Consuming Some Mono- and Oligosaccharides

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In the preceding paper [1] we examined the remaining sugars in the culture liquid of 20 strains of *E. coli*, when a sugar mixture obtained by extracting defatted soybean meal with hot ethanol and cold water was included in the medium. We could not find any spots corresponding to monosaccharides or other sugars than the three main oligosaccharides origanally extracted (sucrose, raffinose, and stachyose). This may be either due to scarceness of such sugars or due to disappearance or further change to nonsugar molecules.

Now each pure sugar solution is used instead of the sugar mixture extracted from defatted soybeans. The sugars examined are sucrose, melibiose, raffinose, and stachyose as oligosaccharides, and glucose, galactose, and fructose as related monosaccharides.

### 1. Experimental Procedures

Eighteen strains of *E. coli* were used for comparison, since the strains No. 9 and No. 18 in Table 1 of Part II were dead during storage.

**1.1.** Culture medium

This was essentially the same as in Part II, but each pure sugar was used instead of the sugar mixture from defatted soybean meal. See Table 1.

Table 1. Culture medium of **1.1.**

| | |
|---|---|
| Well water containing | |
| $(NH_4)_2SO_4$ | 0.2 |
| $K_2HPO_4$ | 0.7 |
| $KH_2PO_4$ | 0.3 |
| Peptone | 0.5 |
| $MgSO_4.7H_2O$ | 0.02 |
| Pure sugar* | 0.5 |

* Sucrose, melibiose, raffinose, stachyose; glucose, galactose, or fructose.

**1.2.** Main cultivation

The culture medium (5 ml) containing 0.5% any one of sucrose, melibiose, raffinose, stachyose, glucose, galactose, and fructose taken into test tubes (19 for each sugar to examine 18 strains and to use as the blank, or $19 \times 7 = 133$ test tubes in total) was sterilized with an autoclave. Add 0.5 ml seed culture pre-

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pared as described in **1.3** of Part II. [1] Cultivate at 37° by shaking.

Measure total sugar after 0, 12, 24, and 48 hours for oligosaccharides, and after 4, 8, and 12 hours for monosaccharides. Also the seed culture liquid (without sugar) was cultivated for 48 hours and the total sugar was measured.

**1.3.** Determination of total sugar

Take 0.1 ml culture liquid from each test tube with a pipet sterilized with 70% ethanol. Add 10 ml water. Centrifuge at 4000 rpm for 5 min (the bacteria could not be precipitated completely). Add 1 ml 4% phenol and 5 ml concentrated sulfuric acid to 1 ml supernatant. Cool with water after 10 min. Measure the absorbance at 480 mμ. Develop the color with 1 ml aqueous solution containing 0, 10, 30, 50, 70, 90, and 100 γ glucose with phenol-sulfuric acid reagent similarly to use as the blank and control.

**1.4.** Measurement of bacterial growth

The bacterial growth was measured by the absorbance at 620 mμ. Dilute 2 ml of the culture liquid after 48 hours in case of oligosaccharides (12 hours in case of monosaccharides) with 2 ml of water. Measure the absorbance (620 mμ) by using the liquid of seed culture (without sugar) uninoculated as the blank.

## 2. Results and Discussion

**2.1.** Sugar consumption

See Table 2 and Figs. 1-18 for oligosaccharides. The abscissa shows the time of cultivation and the ordinate is the relative amount of sugar expressed as the absorbacne at 480 mμ.

Table 2 Amounts of total sugar remaining by cultivation of 18 strains of *E. coli* (relative amounts in absorbance)

| No. of strains | Sucrose | | | Melibiose | | | Raffinose | | | Stachyose | | | hr |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 12 | 24 | 48 | 12 | 24 | 48 | 12 | 24 | 48 | 12 | 24 | 48 | |
| 1 | 0.49 | 0.50 | 0.49 | 0.47 | 0.48 | 0.47 | 0.48 | 0.45 | 0.03 | 0.45 | 0.46 | 0.10 | |
| 2 | .49 | .50 | .50 | .47 | .46 | .46 | .48 | .45 | .45 | .43 | .44 | .07 | |
| 3 | .51 | .51 | .50 | .27 | .20 | .13 | .48 | .48 | .48 | .44 | .43 | .46 | |
| 4 | .49 | .50 | .49 | .35 | .20 | .16 | .34 | .35 | .35 | .44 | .46 | .45 | |
| 5 | .51 | .50 | .49 | .45 | .15 | .08 | .48 | .48 | .48 | .45 | .47 | .46 | |
| 6 | .04 | .06 | .03 | .47 | .47 | .46 | .48 | .48 | .48 | .45 | .44 | .08 | |
| 7 | .49 | .51 | .52 | .47 | .47 | .48 | .45 | .44 | .35 | .44 | .46 | .46 | |
| 8 | .50 | .45 | .41 | .38 | .08 | .06 | .45 | .45 | .41 | .45 | .45 | .07 | |
| 10 | .49 | .31 | .05 | .38 | .10 | .07 | .48 | .47 | .41 | .46 | .46 | .44 | |
| 11 | .49 | .51 | .50 | .35 | .09 | .06 | .45 | .41 | .20 | .46 | .45 | .09 | |
| 12 | .50 | .51 | .50 | .46 | .46 | .07 | .48 | .46 | .08 | .44 | .45 | .05 | |
| 13 | .07 | .05 | .07 | .07 | .07 | .07 | .13 | .07 | .06 | .45 | .07 | .05 | |
| 14 | .27 | .08 | .03 | .08 | .05 | .07 | .07 | .06 | .02 | .45 | .45 | .44 | |
| 15 | .49 | .50 | .05 | .05 | .07 | .07 | .10 | .03 | .03 | .45 | .37 | .33 | |
| 16 | .05 | .10 | .05 | .05 | .07 | .07 | .09 | .07 | .02 | .43 | .45 | .45 | |
| 17 | .49 | .14 | .04 | .48 | .30 | .07 | .48 | .48 | .42 | .45 | .44 | .39 | |
| 19 | .37 | .31 | .06 | .48 | .35 | .11 | .48 | .47 | .43 | .44 | .38 | .32 | |
| 20 | .03 | .05 | .05 | .07 | .08 | .07 | .10 | .08 | .03 | .46 | .44 | .43 | |

Thus we cannot say that the consumption of galactosides (melibiose, raffinose, and stachyose) by bacteria goes in similar ways. We may classify the 18 strains by the consumption of these oligosaccharides as in Table 3.

Table 3 Classification of *E. coli* strains according to consumption of oligosaccharides

| | Sucrose | Melibiose | Raffinose | Stachyose |
|---|---|---|---|---|
| (1) Not at all or only slightly consumed in 48 hours | 1, 2, 3, 4, 5, 7, 8, 11, 12 | 1, 2, 6, 7 | 2, 3, 4, 5, 6, 7, 8, 10, 17, 19 | 3, 4, 5, 7, 10, 14, 15, 16, 17, 19, 20 |
| (2) Almost completely consumed in 48 hours | 10, 14, 15, 19 | 3, 12, 17, 19 | 1, 11, 12 | 1, 2, 6, 8, 11, 12 |
| (3) Considerably consumed in 24 hours | 17 | 4, 5, 8, 10, 11 | - | 13 |
| (4) Almost completely consumed in 12 hours | 6, 13, 16, 20 | 13, 14, 15, 16, 20 | 13, 14, 15, 16, 20 | - |

Table 4 Amounts of sugar remaining by cultivation of 18 strains of *E. coli* (relative amounts in absorbance)

| No. of strain | Glucose 4 | Glucose 8 | Glucose 12 | Galactose 4 | Galactose 8 | Galactose 12 | Fructose 4 | Fructose 8 | Fructose 12 |
|---|---|---|---|---|---|---|---|---|---|
| Blank | 0.55 | 0.55 | 0.48 | 0.49 | 0.48 | 0.41 | 0.44 | 0.38 | 0.37 |
| 1 | .42 | .06 | .05 | .45 | .07 | .07 | .40 | .07 | .06 |
| 2 | .47 | .08 | .07 | .48 | .03 | .08 | .37 | .04 | .04 |
| 3 | .45 | .17 | .07 | .43 | .18 | .06 | .41 | .14 | .09 |
| 4 | .39 | .14 | .05 | .43 | .07 | .08 | .32 | .17 | .04 |
| 5 | .42 | .08 | .08 | .44 | .07 | .08 | .35 | .05 | .05 |
| 6 | .41 | .10 | .04 | .40 | .11 | .10 | .38 | .06 | .05 |
| 7 | .38 | .07 | .07 | .42 | .03 | .05 | .33 | .06 | .05 |
| 8 | .35 | .07 | .04 | .38 | .05 | .05 | .40 | .06 | .05 |
| 10 | .43 | .09 | .06 | .41 | .10 | .08 | .33 | .05 | .04 |
| 11 | .37 | .08 | .07 | .42 | .09 | .07 | .32 | .05 | .04 |
| 12 | .49 | .10 | .04 | .45 | .05 | .08 | .37 | .05 | .05 |
| 13 | .37 | .09 | .06 | .49 | .06 | .07 | .40 | .10 | .10 |
| 14 | .35 | .09 | .05 | .40 | .06 | .07 | .34 | .05 | .04 |
| 15 | .33 | .10 | .04 | .40 | .09 | .07 | .43 | .15 | .05 |
| 16 | .32 | .09 | .03 | .40 | .06 | .06 | .33 | .12 | .03 |
| 17 | .32 | .05 | .05 | .42 | .09 | .09 | .35 | .11 | .04 |
| 19 | .55 | .40 | .21 | .46 | .50 | .04 | .38 | .25 | .18 |
| 20 | .12 | .08 | .04 | .19 | .14 | .07 | .17 | .08 | .05 |

See Table 4 for monosaccharides. Thus all the monosaccharides examined (glucose, galactose, and fructose) were consumed equally very rapidly (in 8 hours) by all the strains examined, except by the strain No. 19, in which the consumptions were slower.

Thus if oligosaccharides are hydrolyzed to monosaccharides, the latter will soon be consumed.

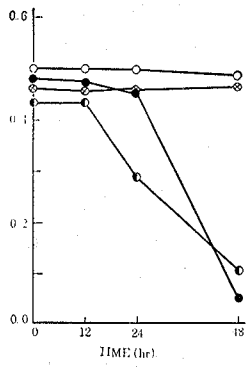


Fig. 1. Strain No. 1.

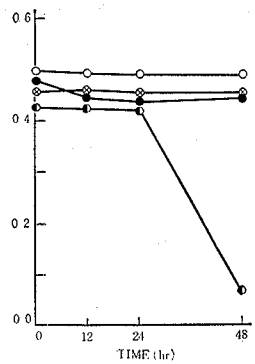


Fig. 2. Strain No. 2.

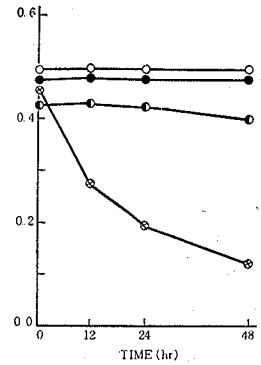


Fig. 3. Strain No. 3.

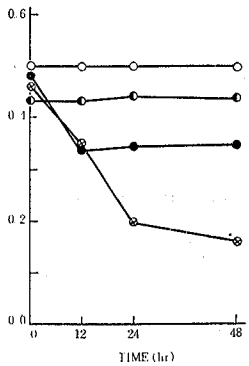


Fig. 4. Strain No. 4.

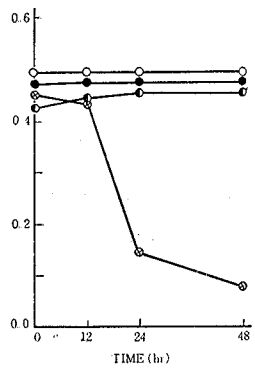


Fig. 5. Strain No. 5.

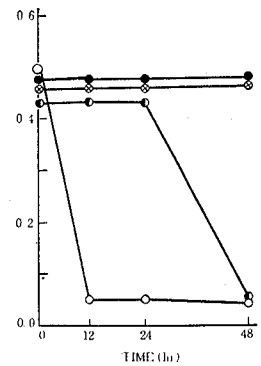


Fig. 6. Strain No. 6.

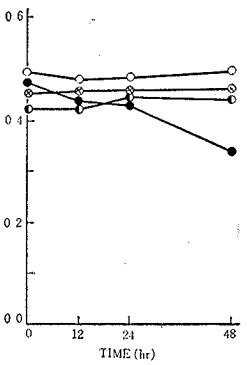


Fig. 7. Strain No. 7.

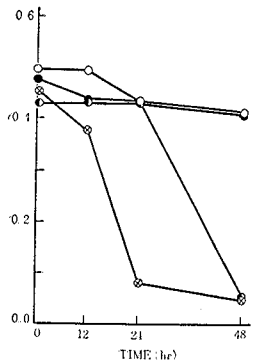


Fig. 8. Strain No. 8.

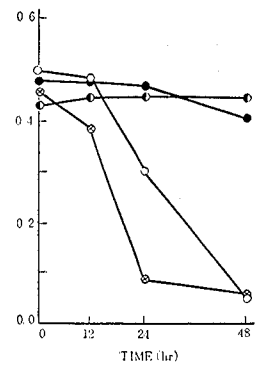


Fig. 9. Strain No. 10.


Figs. 1-9. Decomposition of 4 oligosaccharides by strains Nos. 1-8 and No. 10 of *E. coli*. See the text **2.1.** for explanation.

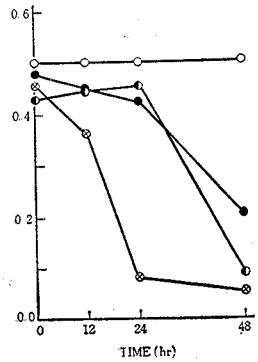


Fig. 10. Strain No. 11 (K-12).

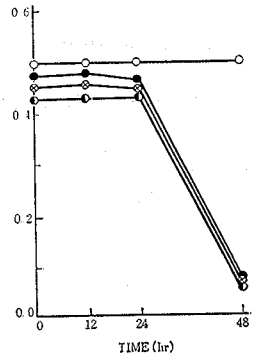


Fig. 11. rtrain No. 12 (B)

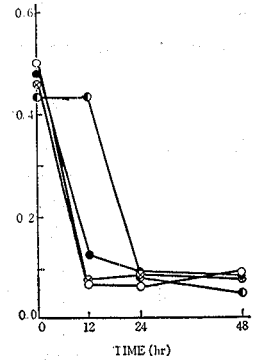


Fig 12. Strain No. 13 (subsp. *communior*)

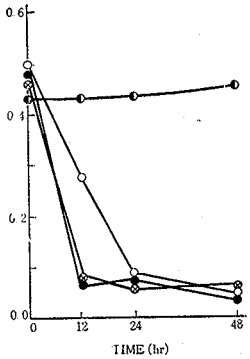


Fig. 13. Strain No. 14 (subsp. *communior*).

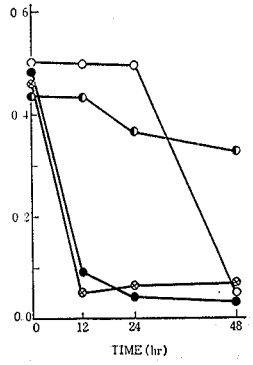


Fig. 14. Strain No. 15 (subsp. *communior*).

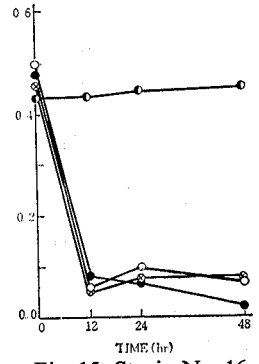


Fig. 15. Strain No. 16.

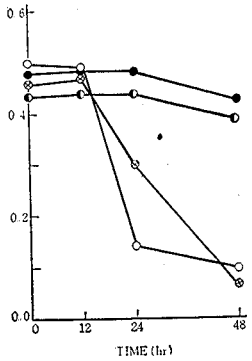


Fig. 16. Strain No. 17.

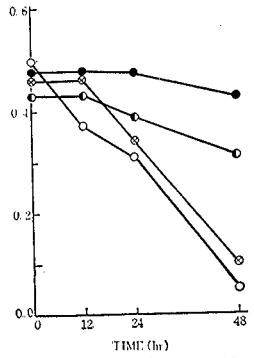


Fig. 17. Strain No. 19.

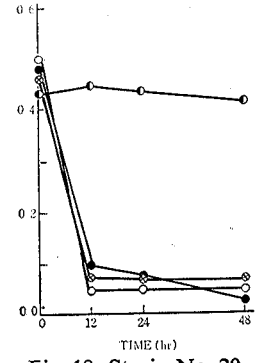


Fig. 18. Strain No. 20.


Figs. 10-18. Decomposition of 4 oligosoccharides by strains Nos. 11-17 and 19-20 of *E. coil*. See the text **2.1.** for explanation.

**2.2.** Bacterial growth

Table 5 shows the absorbance at 620 m$\mu$ of the mixture with one of oligosaccharides after 48 hours'

Table 5 Growth of bacteria on oligoscacharides in 48 hours (absorbance at 620 m$\mu$)

| No. of strain | Blank | Sucrose | Melibiose | Raffinose | Stachyose |
|---|---|---|---|---|---|
| 1 | 0.32 | −0.02 | 0.16 | 1.69 | 1.50 |
| 2 | 0.01 | 0.17 | 0.13 | 0.33 | 1.45 |
| 3 | 0.10 | 0.16 | 0.10 | 0.59 | 0.35 |
| 4 | 0.05 | 0.05 | 0.84 | 1.14 | 0.50 |
| 5 | 0.46 | 0.58 | 1.50 | 0.95 | 0.48 |
| 6 | 0.08 | 1.50 | 0.29 | 0.44 | 1.55 |
| 7 | 0.18 | 0.38 | 0.25 | 1.80 | 0.15 |
| 8 | 0.18 | 0.14 | 1.42 | 0.67 | 1.16 |
| 10 | 0.07 | 0.14 | 1.08 | 0.35 | 0.21 |
| 11 | 0.08 | 0.14 | 1.08 | 0.45 | 1.30 |
| 12 | 0.17 | 0.11 | 1.40 | 1.39 | 1.33 |
| 13 | 0.02 | 1.80 | 1.38 | 1.57 | 1.50 |
| 14 | 0.15 | 1.70 | 1.40 | 1.72 | 0.50 |
| 15 | 0.07 | 1.50 | 1.18 | 1.68 | 0.22 |
| 16 | 0.10 | 1.80 | 1.30 | 1.55 | 0.57 |
| 17 | 0.15 | 1.50 | 1.40 | 1.01 | 0.52 |
| 19 | −0.03 | 1.80 | 1.17 | 0.82 | 0.12 |
| 20 | 0.18 | 0.18 | 1.30 | 1.75 | 0.59 |

Blank means the absorbance of culture liquid without sugar. The values given are in approximate proportion with multiplication of bacteria. Negative values means that the culture liquid was more transparent than the control, or culture medium without bacteria (without sugar).

Table 6 Growth of bacteria on monosaccharides in 12 hours (absorbance at 620 m$\mu$)

| No. of strain | Glucose | Galactose | Fructose |
|---|---|---|---|
| 1 | 0.98 | 0.98 | 0.95 |
| 2 | 0.38 | 0.96 | 0.45 |
| 3 | 0.63 | 0.52 | 0.66 |
| 4 | 0.64 | 0.94 | 0.58 |
| 5 | 0.86 | 0.88 | 0.93 |
| 6 | 0.68 | 0.75 | 1.00 |
| 7 | 1.01 | 1.07 | 1.04 |
| 8 | 0.98 | 0.92 | 1.05 |
| 10 | 1.04 | 0.86 | 1.00 |
| 11 | 0.92 | 0.67 | 0.89 |
| 12 | 0.98 | 0.94 | 0.94 |
| 13 | 1.00 | 0.92 | 1.05 |
| 14 | 0.97 | 0.62 | 0.95 |
| 15 | 0.94 | 0.74 | 0.90 |
| 16 | 0.97 | 0.90 | 0.91 |
| 17 | 0.90 | 0.87 | 0.90 |
| 19 | 0.45 | 0.49 | 0.45 |
| 20 | 1.15 | 0.98 | 1.12 |

cultivation. Table 6 shows the absorbance at 620 mμ with monosaccharides after 12 hours. There were no large difference among the strains nor among the sugars, except for the strains Nos. 2, 3, 4, and 19, the growths of which were slower.

## 3. Summary

All the monosaccharides examined (glucose, galactose, and fructose) were consumed equally very rapidly (in 8 hours) by all the strains (except No. 19). Oilgosaccharides (sucrose, melibiose, raffinose, and stachyose) showed different consumability by 18 strains of *E. coli* (Figs. 1-18 and Table 3).

The growth of bacteria on each sugar was also compared among the strains. In general the strains consuming some sugar rapidly grew well on that sugar.

## Acknowledgments

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A brief summary was presented to the 7th International Congress of Biochemistry, Tokyo, 1967,[2] and an outline of this study was presented before the Annual Meeting of Japanese Biochemical Society at Sakai, Osaka, 1967.[3]

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# 大腸菌による大豆少糖類の分解

## Ⅲ 数種の単糖類および少糖類に対する18株の *Escherichia coli* の比較

川村信一郎，笠井 忠


### 要 旨

研究に用いた単糖類（グルコース，ガラクトース，フルクトース）はすべて一様にすみやかに（8時間振とう）すべての株により分解消費された。(No. 19 ではすこしおそかった.)

研究に用いた少糖類（サッカロース，メリビオース，ラフィノース，スタキオース）は *E.coli* の株により分解能はいろいろちがっていた。図1—18に示してあるが，まとめると第3表のようであった.

なお菌の増殖も比較したが，ある糖をよく分解する株はその糖を含む培地によく増殖した.


この研究は米国農務省委託研究の一部である．菌株についてお世話になった東京大学の有馬啓教授と飯塚広教授，また研究上助言を与えられた本学部の楢崎丁市教授と鈴木裕助教授に感謝する.

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